Aspects of Biological and Organic Chemistry, Particularly Amino Acid, Cyclodextrin, and Free Radical Chemistry

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Abstract

The research described in this thesis comprises mainly aspects of amino acid and free radical chemistry, cyclodextrin chemistry, lipid chemistry and 1,3-dipolar cycloaddition chemistry.

The research on free radical reactions of amino acids and their derivatives has involved studying fundamental aspects of free radical chemistry. The importance of product radical stability and steric, polar and stereoelectronic effects, on the regio- and stereo-selectivity of atom transfer reactions of amino acid derivatives has been delineated. Highlights of this work include an explanation for the anomalous preferential reactivity of glycine derivatives in chemical and biological free radical reactions of amino acid derivatives, and the development of procedures to control the regioselectivity of amino acid functionalisation. The research has resulted in the development of procedures for the regio- and stereo-selective synthesis of amino acid derivatives, including cross-linked, *N*-methylated, deuterated, allylated, β-nitro, α,β-dehydro, methano and hydroxy amino acid derivatives. New and unusual types of neighbouring group effects have been discovered in free radical and ionic reactions of amino acid derivatives.

The chemistry with cyclodextrins has involved their modification, and studies of the use of natural and modified cyclodextrins as hosts in the formation of host-guest complexes. It has been established that host-guest interactions may be altered to meet specific requirements in the complex. Cooperative binding by linked cyclodextrins also leads to host-guest complexes of greater stability, as does simultaneous complexation of the cyclodextrin and the guest to a metal centre. Applications of the modified cyclodextrins have included the development of procedures for their use in the administration of pharmaceuticals, in chiral discrimination, and as templates and molecular reactors.

The lipid chemistry has involved the development of procedures for the identification and characterisation of lipid species, some of which are characteristic of metabolic disorders, and the synthesis of compounds of this type.

Cycloaddition reactions of nitrile oxides have been studied in order to develop synthetic methods, based on controlling the regioselectivity of cycloaddition, limiting side reactions of the nitrile oxides, and finding new procedures for elaboration of the isoxazole and isoxazoline cycloadducts.

Statement

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

Christopher J. Easton, Ph.D.

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List of publications arranged in chronological order

1. "A Stereoelectronic Effect in Hydrogen Atom Abstraction from a Substituted Cyclohexyl Radical", A. L. J. Beckwith and C. Easton, *J. Amer. Chem. Soc.*, **100** (1978), 2913-2914.

2. "Some Guidelines for Radical Reactions", A. L. J. Beckwith, C. J. Easton and A. K. Serelis, *J. Chem. Soc., Chem. Commun.*, (1980), 482-483.

3. "Stereoelectronic Effects in Hydrogen Atom Abstraction from Substituted 1,3-Dioxanes", A. L. J. Beckwith and C. J. Easton, *J. Amer. Chem. Soc.*, **103** (1981), 615-619.

4. "Inhibition of the RTEM β-Lactamase from *Escherichia coli*. Interaction of the Enzyme with Derivatives of Olivanic Acid", C. J. Easton and J. R. Knowles, *Biochemistry*, **21** (1982), 2857-2862.

5. "Reactions of Methyl-Substituted Hex-5-enyl and Pent-4-enyl Radicals", A. L. J. Beckwith, C. J. Easton, T. Lawrence and A. K. Serelis, *Aust. J. Chem.*, **36** (1983), 545-556.

6. "Stereoelectronic Effects in Hydrogen-atom Transfer Reactions of Substituted Cyclohexyl Radicals", A. L. J. Beckwith and C. J. Easton, *J. Chem. Soc., Perkin Trans. II*, (1983), 661-668.

7. "Regioselective Chlorination of *N*-Benzoylvaline Methyl Ester", C. J. Easton and N. J. Bowman, *J. Chem. Soc., Chem. Commun.*, (1983), 1193-1194.

8. "Rearrangement of an Isothiazolidinone to a β-Lactam. A Model for Penicillin Biosynthesis", C. J. Easton, *J. Chem. Soc., Chem. Commun.*, (1983), 1349-1350.

9. "Stereoelectronic Effects in Free-radical Reactions", C. J. Easton, *Science Progress, Oxf.*, **68** (1983), 503-517.

10. "Formation of β-Lactams from 3-Phenylthiopropionamide Derivatives", A. L. J. Beckwith and C. J. Easton, *Tetrahedron*, **39** (1983), 3995-4001.

11. "Correlation of the Effect of β-Lactamase Inhibitors on the β-Lactamase in Growing Cultures of Gram-Negative Bacteria with Their Effect on the Isolated β-Lactamase", C. J. Easton and J. R. Knowles, *Antimicrob. Agents Chemother.*, **26** (1984), 358-363.

12. "Syntheses of β-Lactams by Ring Contraction of Isothiazolidinones", C. J. Easton, *J. Chem. Soc., Perkin Trans. I*, (1985), 153-157.

13. "Regioselectivity in Formations of Amidocarboxy-substituted Free Radicals", C. J. Easton and M. P. Hay, *J. Chem. Soc., Chem. Commun.*, (1985), 425-427.

14. "Synthesis of (2*R*)- and (2*S*)-[3-^{2}H]-Valine", C. J. Easton and N. G. Findlay, *J. Labelled Compounds and Radiopharmaceuticals*, **22** (1985), 667-672.

15. "Preferential Reactivity of Glycine Residues in Free Radical Reactions of Amino Acid Derivatives", C. J. Easton and M. P. Hay, *J. Chem. Soc., Chem. Commun.*, (1986), 55-57.

16. "Direct Introduction of a Benzoyloxy Substituent at the C-4 Position of β-Lactams", C. J. Easton and S. G. Love, *Tetrahedron Lett.*, **27** (1986), 2315-2318.

17. "Selective γ-Hydrogen Atom Abstraction in Reactions of *N*-Acetylamino Acids and *N*-Alkylacetamides with Titanous Ion and Hydrogen Peroxide", V. A. Burgess and C. J. Easton, *Tetrahedron Lett.*, **28** (1987), 2747-2750.

18. "Occurrence of Unusual Molecular Species of Sphingomyelin Containing 28-34-Carbon Polyenoic Fatty Acids in Ram Spermatozoa", A. Poulos, D. W. Johnson, K. Beckman, I. G. White and C. Easton, *Biochem. J.*, **248** (1987), 961-964.

19. "Determination of Phospholipid Base Structure by CA MIKES Mass Spectrometry", C. Easton, D. W. Johnson and A. Poulos, *J. Lipid Res.*, **29** (1988), 109-111.

20. "Regioselective Chlorination of Valine Derivatives", N. J. Bowman, M. P. Hay, S. G. Love and C. J. Easton, *J. Chem. Soc., Perkin Trans. I*, (1988), 259-264.

21. "Regioselective Formation of Amidocarboxy-substituted Free Radicals", C. J. Easton, M. P. Hay and S. G. Love, *J. Chem. Soc., Perkin Trans. I*, (1988), 265-268.

22. "Selective Modification of Glycine Residues in Dipeptides", C. J. Easton, I. M. Scharfbillig and E. W. Tan, *Tetrahedron Lett.*, **29** (1988), 1565-1568.

23. "Dimerization of Glycine Derivatives", V. A. Burgess, C. J. Easton, M. P. Hay and P. J. Steel, *Aust. J. Chem.*, **41** (1988), 701-710.

24. "Reactions of *N*-Methyl-γ-, δ- and ε-lactams with t-Butyl Perbenzoate", C. J. Easton, S. C. Peters and S. G. Love, *Heterocycles*, **27** (1988), 2305-2308.

25. "The Occurrence of Polyenoic Very Long Chain Fatty Acids with Greater than 32 Carbon Atoms in Molecular Species of Phosphatidylcholine in Normal and Peroxisome-Deficient (Zellweger's Syndrome) Brain", A. Poulos, P. Sharp, D. Johnson and C. Easton, *Biochem. J.*, **253** (1988), 645-650.

26. "Reaction of *N*-Benzoyl-2-bromoglycine Methyl Ester with Deprotonated Nitroalkanes: Synthesis of β-Nitro and α,β-Dehydro Amino Acid Derivatives", V. A. Burgess and C. J. Easton, *Aust. J. Chem.*, **41** (1988), 1063-1070.

27. "Selective Reaction of Glycine Residues in Hydrogen Atom Transfer from Amino Acid Derivatives", V. A. Burgess, C. J. Easton and M. P. Hay, *J. Amer. Chem. Soc.*, **111** (1989), 1047-1052.

28. "Bromination of *N*-Phthaloylamino Acid Derivatives", C. J. Easton, E. W. Tan and M. P. Hay, *J. Chem. Soc., Chem. Commun.*, (1989), 385-386.

29. "Crystal and Molecular Structure of *N-p*-Fluorobenzoyl-*L*-valine", J. H. Coates, D. R. Coghlan, C. J. Easton, B. F. Hoskins, S. F. Lincoln and E. R. T. Tiekink, *Z. Krist.*, **188** (1989), 131-138.

30. "Homolytic Allyl Transfer Reactions of 1- and 3-Alkyl-Substituted Allyltributylstannanes", C. J. Easton and I. M. Scharfbillig, *J. Org. Chem.*, **55** (1990), 384-386.

31. "Synthesis of Novel Cross-Linked Amino Acid Derivatives", C. J. Easton and S. C. Peters, *Aust. J. Chem.*, **43** (1990), 87-97.

32. "Acyloxylation at the 4-Position of Azetidin-2-ones", C. J. Easton, S. G. Love and P. Wang, *J. Chem. Soc., Perkin Trans. 1*, (1990), 277-282.

33. "Exocyclic Bromination of *N*-Substituted β- and γ-Lactams", C. J. Easton and M. J. Pitt, *Tetrahedron Lett.*, **31** (1990), 3471-3474.

34. "Monosubstitution of Symmetric Piperazine-2,5-dione Derivatives", T. W. Badran and C. J. Easton, *Aust. J. Chem.*, **43** (1990), 1455-1459.

35. "Methyl (*E*)-3-Benzamido-2-bromoacrylate", C. J. Easton, G. Rositano and E. R. T. Tiekink, *Acta Cryst.*, **C46** (1990), 1121-1122.

36. "A New Synthesis of Cyclodextrin Dimers", J. H. Coates, C. J. Easton, S. J. van Eyk, S. F. Lincoln, B. L. May, C. B. Whalland and M. L. Williams, *J. Chem. Soc., Perkin Trans. 1*, (1990), 2619-2620.

37. "Synthesis of Homochiral Hydroxy-α-Amino Acid Derivatives", C. J. Easton, C. A. Hutton, E. W. Tan and E. R. T. Tiekink, *Tetrahedron Lett.*, **31** (1990), 7059-7062.

38. "Synthesis and Molecular Structure of Stable Derivatives of (*E*)- and (Z)-Dehydrophenylalanine", C. J. Easton, C. A. Hutton, P. D. Roselt and E. R. T. Tiekink, *Aust. J. Chem.*, **44** (1991), 687-694.

39. "Cyclodextrin Inclusion Complexes of Two Non-Steroidal Antiinflammatory Drugs and of an Analgesic Drug", S. E. Brown, J. H. Coates, C. J. Easton, S. F. Lincoln, Y. Luo and A. K. W. Stephens, *Aust. J. Chem.*, **44** (1991), 855-862.

40. "Chiral Differentiation in the Deacylation of 6^{A}-*O*-(2-(4-(2-Methylpropyl)-phenyl)-propanoyl)-β-cyclodextrin", J. H. Coates, C. J. Easton, S. J. van Eyk, B. L. May, P. Singh and S. F. Lincoln, *J. Chem. Soc., Chem. Commun.*, (1991), 759-760.

41. "Chiral Molecular Recognition. A ^{19}F Nuclear Magnetic Resonance Study of the Diastereomer Inclusion Complexes formed between Fluorinated Amino Acid Derivatives and α-Cyclodextrin in Aqueous Solution", S. E. Brown, J. H. Coates, S. F. Lincoln, D. R. Coghlan and C. J. Easton, *J. Chem. Soc., Faraday Trans.*, **87** (1991), 2699-2703.

42. "Regioselective Functionalization of *N*-Phthaloyl-Substituted Amino Acid and Peptide Derivatives", C. J. Easton, C. A. Hutton, G. Rositano and E. W. Tan, *J. Org. Chem.*, **56** (1991), 5614-5618.

43. "An EPR Study of Polar Effects in Radical Reactions of *N*-Acylamino Acid Derivatives", V. A. Burgess and C. J . Easton, *Spectroscopy Lett.*, **24** (1991), 1059-1070.

44. "*N*-Methylation of Carbamate Derivatives of α-Amino Acids", C. J. Easton, K. Kociuba and S. C. Peters, *J. Chem. Soc., Chem. Commun.*, (1991), 1475-1476.

45. "α-Carbon-Centered Radicals from Amino Acids and Their Derivatives", C. J. Easton, in "Advances in Detailed Reaction Mechanisms", Vol. 1, ed. J. M. Coxon, JAI Press, 1991, pp. 83-126.

46. "Crystal Structure of (Z)-1-Methoxycarbonyl-2-phenyl-1-phthalimidocyclopropane", C. J. Easton, I. B. Mahadevan, E. R. T. Tiekink and C. M. Ward, *Z. Krist.*, **198** (1992), 142-144.

47. "Crystal Structure of *meso*-Bis(1,4-dimethylpiperazine-2,5-dione-3-yl)", C. J. Easton, G. Rositano and E. R. T. Tiekink, *Z. Krist.*, **198** (1992), 145-147.

48. "Crystal Structure of Methyl 2-Phthalimido-3-methylbut-2-enoate", C. J. Easton, E. W. Tan and E. R. T. Tiekink, *Z. Krist.*, **198** (1992), 155-157.

49. "Synthesis of Cyclopropyl Amino Acid Derivatives", C. J. Easton, E. W. Tan and C. M. Ward, *Aust. J. Chem.*, **45** (1992), 395-402.

50. "Crystal and Molecular Structure of *N*-Ethyl-1,8-naphthalimide", C. J. Easton, J. M. Gulbis, B. F. Hoskins, I. M. Scharfbillig and E. R. T. Tiekink, *Z. Krist.*, **199** (1992), 249-254.

51. "Reactions of α-Substituted Glycine Derivatives with Stannanes", C. J. Easton and S. C. Peters, *Tetrahedron Lett.*, **33** (1992), 5581-5584.

52. "Stereoelectronic Effects in the Halogenation of *N*-Alkylnaphthalimides", C. J. Easton, I. M. Scharfbillig and E. R. T. Tiekink, *Tetrahedron Lett.*, **33** (1992), 5613-5616.

53. "Nickel Peroxide as a Glycine-selective Chemical Model of Peptidylglycine α-Amidating Monooxygenase", C. J. Easton, S. K. Eichinger and M. J. Pitt, *J. Chem. Soc., Chem. Commun.*, (1992), 1295-1296.

54. "Crystal Structure of 2-Benzamido-4-hydroxy-2,4,4-trimethylbutanoic Acid γ-Lactone", D. R. Coghlan, C. J. Easton and E. R. T. Tiekink, *Z. Krist.*, **203** (1993), 307-309.

55. "Crystal Structure of *N*-Phthaloyl-β-phenylserine Methyl Ester", C. J. Easton, C. A. Hutton and E. R. T. Tiekink, *Z. Krist.*, **203** (1993), 310-312.

56. "A New α-Haloglycine Template for the Asymmetric Synthesis of Amino Acid Derivatives", T. W. Badran, C. J. Easton, E. Horn, K. Kociuba, B. L. May, D. M. Schliebs and E. R. T. Tiekink, *Tetrahedron: Asymmetry*, **4** (1993), 197-200.

57. "Substituent Effects and Chiral Discrimination in the Complexation of Benzoic, 4-Methylbenzoic and (*RS*)-2-Phenylpropanoic Acids and Their Conjugate Bases by β-Cyclodextrin and 6^A-Amino-6^A-deoxy-β-cyclodextrin in Aqueous Solution: Potentiometric Titration and ^{1}H Nuclear Magnetic Resonance Spectroscopic Study", S. E. Brown, J. H. Coates, P. A. Duckworth, S. F. Lincoln, C. J. Easton and B. L. May, *J. Chem. Soc., Faraday Trans.*, **89** (1993), 1035-1040.

58. "Synthesis and Properties of 6^A-Amino-6^A-deoxy-α- and -β-cyclodextrin", S. E. Brown, J. H. Coates, D. R. Coghlan, C. J. Easton, S. J. van Eyk, W. Janowski, A. Lepore, S. F. Lincoln, Y. Luo, B. L. May, D. S. Schiesser, P. Wang and M. L. Williams, *Aust. J. Chem.*, **46** (1993), 953-958.

59. "Crystal Structure of 1,4-Dimethyl-3-phthalimidopiperazine-2,5-dione", C. J. Easton, I. M. Scharfbillig and E. R. T. Tiekink, *Z. Krist.*, **205** (1993), 137-139.

60. "Synthesis of Very Long Chain Fatty Acid Methyl Esters", M. R. Kling, C. J. Easton and A. Poulos, *J. Chem. Soc., Perkin Trans. 1*, (1993), 1183-1189.

61. "Formic Acid is a Product of the α-Oxidation of Fatty Acids by Human Skin Fibroblasts: Deficiency of Formic Acid Production in Peroxisome-Deficient

Fibroblasts", A. Poulos, P. Sharp, H. Singh, D. W. Johnson, W. F. Carey and C. Easton, *Biochem. J.*, **292** (1993), 457-461.

62. "Crystal Structure of 1-Acetyl-6-(1-methylethyl)-3-(prop-2-enyl)-2,5-piperazinedione", E. Horn, T. W. Badran, C. J. Easton, D. M. Schliebs and E. R. T. Tiekink, *Z. Krist.*, **207** (1993), 296-298.

63. "Tryptophan Anion Complexes of β–Cyclodextrin (Cyclomaltaheptaose), an Aminopropylamino-β-cyclodextrin and its Enantioslective Nickel(II) Complex", S. E. Brown, J. H. Coates, C. J. Easton, S. J. van Eyk, S. F. Lincoln, B. L. May, M. A. Stile, C. B. Whalland and M. L. Williams, *J. Chem. Soc., Chem. Commun.*, (1994), 47.

64. "Metallocyclodextrins of 6^A-(3-Aminopropylamino)-6^A-deoxy-β-cylodextrin: Their Formation and Enantioselective Complexation of (*R*)- and (*S*)-Tryptophan Anions in Aqueous Solution", S. E. Brown, J. H. Coates, C. J. Easton and S. F. Lincoln, *J. Chem. Soc., Faraday Trans.*, **90** (1994), 739-743.

65. "Reactions of α–Substituted Glycine Derivatives with Stannanes in the Presence of Disulfides", C. J. Easton and S. C. Peters, *Aust. J. Chem.*, **47** (1994), 859-868.

66. "Reversal of Regiochemistry in the Synthesis of Isoxazoles by Nitrile Oxide Cycloadditions", C. J. Easton, C. M. Hughes, E. R. T. Tiekink, C. E. Lubin, G. P. Savage and G. W. Simpson, *Tetrahedron Lett.*, **35** (1994), 3589-3592.

67. "Stereocontrolled Synthesis of β-Hydroxyphenylalanine and β-Hydroxy-tyrosine Derivatives", C. J. Easton, C. A. Hutton, P. D. Roselt and E. R. T. Tiekink, *Tetrahedron*, **50** (1994), 7327-7340.

68. "Yeast-catalysed Reductive Ring-opening of Isoxazoles", C. J. Easton, C. M. Hughes, K. D. Kirby, G. P. Savage, G. W. Simpson and E. R. T. Tiekink, *J. Chem. Soc., Chem. Commun.*, (1994), 2035.

69. "Complementary Diastereoselectivity in the Synthesis and Hydrolysis of Acylated Cyclodextrins", J. H. Coates, C. J. Easton, N. L. Fryer and S. F. Lincoln, *Chem. Lett.*, (1994), 1153-1156.

70. "Crystal Structure of 7-(2,6-Dichlorophenyl)-8-aza-9-oxa-[4.3.0]-bicyclonon-1,7-diene-2-one", C. J. Easton, C. M. Hughes, E. R. T. Tiekink, G. P. Savage and G. W. Simpson, *Z. Krist.*, **209** (1994), 767-768.

71. "Crystal Structure of 9-(2,6-Dichlorophenyl)-8-aza-9-oxa-[4.3.0]-bicyclonon-1,8-diene-2-one", C. J. Easton, C. M. Hughes, E. R. T. Tiekink, G. P. Savage and G. W. Simpson, *Z. Krist.*, **209** (1994), 771-772.

72. "Synthesis of Each Stereoisomer of [3-$^2H_1$]-Phenylalanine and Evaluation of the Stereochemical Course of the Reaction of (*R*)-Phenylalanine with (*S*)-Phenylalanine Ammonia Lyase", C. J. Easton and C. A. Hutton, *J. Chem. Soc., Perkin Trans. 1*, (1994), 3545-3548.

73. "Cycloaddition Reactions of Nitrile Oxides with Alkenes", C. J. Easton, C. M. M. Hughes, G. P. Savage and G. W. Simpson, *Adv. Het. Chem.*, 60 (1994), 261-327.

74. "Aryl Nitrile Oxide Cycloaddition Reactions in the Presence of Baker's Yeast and β-Cyclodextrin", C. J. Easton, C. M. M. Hughes, E. R. T. Tiekink, G. P. Savage and G. W. Simpson, *Tetrahedron Lett.*, **36** (1995), 629-632.

75. "Complexes of Fluorinated Amino Acid Derivatives and Hexakis(2,3,6-tri-*O*-methyl)-α-cyclodextrin in Aqueous Solution. A Fluorine-19 Nuclear Magnetic Resonance Study", S. E. Brown, C. J. Easton and S. F. Lincoln, *J. Chem. Res. (S)*, (1995) 2; *J. Chem. Res. (M)* (1995), 173-184.

76. "Amino Substituents as a Probe of Reactions of Phenyl Acetates with Cyclodextrins", C. J. Easton, S. Kassara, S. F. Lincoln and B. L. May, *Aust. J. Chem.*, **48** (1995), 267-277.

77. "Complexation of Fluorinated Amino Acid Derivatives by β-and γ-Cyclodextrin in Aqueous Solution. A ^{19}F Nuclear Magnetic Resonance Study", S. E. Brown, C. J. Easton and S. F. Lincoln, *Aust. J. Chem.*, **48** (1995), 505-513.

78. "Complexation of Phenylalanine and Histidine by β-Cyclodextrin, 6^A-(3-Aminopropylamino)-6^A-deoxy-β-cyclodextrin and its Metallocyclodextrins in Aqueous Solution", S. E. Brown, C. A. Haskard, C. J . Easton and S. F. Lincoln, *J. Chem. Soc., Faraday Trans.*, **91** (1995), 1013-1018.

79. "Crystal Structure of α-Amino-α-(2,6-dichlorophenyl)-cyclohexane-2,6-dione", C. J. Easton, C. M. Hughes, E. R. T. Tiekink, K. D. Kirby, G. P. Savage and G. W. Simpson, *Z. Krist.*, **210** (1995), 231-232.

80. "Crystal Structure of (*Z*)-*N*-Phthaloyl-2,3-dehydrophenylalanine Methyl Ester", C. J. Easton, C. A. Hutton, P. D. Roselt and E. R. T. Tiekink, *Z. Krist.*, **210** (1995), 233-234.

81. "Copper-catalysed Reactions of Penicillin Derivatives with *t*-Butyl Perbenzoate", B. M. Clark, C. J. Easton and S. K. Watkins, *Aust. J. Chem.*, **48** (1995), 1065-1070.

82. "Complexation of Benzoic, 4-Methylbenzoic, and (*R*)- and (*S*)-2-Phenylpropanoic Acids and Their Conjugate Bases by 3^A-Amino-3^A-deoxy-(2^AS,3^AS)-β-cyclodextrin in Aqueous Solution", R. Dhillon, C. J. Easton, S. F. Lincoln and J. Papageorgiou, *Aust. J. Chem.*, **48** (1995), 1117-1124.

83. "Cyclodextrin and Termethylated Cyclodextrin Complexation of Aromatic Carboxylic Acids and Their Conjugate Bases in Aqueous Solution. The Effect of Size, Hydrophobicity and Charge", K. Hendrickson, C. J. Easton and S. F. Lincoln, *Aust. J. Chem.*, **48** (1995), 1125-1132.

84. "High Enantioselectivity in the Reactions of (*R*)- and (*S*)-1-Phenylethylamine with 6^A-Deoxy-6^A-iodo-β-cyclodextrin", C. J. Easton, S. F. Lincoln and D. M. Schliebs, *J. Chem. Soc., Chem. Commun.*, (1995), 1167-1168.

85. "Synthesis of Side-chain Functionalised Amino Acid Derivatives Through Reaction of Alkyl Nitronates with α-Bromoglycine Derivatives", C. J. Easton, P. D. Roselt and E. R. T. Tiekink, *Tetrahedron*, **51** (1995), 7809-7822.

86. "Directing Bromination of Piperazine-2,5-diones", T. W. Badran, C. L. L. Chai, C. J. Easton, J. B. Harper and D. M. Page, *Aust. J. Chem.*, **48** (1995), 1379-1384.

87. "Crystal Structure of 4,5-Bis(2,6-dichlorophenyl)-1-oxide-2-oxa-1,3-diazole", C. J. Easton, C. M. Hughes, E. R. T. Tiekink, G. P. Savage and G. W. Simpson, *Z. Krist.*, **210** (1995), 625-626.

88. "Complexes of 2- and 4-Fluorobenzoate Anions and the Corresponding Methyl Esters with β-Cyclodextrin and the Conjugate Acids of 6^A-Amino-6^A-deoxy-β-cyclodextrin and 3^A-Amino-3^A-deoxy-(2^AS,3^AS)-β-cyclodextrin in Aqueous Solution: A Fluorine-19 Nuclear Magnetic Resonance Study", C. J. Easton, S. F. Lincon, J. Papageorgiou and D. M. Schliebs, *J. Chem. Res. (S)*, (1995), 381; *J. Chem. Res. (M)*, (1995), 2337-2346.

89. "Use of Cyclodextrins to Limit Product Inhibition of (*S*)-Phenylalanine Ammonia Lyase", C. J. Easton, J. B. Harper and S. F. Lincoln, *J. Chem. Soc., Perkin Trans I*, (1995), 2525-2526.

90. "Crystal Structure of 5-(2,6-Dichlorophenyl)-4-ethoxycarbonyl-3-phenyl-Δ^2-isoxazoline", C. J. Easton, C. M. M. Hughes, E. R. T. Tiekink, G. P. Savage and G. W. Simpson, *Z. Krist.*, **210** (1995), 699-700.

91. "Athelstan L. J. Beckwith", C. J. Easton, *Aust. J. Chem.*, **48** (1995), 153-154.

92. "Functionalisation of Pyrrolidin-2-ones at C4 and C5", C. J. Easton, M. J. Pitt, and C. M. Ward, *Tetrahedron*, **51** (1995), 12781-12790.

93. Formation of Metallo-6^A-((2-(bis(2-aminoethyl)amino)ethyl)amino)-6^A-deoxy-β-cyclodextrins and Their Complexation of Tryptophan in Aqueous Solution", C. A. Haskard, C. J. Easton, B. L. May and S. F. Lincoln, *Inorg. Chem.*, **35** (1996), 1059-1064.

94. "Crystal Structure of 9-(2,6-Dichlorophenyl)-8-aza-7-oxo-[4.3.0]-bicyclonon-1,7-diene-2-one", C. J. Easton, C. M. M. Hughes, E. R. T. Tiekink, G. P. Savage and G. W. Simpson, *Z. Krist.*, **211** (1996), 193-194.

95. "Crystal Structure of Methyl (Z)-2-Benzamido-3-nitroprop-2-enoate", C. J. Easton, P. D. Roselt and E. R. T. Tiekink, *Z. Krist.*, **211** (1996), 195.

96. "Crystal Structure of Methyl (*Z*)-3-Aza-2-benzamido-3-benzoyl-4-methoxycarbonyl-5-methylhept-4-enoate", C. J. Easton, P. D. Roselt and E. R. T. Tiekink, *Z. Krist.*, **211** (1996), 196-197.

97. "Crystal Structure of 3,6-Diethoxycarbonyl-1,4-dimethyl-3,6-epitetrathia-2,5-piperazinedione", C. J. Easton, B. L. May, G. Mercorella and E. R. T. Tiekink, *Z. Krist.*, **211** (1996), 198-199.

98. "Anchimeric Assistance in Hydrogen Atom Transfer Reactions on the Side Chains of Amino Acid Derivatives, C. J. Easton and M. C. Merrett, J. *Amer. Chem. Soc.*, **118** (1996), 3035-3036.

99. "Neighbouring Group Effects Promote Substitution Reactions over Elimination and Provide a Stereocontrolled Route to Chloramphenicol", C. J. Easton, C. A. Hutton, M. C. Merrett and E. R. T. Tiekink, *Tetrahedron*, **52** (1996), 7025-7036.

100. "Crystal Structure of Methyl (Z)-2-Benzamido-3-nitrobut-2-enoate", C. J. Easton, P. D. Roselt and E. R. T. Tiekink, *Z. Krist.*, **211** (1996), 286.

101. "Crystal Structure of (2*RS*,3*RS*)-3-Bromo-*N*-*tert*-butyl-N^{α}-phthaloyl-*p*-nitrophenylalaninamide", C. J. Easton, M. C. Merrett, C. A. Hutton and E. R. T. Tiekink, *Z. Krist.*, **211** (1996), 287-288.

102. "Crystal Structure of (*S*)-3-Hydroxy-*N*-*tert*-butyl-N^{α}-phthaloylvalinamide", C. J. Easton, M. C. Merrett, C. A. Hutton and E. R. T. Tiekink, *Z. Krist.*, **211** (1996), 289-290.

103. "Crystal Structure of (2*S*,3*S*)-3-Bromo-*N*-phthaloyl-*p*-nitrophenylalanine Methyl Ester", C. J. Easton, M. C. Merrett, C. A. Hutton and E. R. T. Tiekink, *Z. Krist.*, **211** (1996), 291-292.

104. "PVC in Perspective", J. O'Connell, C. Easton and G. Simpson, *Search*, **27** (1996), 176-179.

105. "Cooperative Binding of 6-(*p*-Toluidinyl)naphthalene-2-sulfonate by β-Cyclodextrin Dimers", C. A. Haskard, C. J. Easton, B. L. May and S. F. Lincoln, *J. Phys. Chem.*, **100** (1996), 14457-14461.

106. "Chiral Discrimination by Modified Cyclodextrins", C. J. Easton and S. F. Lincoln, *Chem. Soc. Rev.*, (1996), 163-170.

107. "Stereoselective Synthesis of (2*S*,3*S*)-γ-Hydroxyvaline Utilising an Asymmetric Radical Hydrogen Bromide Addition", C. J. Easton and M. C. Merrett, *Tetrahedron*, **53** (1997), 1151-1156.

108. "Free Radical Reactions in the Synthesis of α-Amino Acids and Derivatives", C. J. Easton, *Chem. Rev.*, **97** (1997), 53-82.

109. "Free-Radical Reactions for the Stereoselective Synthesis of Amino Acid Derivatives", C. J. Easton, *Pure Appl. Chem.*, **69** (1997), 489-494.

110. "A Versatile Synthesis of Linked Cyclodextrins", C. J. Easton, S. J. van Eyk, S. F. Lincoln, B. L. May, J. Papageorgiou and M. L. Williams, *Aust. J. Chem.*, **50** (1997), 9-12.

111. "Complexation of Methyl Orange and Tropaeolin 000 No. 2 by β-Cyclodextrin Dimers", C. A. Haskard, B. L. May, T. Kurucsev, S. F. Lincoln and C. J. Easton, *J. Chem. Soc., Faraday Trans.*, **93** (1997), 279-282.

112. "Exploiting the 1,3-Dithiane of 2-Oxopropanenitrile Oxide to Limit Competing Dimerization in 1,3-Dipolar Cycloaddition Reactions", S. J. Barrow, C. J. Easton, G. P. Savage and G. W. Simpson, *Tetrahedron Lett.*, **38** (1997), 2175-2178.

113. "Nitrate Esters in the Generation of Amino Acid Radicals", C. J. Easton, A. J. Ivory and C. A. Smith, *J. Chem. Soc., Perkin Trans. 2*, (1997), 503-507.

114. "Glycine-selective α-Carbon-Nitrogen Bond Cleavage of Dipeptides by Nickel Peroxide", C. J. Easton, S. K. Eichinger and M. J. Pitt, *Tetrahedron*, **53** (1997), 5609-5616.

115. "Cyclodextrins and Modified Forms in Chemistry and Industry", C. J. Easton and S. F. Lincoln, *Chem. in Aust.*, (1997), 3-5 (May).

116. "Complexes of Naproxen and Ibuprofen with 6^{A}-Amino-6^{A}-deoxy-β-cyclodextrin", A. Ashnagar, P. T. Culnane, C. J. Easton, J. B. Harper and S. F. Lincoln, *Aust J. Chem.*, **50** (1997), 447-450.

117. "Inclusion Complexes of the Cyclodextrins", S. F. Lincoln and C. J. Easton, in "Structural Diversity and Functional Versatility of Polysaccharides", ed. S. Dumitriu, Marcel Dekker, Inc., 1997, in press.

118. "Reactions of Amino-substituted Cyclodextrins with 2-Arylpropanoic Acid Derivatives", C. J. Easton, S. F. Lincoln, B. L. May and J. Papageorgiou, *Aust. J. Chem.*, **50** (1997), 451-456.

119. "A Cyclodextrin to Reverse the Regiochemistry in Nitrile Oxide Cycloaddition to a Terminal Alkene", A. G. Meyer, C. J. Easton, S. F. Lincoln and G. W. Simpson, *J. Chem. Soc., Chem. Commun.*, (1997), 1517-1518.

120. "Synthesis of Polyunsaturated β-Oxa Fatty Acids *via* Rhodium Mediated Carbenoid Insertion", M. J. Pitt, C. J. Easton, C. J. Moody, A. Ferrante, A. Poulos and D. A. Rathjen, *Synthesis*, in press.

121. "Preparation and Characterization of 6^A-Polyamine-mono-substituted β-Cyclodextrins", B. L. May, S. D. Kean, C. J. Easton and S. F. Lincoln, *J. Chem. Soc., Perkin Trans. 1*, in press.

List of patent applications

"Compositions and Methods for Drug Delivery and Chromatography", J. H. Coates, C. J. Easton, S. F. Lincoln, S. J. van Eyk, B. L. May, P. Singh, M. L. Williams and M. A. Stile, International Patent Application No. PCT/AU89/00359-WO90/02141;
incorporating Australian Provisional Patent Applications Nos. PJ 0165, PJ 0189, PJ 0618, PJ 1053, PJ 1198, PJ 1417, PJ 4909, PJ 4894, PJ 5034, PJ 5278, PJ 5354, PJ 5576, PJ 5641, PJ 5682 and PJ 8277.
"Compositions and Methods for Drug Delivery and Chromatography", J. H. Coates, C. J. Easton, S. F. Lincoln, S. J. van Eyk, B. L. May, P. Singh, M. L. Williams and M. A. Stile, United States Patent No. 5,324,750 A (1994); BR 89/07625 A; CN 1040987 A; DD 297833 A5; EP 431080 A1; JP 04/500229; ZA 89/06624 A.

"Cyclodextrin Compositions and Methods for Pharmaceutical and Industrial Application", J. H. Coates, C. J. Easton, S. F. Lincoln, S. J. van Eyk, B. L. May, M. L. Williams, S. E. Brown, A. Lepore, M.-L. Liao, Y. Luo, V. Macolino, D. S. Schiesser, P. Singh, C. B. Whalland and I. McKensie, International Patent Application No. PCT/AU91/00071-WO91/13100;
incorporating Australian Provisional Patent Applications Nos. PJ 8899, PJ 8993, PJ 9344, PJ 9373, PJ 9756, PK 1538, PK 1755, PK 2269, PK 3596, PK 3624, PK 4284, PK 4603 and PK 4856.

"New Cyclodextrins and New Formulated Drugs", J. H. Coates, C. J. Easton, S. F. Lincoln, P. A. Duckworth, P. Wang, D. R. Coghlan, W. Janowski, S. E. Brown, B. L. May, D. S. Schiesser, Y. Luo, A. Lepore, M. L. Williams, S. J. van Eyk, C. F. Palmer, P. Ho and N. Dennis, International Patent Application No. PCT/AU93/00100 (1993)-WO93/17711;
incorporating Australian Provisional Patent Applications Nos. PL 1288, PL 1915, PL 2182, PL 3612, PL 3673, PL 3674, PL 3836, PL 4119, PL 4409, PL 4747 and PL 7061.

"New Procedures for the Covalent Attachment of Dyes to Wool Fibre", C. J. Easton and I. M. Scharfbillig, Australian Provisional Patent Application No. PL 8927 (1993).

"Modified Polyunsaturated Fatty Acids", A. Ferrante, A. Poulos, C. J. Easton, M. J. Pitt, T. A. Robertson and D. A. Rathjen, International Patent Application No. PCT/AU95/00677 (1995)-WO96/11908;
incorporating Australian Provisional Patent Application Nos. PM 8781 and PM 9066.

"Methods of Treating Immunopathologies Using Polyunsaturated Fatty Acids", A. Ferrante, A. Poulos, M. J. Pitt, C. J. Easton, M. J. Sleigh, D. A. Rathjen and F. Widmer, International Patent Application (1997);
incorporating Australian Provisional Patent Application Nos. PN 9250 and PN 9538.

List of publications and patent applications classified according to research fields

* Denotes corresponding author (not applicable to patent applications)

Amino Acid and Free Radical Chemistry

* 7. "Regioselective Chlorination of *N*-Benzoylvaline Methyl Ester", C. J. Easton and N. J. Bowman, *J. Chem. Soc., Chem. Commun.*, (1983), 1193-1194.

* 8. "Rearrangement of an Isothiazolidinone to a β-Lactam. A Model for Penicillin Biosynthesis", C. J. Easton, *J. Chem. Soc., Chem. Commun.*, (1983), 1349-1350.

* 9. "Stereoelectronic Effects in Free-radical Reactions", C. J. Easton, *Science Progress, Oxf.*, **68** (1983), 503-517.

* 12. "Syntheses of β-Lactams by Ring Contraction of Isothiazolidinones", C. J. Easton, *J. Chem. Soc., Perkin Trans. I,* (1985), 153-157.

* 13. "Regioselectivity in Formations of Amidocarboxy-substituted Free Radicals", C. J. Easton and M. P. Hay, *J. Chem. Soc., Chem. Commun.*, (1985), 425-427.

* 14. "Synthesis of (2*R*)- and (2*S*)-[3-^{2}H]-Valine", C. J. Easton and N. G. Findlay, *J. Labelled Compounds and Radiopharmaceuticals*, **22** (1985), 667-672.

* 15. "Preferential Reactivity of Glycine Residues in Free Radical Reactions of Amino Acid Derivatives", C. J. Easton and M. P. Hay, *J. Chem. Soc., Chem. Commun.*, (1986), 55-57.

* 16. "Direct Introduction of a Benzoyloxy Substituent at the C-4 Position of β-Lactams", C. J. Easton and S. G. Love, *Tetrahedron Lett.*, **27** (1986), 2315-2318.

* 17. "Selective γ-Hydrogen Atom Abstraction in Reactions of *N*-Acetylamino Acids and *N*-Alkylacetamides with Titanous Ion and Hydrogen Peroxide", V. A. Burgess and C. J. Easton, *Tetrahedron Lett.*, **28** (1987), 2747-2750.

* 20. "Regioselective Chlorination of Valine Derivatives", N. J. Bowman, M. P. Hay, S. G. Love and C. J. Easton, *J. Chem. Soc., Perkin Trans. I*, (1988), 259-264.

* 21. "Regioselective Formation of Amidocarboxy-substituted Free Radicals", C. J. Easton, M. P. Hay and S. G. Love, *J. Chem. Soc., Perkin Trans. I*, (1988), 265-268.

* 22. "Selective Modification of Glycine Residues in Dipeptides", C. J. Easton, I. M. Scharfbillig and E. W. Tan, *Tetrahedron Lett.*, **29** (1988), 1565-1568.

* 23. "Dimerization of Glycine Derivatives", V. A. Burgess, C. J. Easton, M. P. Hay and P. J. Steel, *Aust. J. Chem.*, **41** (1988), 701-710.

* 24. "Reactions of *N*-Methyl-γ-, δ- and ε-lactams with t-Butyl Perbenzoate", C. J. Easton, S. C. Peters and S. G. Love, *Heterocycles*, **27** (1988), 2305-2308.

* 26. "Reaction of *N*-Benzoyl-2-bromoglycine Methyl Ester with Deprotonated Nitroalkanes: Synthesis of β-Nitro and α,β-Dehydro Amino Acid Derivatives", V. A. Burgess and C. J. Easton, *Aust. J. Chem.*, **41** (1988), 1063-1070.

* 27. "Selective Reaction of Glycine Residues in Hydrogen Atom Transfer from Amino Acid Derivatives", V. A. Burgess, C. J. Easton and M. P. Hay, *J. Amer. Chem. Soc.*, **111** (1989), 1047-1052.

* 28. "Bromination of *N*-Phthaloylamino Acid Derivatives", C. J. Easton, E. W. Tan and M. P. Hay, *J. Chem. Soc., Chem. Commun.*, (1989), 385-386.

* 30. "Homolytic Allyl Transfer Reactions of 1- and 3-Alkyl-Substituted Allyltributylstannanes", C. J. Easton and I. M. Scharfbillig, *J. Org. Chem.*, **55** (1990), 384-386.

* 31. "Synthesis of Novel Cross-Linked Amino Acid Derivatives", C. J. Easton and S. C. Peters, *Aust. J. Chem.*, **43** (1990), 87-97.

* 32. "Acyloxylation at the 4-Position of Azetidin-2-ones", C. J. Easton, S. G. Love and P. Wang, *J. Chem. Soc., Perkin Trans. 1*, (1990), 277-282.

* 33. "Exocyclic Bromination of *N*-Substituted β- and γ-Lactams", C. J. Easton and M. J. Pitt, *Tetrahedron Lett.*, **31** (1990), 3471-3474.

* 34. "Monosubstitution of Symmetric Piperazine-2,5-dione Derivatives", T. W. Badran and C. J. Easton, *Aust. J. Chem.*, **43** (1990), 1455-1459.

* 37. "Synthesis of Homochiral Hydroxy-α-Amino Acid Derivatives", C. J. Easton, C. A. Hutton, E. W. Tan and E. R. T. Tiekink, *Tetrahedron Lett.*, **31** (1990), 7059-7062.

* 38. "Synthesis and Molecular Structure of Stable Derivatives of (*E*)- and (*Z*)-Dehydrophenylalanine", C. J. Easton, C. A. Hutton, P. D. Roselt and E. R. T. Tiekink, *Aust. J. Chem.*, **44** (1991), 687-694.

* 42. "Regioselective Functionalization of *N*-Phthaloyl-Substituted Amino Acid and Peptide Derivatives", C. J. Easton, C. A. Hutton, G. Rositano and E. W. Tan, *J. Org. Chem.*, **56** (1991), 5614-5618.

* 43. "An EPR Study of Polar Effects in Radical Reactions of *N*-Acylamino Acid Derivatives", V. A. Burgess and C. J . Easton, *Spectroscopy Lett.*, **24** (1991), 1059-1070.

* 44. "*N*-Methylation of Carbamate Derivatives of α-Amino Acids", C. J. Easton, K. Kociuba and S. C. Peters, *J. Chem. Soc., Chem. Commun.*, (1991), 1475-1476.

* 45. "α-Carbon-Centered Radicals from Amino Acids and Their Derivatives", C. J. Easton, in "Advances in Detailed Reaction Mechanisms", Vol. 1, ed. J. M. Coxon, JAI Press, 1991, pp. 83-126.

* 49. "Synthesis of Cyclopropyl Amino Acid Derivatives", C. J. Easton, E. W. Tan and C. M. Ward, *Aust. J. Chem.*, **45** (1992), 395-402.

* 51. "Reactions of α-Substituted Glycine Derivatives with Stannanes", C. J. Easton and S. C. Peters, *Tetrahedron Lett.*, **33** (1992), 5581-5584.

* 52. "Stereoelectronic Effects in the Halogenation of *N*-Alkylnaphthalimides", C. J. Easton, I. M. Scharfbillig and E. R. T. Tiekink, *Tetrahedron Lett.*, **33** (1992), 5613-5616.

* 53. "Nickel Peroxide as a Glycine-selective Chemical Model of Peptidylglycine α-Amidating Monooxygenase", C. J. Easton, S. K. Eichinger and M. J. Pitt, *J. Chem. Soc., Chem. Commun.*, (1992), 1295-1296.

* 56. "A New α-Haloglycine Template for the Asymmetric Synthesis of Amino Acid Derivatives", T. W. Badran, C. J. Easton, E. Horn, K. Kociuba, B. L. May,

D. M. Schliebs and E. R. T. Tiekink, *Tetrahedron: Asymmetry*, **4** (1993), 197-200.

* 65. "Reactions of α–Substituted Glycine Derivatives with Stannanes in the Presence of Disulfides", C. J. Easton and S. C. Peters, *Aust. J. Chem.*, **47** (1994), 859-868.

* 67. "Stereocontrolled Synthesis of β-Hydroxyphenylalanine and β-Hydroxytyrosine Derivatives", C. J. Easton, C. A. Hutton, P. D. Roselt and E. R. T. Tiekink, *Tetrahedron*, **50** (1994), 7327-7340.

* 72. "Synthesis of Each Stereoisomer of [3-$^2H_1$]-Phenylalanine and Evaluation of the Stereochemical Course of the Reaction of (*R*)-Phenylalanine with (*S*)-Phenylalanine Ammonia Lyase", C. J. Easton and C. A. Hutton, *J. Chem. Soc., Perkin Trans. 1*, (1994), 3545-3548.

* 81. "Copper-catalysed Reactions of Penicillin Derivatives with *t*-Butyl Perbenzoate", B. M. Clark, C. J. Easton and S. K. Watkins, *Aust. J. Chem.*, **48** (1995), 1065-1070.

* 85. "Synthesis of Side-chain Functionalised Amino Acid Derivatives Through Reaction of Alkyl Nitronates with α-Bromoglycine Derivatives", C. J. Easton, P. D. Roselt and E. R. T. Tiekink, *Tetrahedron*, **51** (1995), 7809-7822.

86. "Directing Bromination of Piperazine-2,5-diones", T. W. Badran, C. L. L. Chai, C. J. Easton, J. B. Harper and D. M. Page, *Aust. J. Chem.*, **48** (1995), 1379-1384.

* 92. "Functionalisation of Pyrrolidin-2-ones at C4 and C5", C. J. Easton, M. J. Pitt, and C. M. Ward, *Tetrahedron*, **51** (1995), 12781-12790.

* 98. "Anchimeric Assistance in Hydrogen Atom Transfer Reactions on the Side Chains of Amino Acid Derivatives, C. J. Easton and M. C. Merrett, *J. Amer. Chem. Soc.*, **118** (1996), 3035-3036.

* 99. "Neighbouring Group Effects Promote Substitution Reactions over Elimination and Provide a Stereocontrolled Route to Chloramphenicol", C. J. Easton, C. A. Hutton, M. C. Merrett and E. R. T. Tiekink, *Tetrahedron*, **52** (1996), 7025-7036.

* 107. "Stereoselective Synthesis of (2*S*,3*S*)-γ-Hydroxyvaline Utilising an Asymmetric Radical Hydrogen Bromide Addition", C. J. Easton and M. C. Merrett, *Tetrahedron*, **53** (1997), 1151-1156.

* 108. "Free Radical Reactions in the Synthesis of α-Amino Acids and Derivatives", C. J. Easton, *Chem. Rev.*, **97** (1997), 53-82.

* 109. "Free-Radical Reactions for the Stereoselective Synthesis of Amino Acid Derivatives", C. J. Easton, *Pure Appl. Chem.*, **69** (1997), 489-494.

* 113. "Nitrate Esters in the Generation of Amino Acid Radicals", C. J. Easton, A. J. Ivory and C. A. Smith, *J. Chem. Soc., Perkin Trans. 2*, (1997), 503-507.

* 114. "Glycine-selective α-Carbon-Nitrogen Bond Cleavage of Dipeptides by Nickel Peroxide", C. J. Easton, S. K. Eichinger and M. J. Pitt, *Tetrahedron*, **53** (1997), 5609-5616.

"New Procedures for the Covalent Attachment of Dyes to Wool Fibre", C. J. Easton and I. M. Scharfbillig, Australian Provisional Patent Application No. PL 8927 (1993).

Cyclodextrin Chemistry

* 36. "A New Synthesis of Cyclodextrin Dimers", J. H. Coates, C. J. Easton, S. J. van Eyk, S. F. Lincoln, B. L. May, C. B. Whalland and M. L. Williams, *J. Chem. Soc., Perkin Trans. 1*, (1990), 2619-2620.

39. "Cyclodextrin Inclusion Complexes of Two Non-Steroidal Antiinflammatory Drugs and of an Analgesic Drug", S. E. Brown, J. H. Coates, C. J. Easton, S. F. Lincoln, Y. Luo and A. K. W. Stephens, *Aust. J. Chem.*, **44** (1991), 855-862.

* 40. "Chiral Differentiation in the Deacylation of 6^A-*O*-(2-(4-(2-Methylpropyl)-phenyl)-propanoyl)-β-cyclodextrin", J. H. Coates, C. J. Easton, S. J. van Eyk, B. L. May, P. Singh and S. F. Lincoln, *J. Chem. Soc., Chem. Commun.*, (1991), 759-760.

41. "Chiral Molecular Recognition. A ^{19}F Nuclear Magnetic Resonance Study of the Diastereomer Inclusion Complexes formed between Fluorinated Amino Acid Derivatives and α-Cyclodextrin in Aqueous Solution", S. E. Brown, J. H. Coates, S. F. Lincoln, D. R. Coghlan and C. J. Easton, *J. Chem. Soc., Faraday Trans.*, **87** (1991), 2699-2703.

57. "Substituent Effects and Chiral Discrimination in the Complexation of Benzoic, 4-Methylbenzoic and (*RS*)-2-Phenylpropanoic Acids and Their Conjugate Bases by β-Cyclodextrin and 6^A-Amino-6^A-deoxy-β-cyclodextrin in Aqueous Solution: Potentiometric Titration and ^1H Nuclear Magnetic Resonance Spectroscopic Study", S. E. Brown, J. H. Coates, P. A. Duckworth, S. F. Lincoln, C. J. Easton and B. L. May, *J. Chem. Soc., Faraday Trans.*, **89** (1993), 1035-1040.

* 58. "Synthesis and Properties of 6^A-Amino-6^A-deoxy-α- and -β-cyclodextrin", S. E. Brown, J. H. Coates, D. R. Coghlan, C. J. Easton, S. J. van Eyk, W. Janowski, A. Lepore, S. F. Lincoln, Y. Luo, B. L. May, D. S. Schiesser, P. Wang and M. L. Williams, *Aust. J. Chem.*, **46** (1993), 953-958.

* 63. "Tryptophan Anion Complexes of β–Cyclodextrin (Cyclomaltaheptaose), an Aminopropylamino-β-cyclodextrin and its Enantioslective Nickel(II) Complex", S. E. Brown, J. H. Coates, C. J. Easton, S. J. van Eyk, S. F. Lincoln, B. L. May, M. A. Stile, C. B. Whalland and M. L. Williams, *J. Chem. Soc., Chem. Commun.*, (1994), 47.

64. "Metallocyclodextrins of 6^A-(3-Aminopropylamino)-6^A-deoxy-β-cylodextrin: Their Formation and Enantioselective Complexation of (*R*)- and (*S*)-Tryptophan Anions in Aqueous Solution", S. E. Brown, J. H. Coates, C. J. Easton and S. F. Lincoln, *J. Chem. Soc., Faraday Trans.*, **90** (1994), 739-743.

* 69. "Complementary Diastereoselectivity in the Synthesis and Hydrolysis of Acylated Cyclodextrins", J. H. Coates, C. J. Easton, N. L. Fryer and S. F. Lincoln, *Chem. Lett.*, (1994), 1153-1156.

75. "Complexes of Fluorinated Amino Acid Derivatives and Hexakis(2,3,6-tri-*O*-methyl)-α-cyclodextrin in Aqueous Solution. A Fluorine-19 Nuclear Magnetic Resonance Study", S. E. Brown, C. J. Easton and S. F. Lincoln, *J. Chem. Res. (S)*, (1995) 2; *J. Chem. Res. (M)* (1995), 173-184.

* 76. "Amino Substituents as a Probe of Reactions of Phenyl Acetates with Cyclodextrins", C. J. Easton, S. Kassara, S. F. Lincoln and B. L. May, *Aust. J. Chem.*, **48** (1995), 267-277.

77. "Complexation of Fluorinated Amino Acid Derivatives by β-and γ-Cyclodextrin in Aqueous Solution. A ^{19}F Nuclear Magnetic Resonance Study", S. E. Brown, C. J. Easton and S. F. Lincoln, *Aust. J. Chem.*, **48** (1995), 505-513.

78. "Complexation of Phenylalanine and Histidine by β-Cyclodextrin, 6^A-(3-Aminopropylamino)-6^A-deoxy-β-cyclodextrin and its Metallocyclodextrins in Aqueous Solution", S. E. Brown, C. A. Haskard, C. J . Easton and S. F. Lincoln, *J. Chem. Soc., Faraday Trans.*, **91** (1995), 1013-1018.

82. "Complexation of Benzoic, 4-Methylbenzoic, and (*R*)- and (*S*)-2-Phenylpropanoic Acids and Their Conjugate Bases by 3^A-Amino-3^A-deoxy-(2^AS,3^AS)-β-cyclodextrin in Aqueous Solution", R. Dhillon, C. J. Easton, S. F. Lincoln and J. Papageorgiou, *Aust. J. Chem.*, **48** (1995), 1117-1124.

83. "Cyclodextrin and Termethylated Cyclodextrin Complexation of Aromatic Carboxylic Acids and Their Conjugate Bases in Aqueous Solution. The Effect of Size, Hydrophobicity and Charge", K. Hendrickson, C. J. Easton and S. F. Lincoln, *Aust. J. Chem.*, **48** (1995), 1125-1132.

* 84. "High Enantioselectivity in the Reactions of (*R*)- and (*S*)-1-Phenylethylamine with 6^A-Deoxy-6^A-iodo-β-cyclodextrin", C. J. Easton, S. F. Lincoln and D. M. Schliebs, *J. Chem. Soc., Chem. Commun.*, (1995), 1167-1168.

* 88. "Complexes of 2- and 4-Fluorobenzoate Anions and the Corresponding Methyl Esters with β-Cyclodextrin and the Conjugate Acids of 6^A-Amino-6^A-deoxy-β-cyclodextrin and 3^A-Amino-3^A-deoxy-(2^AS,3^AS)-β-cyclodextrin in Aqueous Solution: A Fluorine-19 Nuclear Magnetic Resonance Study", C. J. Easton, S. F. Lincon, J. Papageorgiou and D. M. Schliebs, *J. Chem. Res. (S)*, (1995), 381; *J. Chem. Res. (M)*, (1995), 2337-2346.

* 89. "Use of Cyclodextrins to Limit Product Inhibition of (*S*)-Phenylalanine Ammonia Lyase", C. J. Easton, J. B. Harper and S. F. Lincoln, *J. Chem. Soc., Perkin Trans I*, (1995), 2525-2526.

93. Formation of Metallo-6^A-((2-(bis(2-aminoethyl)amino)ethyl)amino)-6^A-deoxy-β-cyclodextrins and Their Complexation of Tryptophan in Aqueous Solution", C. A. Haskard, C. J. Easton, B. L. May and S. F. Lincoln, *Inorg. Chem.*, **35** (1996), 1059-1064.

105. "Cooperative Binding of 6-(*p*-Toluidinyl)naphthalene-2-sulfonate by β-Cyclodextrin Dimers", C. A. Haskard, C. J. Easton, B. L. May and S. F. Lincoln, *J. Phys. Chem.*, **100** (1996), 14457-14461.

* 106. "Chiral Discrimination by Modified Cyclodextrins", C. J. Easton and S. F. Lincoln, *Chem. Soc. Rev.*, (1996), 163-170.

* 110. "A Versatile Synthesis of Linked Cyclodextrins", C. J. Easton, S. J. van Eyk, S. F. Lincoln, B. L. May, J. Papageorgiou and M. L. Williams, *Aust. J. Chem.*, **50** (1997), 9-12.

111. "Complexation of Methyl Orange and Tropaeolin 000 No. 2 by β-Cyclodextrin Dimers", C. A. Haskard, B. L. May, T. Kurucsev, S. F. Lincoln and C. J. Easton, *J. Chem. Soc., Faraday Trans.*, **93** (1997), 279-282.

* 115. "Cyclodextrins and Modified Forms in Chemistry and Industry", C. J. Easton and S. F. Lincoln, *Chem. in Aust.*, (1997), 3-5 (May).

* 116. "Complexes of Naproxen and Ibuprofen with 6^A-Amino-6^A-deoxy-β-cyclodextrin", A. Ashnagar, P. T. Culnane, C. J. Easton, J. B. Harper and S. F. Lincoln, *Aust J. Chem.*, **50** (1997), 447-450.

117. "Inclusion Complexes of the Cyclodextrins", S. F. Lincoln and C. J. Easton, in "Structural Diversity and Functional Versatility of Polysaccharides", ed. S. Dumitriu, Marcel Dekker, Inc., 1997, in press.

* 118. "Reactions of Amino-substituted Cyclodextrins with 2-Arylpropanoic Acid Derivatives", C. J. Easton, S. F. Lincoln, B. L. May and J. Papageorgiou, *Aust. J. Chem.*, **50** (1997), 451-456.

* 119. "A Cyclodextrin to Reverse the Regiochemistry in Nitrile Oxide Cycloaddition to a Terminal Alkene", A. G. Meyer, C. J. Easton, S. F. Lincoln and G. W. Simpson, *J. Chem. Soc., Chem. Commun.*, (1997), 1517-1518.

121. "Preparation and Characterization of 6^A-Polyamine-mono-substituted β-Cyclodextrins", B. L. May, S. D. Kean, C. J. Easton and S. F. Lincoln, *J. Chem. Soc., Perkin Trans. 1*, in press.

"Compositions and Methods for Drug Delivery and Chromatography", J. H. Coates, C. J. Easton, S. F. Lincoln, S. J. van Eyk, B. L. May, P. Singh, M. L. Williams and M. A. Stile, International Patent Application No. PCT/AU89/00359-WO90/02141; incorporating Australian Provisional Patent Applications Nos. PJ 0165, PJ 0189, PJ 0618, PJ 1053, PJ 1198, PJ 1417, PJ 4909, PJ 4894, PJ 5034, PJ 5278, PJ 5354, PJ 5576, PJ 5641, PJ 5682 and PJ 8277.
"Compositions and Methods for Drug Delivery and Chromatography", J. H. Coates, C. J. Easton, S. F. Lincoln, S. J. van Eyk, B. L. May, P. Singh, M. L. Williams and M. A. Stile, United States Patent No. 5,324,750 A (1994); BR 89/07625 A; CN 1040987 A; DD 297833 A5; EP 431080 A1; JP 04/500229; ZA 89/06624 A.

"Cyclodextrin Compositions and Methods for Pharmaceutical and Industrial Application", J. H. Coates, C. J. Easton, S. F. Lincoln, S. J. van Eyk, B. L. May, M. L. Williams, S. E. Brown, A. Lepore, M.-L. Liao, Y. Luo, V. Macolino, D. S. Schiesser, P. Singh, C. B. Whalland and I. McKensie, International Patent Application No. PCT/AU91/00071-WO91/13100;
incorporating Australian Provisional Patent Applications Nos. PJ 8899, PJ 8993, PJ 9344, PJ 9373, PJ 9756, PK 1538, PK 1755, PK 2269, PK 3596, PK 3624, PK 4284, PK 4603 and PK 4856.

"New Cyclodextrins and New Formulated Drugs", J. H. Coates, C. J. Easton, S. F. Lincoln, P. A. Duckworth, P. Wang, D. R. Coghlan, W. Janowski, S. E. Brown, B. L. May, D. S. Schiesser, Y. Luo, A. Lepore, M. L. Williams, S. J. van Eyk, C. F. Palmer, P. Ho and N. Dennis, International Patent Application No. PCT/AU93/00100 (1993)-WO93/17711;
incorporating Australian Provisional Patent Applications Nos. PL 1288, PL 1915, PL 2182, PL 3612, PL 3673, PL 3674, PL 3836, PL 4119, PL 4409, PL 4747 and PL 7061.

Lipid Chemistry

18. "Occurrence of Unusual Molecular Species of Sphingomyelin Containing 28-34-Carbon Polyenoic Fatty Acids in Ram Spermatozoa", A. Poulos, D. W. Johnson, K. Beckman, I. G. White and C. Easton, *Biochem. J.*, **248** (1987), 961-964.

* 19. "Determination of Phospholipid Base Structure by CA MIKES Mass Spectrometry", C. Easton, D. W. Johnson and A. Poulos, *J. Lipid Res.*, **29** (1988), 109-111.

25. "The Occurrence of Polyenoic Very Long Chain Fatty Acids with Greater than 32 Carbon Atoms in Molecular Species of Phosphatidylcholine in Normal and Peroxisome-Deficient (Zellweger's Syndrome) Brain", A. Poulos, P. Sharp, D. Johnson and C. Easton, *Biochem. J.*, **253** (1988), 645-650.

* 60. "Synthesis of Very Long Chain Fatty Acid Methyl Esters", M. R. Kling, C. J. Easton and A. Poulos, *J. Chem. Soc., Perkin Trans. 1*, (1993), 1183-1189.

61. "Formic Acid is a Product of the α-Oxidation of Fatty Acids by Human Skin Fibroblasts: Deficiency of Formic Acid Production in Peroxisome-Deficient Fibroblasts", A. Poulos, P. Sharp, H. Singh, D. W. Johnson, W. F. Carey and C. Easton, *Biochem. J.*, **292** (1993), 457-461.

* 120. "Synthesis of Polyunsaturated β-Oxa Fatty Acids *via* Rhodium Mediated Carbenoid Insertion", M. J. Pitt, C. J. Easton, C. J. Moody, A. Ferrante, A. Poulos and D. A. Rathjen, *Synthesis*, in press.

"Modified Polyunsaturated Fatty Acids", A. Ferrante, A. Poulos, C. J. Easton, M. J. Pitt, T. A. Robertson and D. A. Rathjen, International Patent Application No. PCT/AU95/00677 (1995)-WO96/11908;
incorporating Australian Provisional Patent Application Nos. PM 8781 and PM 9066.

"Methods of Treating Immunopathologies Using Polyunsaturated Fatty Acids", A. Ferrante, A. Poulos, M. J. Pitt, C. J. Easton, M. J. Sleigh, D. A. Rathjen and F. Widmer, International Patent Application (1997);
incorporating Australian Provisional Patent Application Nos. PN 9250 and PN 9538.

1,3-Dipolar Cycloaddition Chemistry

66. "Reversal of Regiochemistry in the Synthesis of Isoxazoles by Nitrile Oxide Cycloadditions", C. J. Easton, C. M. Hughes, E. R. T. Tiekink, C. E. Lubin, G. P. Savage and G. W. Simpson, *Tetrahedron Lett.*, **35** (1994), 3589-3592.

68. "Yeast-catalysed Reductive Ring-opening of Isoxazoles", C. J. Easton, C. M. Hughes, K. D. Kirby, G. P. Savage, G. W. Simpson and E. R. T. Tiekink, *J. Chem. Soc., Chem. Commun.*, (1994), 2035.

* 73. "Cycloaddition Reactions of Nitrile Oxides with Alkenes", C. J. Easton, C. M. M. Hughes, G. P. Savage and G. W. Simpson, *Adv. Het. Chem.*, 60 (1994), 261-327.

74. "Aryl Nitrile Oxide Cycloaddition Reactions in the Presence of Baker's Yeast and β-Cyclodextrin", C. J. Easton, C. M. M. Hughes, E. R. T. Tiekink, G. P. Savage and G. W. Simpson, *Tetrahedron Lett.*, **36** (1995), 629-632.

* 112. "Exploiting the 1,3-Dithiane of 2-Oxopropanenitrile Oxide to Limit Competing Dimerization in 1,3-Dipolar Cycloaddition Reactions", S. J. Barrow, C. J. Easton, G. P. Savage and G. W. Simpson, *Tetrahedron Lett.*, **38** (1997), 2175-2178.

X-Ray Crystallography

29. "Crystal and Molecular Structure of *N*-*p*-Fluorobenzoyl-*L*-valine", J. H. Coates, D. R. Coghlan, C. J. Easton, B. F. Hoskins, S. F. Lincoln and E. R. T. Tiekink, *Z. Krist.*, **188** (1989), 131-138.

35. "Methyl (*E*)-3-Benzamido-2-bromoacrylate", C. J. Easton, G. Rositano and E. R. T. Tiekink, *Acta Cryst.*, **C46** (1990), 1121-1122.

46. "Crystal Structure of (*Z*)-1-Methoxycarbonyl-2-phenyl-1-phthalimidocyclopropane", C. J. Easton, I. B. Mahadevan, E. R. T. Tiekink and C. M. Ward, *Z. Krist.*, **198** (1992), 142-144.

47. "Crystal Structure of *meso*-Bis(1,4-dimethylpiperazine-2,5-dione-3-yl)", C. J. Easton, G. Rositano and E. R. T. Tiekink, *Z. Krist.*, **198** (1992), 145-147.

48. "Crystal Structure of Methyl 2-Phthalimido-3-methylbut-2-enoate", C. J. Easton, E. W. Tan and E. R. T. Tiekink, *Z. Krist.*, **198** (1992), 155-157.

50. "Crystal and Molecular Structure of *N*-Ethyl-1,8-naphthalimide", C. J. Easton, J. M. Gulbis, B. F. Hoskins, I. M. Scharfbillig and E. R. T. Tiekink, *Z. Krist.*, **199** (1992), 249-254.

54. "Crystal Structure of 2-Benzamido-4-hydroxy-2,4,4-trimethylbutanoic Acid γ-Lactone", D. R. Coghlan, C. J. Easton and E. R. T. Tiekink, *Z. Krist.*, **203** (1993), 307-309.

55. "Crystal Structure of *N*-Phthaloyl-β-phenylserine Methyl Ester", C. J. Easton, C. A. Hutton and E. R. T. Tiekink, *Z. Krist.*, **203** (1993), 310-312.

59. "Crystal Structure of 1,4-Dimethyl-3-phthalimidopiperazine-2,5-dione", C. J. Easton, I. M. Scharfbillig and E. R. T. Tiekink, *Z. Krist.*, **205** (1993), 137-139.

62. "Crystal Structure of 1-Acetyl-6-(1-methylethyl)-3-(prop-2-enyl)-2,5-piperazinedione", E. Horn, T. W. Badran, C. J. Easton, D. M. Schliebs and E. R. T. Tiekink, *Z. Krist.*, **207** (1993), 296-298.

70. "Crystal Structure of 7-(2,6-Dichlorophenyl)-8-aza-9-oxa-[4.3.0]-bicyclonon-1,7-diene-2-one", C. J. Easton, C. M. Hughes, E. R. T. Tiekink, G. P. Savage and G. W. Simpson, *Z. Krist.*, **209** (1994), 767-768.

71. "Crystal Structure of 9-(2,6-Dichlorophenyl)-8-aza-9-oxa-[4.3.0]-bicyclonon-1,8-diene-2-one", C. J. Easton, C. M. Hughes, E. R. T. Tiekink, G. P. Savage and G. W. Simpson, *Z. Krist.*, **209** (1994), 771-772.

79. "Crystal Structure of α-Amino-α-(2,6-dichlorophenyl)-cyclohexane-2,6-dione", C. J. Easton, C. M. Hughes, E. R. T. Tiekink, K. D. Kirby, G. P. Savage and G. W. Simpson, *Z. Krist.*, **210** (1995), 231-232.

80. "Crystal Structure of (*Z*)-*N*-Phthaloyl-2,3-dehydrophenylalanine Methyl Ester", C. J. Easton, C. A. Hutton, P. D. Roselt and E. R. T. Tiekink, *Z. Krist.*, **210** (1995), 233-234.

87. "Crystal Structure of 4,5-Bis(2,6-dichlorophenyl)-1-oxide-2-oxa-1,3-diazole", C. J. Easton, C. M. Hughes, E. R. T. Tiekink, G. P. Savage and G. W. Simpson, *Z. Krist.*, **210** (1995), 625-626.

90. "Crystal Structure of 5-(2,6-Dichlorophenyl)-4-ethoxycarbonyl-3-phenyl-Δ^2-isoxazoline", C. J. Easton, C. M. M. Hughes, E. R. T. Tiekink, G. P. Savage and G. W. Simpson, *Z. Krist.*, **210** (1995), 699-700.

94. "Crystal Structure of 9-(2,6-Dichlorophenyl)-8-aza-7-oxo-[4.3.0]-bicyclonon-1,7-diene-2-one", C. J. Easton, C. M. M. Hughes, E. R. T. Tiekink, G. P. Savage and G. W. Simpson, *Z. Krist.*, **211** (1996), 193-194.

95. "Crystal Structure of Methyl (*Z*)-2-Benzamido-3-nitroprop-2-enoate", C. J. Easton, P. D. Roselt and E. R. T. Tiekink, *Z. Krist.*, **211** (1996), 195.

96. "Crystal Structure of Methyl (*Z*)-3-Aza-2-benzamido-3-benzoyl-4-methoxycarbonyl-5-methylhept-4-enoate", C. J. Easton, P. D. Roselt and E. R. T. Tiekink, *Z. Krist.*, **211** (1996), 196-197.

97. "Crystal Structure of 3,6-Diethoxycarbonyl-1,4-dimethyl-3,6-epitetrathia-2,5-piperazinedione", C. J. Easton, B. L. May, G. Mercorella and E. R. T. Tiekink, *Z. Krist.*, **211** (1996), 198-199.

100. "Crystal Structure of Methyl (*Z*)-2-Benzamido-3-nitrobut-2-enoate", C. J. Easton, P. D. Roselt and E. R. T. Tiekink, *Z. Krist.*, **211** (1996), 286.

101. "Crystal Structure of (2*RS*,3*RS*)-3-Bromo-*N*-*tert*-butyl-N^{α}-phthaloyl-*p*-nitrophenylalaninamide", C. J. Easton, M. C. Merrett, C. A. Hutton and E. R. T. Tiekink, *Z. Krist.*, **211** (1996), 287-288.

102. "Crystal Structure of (*S*)-3-Hydroxy-*N*-*tert*-butyl-N^{α}-phthaloylvalinamide", C. J. Easton, M. C. Merrett, C. A. Hutton and E. R. T. Tiekink, *Z. Krist.*, **211** (1996), 289-290.

103. "Crystal Structure of (2*S*,3*S*)-3-Bromo-*N*-phthaloyl-*p*-nitrophenylalanine Methyl Ester", C. J. Easton, M. C. Merrett, C. A. Hutton and E. R. T. Tiekink, *Z. Krist.*, **211** (1996), 291-292.

Miscellaneous

* 91. "Athelstan L. J. Beckwith", C. J. Easton, *Aust. J. Chem.*, **48** (1995), 153-154.

104. "PVC in Perspective", J. O'Connell, C. Easton and G. Simpson, *Search*, **27** (1996), 176-179.

Graduate and Postdoctoral Research

1. "A Stereoelectronic Effect in Hydrogen Atom Abstraction from a Substituted Cyclohexyl Radical", A. L. J. Beckwith and C. Easton, *J. Amer. Chem. Soc.*, **100** (1978), 2913-2914.

2. "Some Guidelines for Radical Reactions", A. L. J. Beckwith, C. J. Easton and A. K. Serelis, *J. Chem. Soc., Chem. Commun.*, (1980), 482-483.

3. "Stereoelectronic Effects in Hydrogen Atom Abstraction from Substituted 1,3-Dioxanes", A. L. J. Beckwith and C. J. Easton, *J. Amer. Chem. Soc.*, **103** (1981), 615-619.

4. "Inhibition of the RTEM β-Lactamase from *Escherichia coli*. Interaction of the Enzyme with Derivatives of Olivanic Acid", C. J. Easton and J. R. Knowles, *Biochemistry*, **21** (1982), 2857-2862.

5. "Reactions of Methyl-Substituted Hex-5-enyl and Pent-4-enyl Radicals", A. L. J. Beckwith, C. J. Easton, T. Lawrence and A. K. Serelis, *Aust. J. Chem.*, **36** (1983), 545-556.

6. "Stereoelectronic Effects in Hydrogen-atom Transfer Reactions of Substituted Cyclohexyl Radicals", A. L. J. Beckwith and C. J. Easton, *J. Chem. Soc., Perkin Trans. II*, (1983), 661-668.

10. "Formation of β-Lactams from 3-Phenylthiopropionamide Derivatives", A. L. J. Beckwith and C. J. Easton, *Tetrahedron*, **39** (1983), 3995-4001.

11. "Correlation of the Effect of β-Lactamase Inhibitors on the β-Lactamase in Growing Cultures of Gram-Negative Bacteria with Their Effect on the Isolated β-Lactamase", C. J. Easton and J. R. Knowles, *Antimicrob. Agents Chemother.*, **26** (1984), 358-363.

Outline of the research, the significance of the publications and patent applications, and percentage contribution

On the preceding pages, publications and patent applications are listed in chronological order. The listing is then repeated under the classifications of amino acid and free radical chemistry, cyclodextrin chemistry, lipid chemistry, 1,3-dipolar cycloaddition chemistry, X-ray crystallography, miscellaneous, and graduate and postdoctoral research. An outline of the research and the significance of the publications and patent applications, and a statement of percentage contribution in each of these areas, are given below.

Amino Acid and Free Radical Chemistry

This area of research has involved studies of free radical reactions of amino acids, small peptides and related compounds, including lactams and imides which have similar functionality. The work has comprised studying fundamental aspects of free radical reactions of these compounds, in order to probe biochemical systems and develop synthetic methods.

The initial research involved the use of model compounds to examine the mechanism of penicillin biosynthesis from Arnstein's tripeptide.[7,8] In the area of lactam chemistry, this resulted in the development of a new and possibly biomimetic method for the synthesis of β-lactams, involving ring-contraction reactions of isothiazolidinones,[8,12] and a procedure for the functionalisation of β- and γ-lactams at endo- and exo-cyclic carbon adjacent to the lactam nitrogen.[16,24,32,33,81,92] Through the latter work, the importance of geometrical constraints on the formation of free radicals in these systems was established. The procedures were shown to be suitable for application in the synthesis of lactam antibiotics.

Free radical reactions of derivatives of valine and other proteinogenic amino acids were examined, initially to establish the factors which contribute to the regioselectivity of carbon-carbon bond formation, at the β-position of the valine residue of Arnstein's

tripeptide, during penicillin biosynthesis.[7] Studies of this type have now delineated the importance of steric, polar and stereoelectronic effects, which often outweigh product radical stability, to determine the regio- and stereo-selectivity of atom transfer reactions of amino acid derivatives.[7,9,13,17,20,21,34,43,52,113]

Based on these observations, an explanation for the anomalous preferential reactivity of glycine derivatives in chemical and biological free radical reactions of amino acid derivatives has been proposed and tested.[15,27] Accordingly the selectivity can be attributed to the ability of glycinyl radicals to adopt planar conformations which are relatively free of non-bonding steric interactions and which allow maximum overlap of the orbitals as required for radical stabilisation. An extension of this hypothesis resulted in the development of the use of *N*-phthaloylamino acid derivatives[28,42] and other imides,[86] to limit the ease of formation of α-carbon-centred amino acid radicals, in order to control the regioselectivity of reaction of small peptides and side chain functionalisation of amino acid derivatives.

Apart from the discovery of fundamental new information about free radical chemistry, particularly that of amino acid derivatives, the research has resulted in the development of procedures for the regio- and stereo-selective functionalisation of these compounds. These have been exploited in further synthesis, using a combination of free radical and ionic methods. Procedures for the synthesis of cross-linked amino acid derivatives have been developed.[23,31,51,65] Compounds of this type are of interest for the preparation of conformationally restricted peptides. Methods to prepare deuterated and allylated glycine derivatives,[30] and β-nitro and α,β-dehydro amino acids,[26,85] from α-haloglycine derivatives, have also been found. When these were combined with the selective reaction of glycine residues in peptides, the other amino acid residues in the peptides served as chiral auxiliaries for asymmetric amino acid synthesis.[22,56] An unusual procedure for the *N*-methylation of amino acid derivatives has also been found.[44]

The ability to prepare side chain halogenated amino acid derivatives from *N*-phthaloyl-substituted proteinogenic amino acids has been exploited, for example to develop regio- and stereo-controlled syntheses of deuterated,[14,72] α,β-dehydro,[38] methano[49] and hydroxy[37,67,107] amino acid derivatives. The corresponding free amino acids, including components of the peptide antibiotics lysobactin and vancomycin,[67] and a synthetic precursor of the antibiotic chloramphenicol,[99] have also been prepared using this methodology. These methods exploit the proteinogenic amino acids as part of the pool of optically active starting materials for asymmetric synthesis. In the products of these reactions, the absolute stereochemistry is determined by that of the starting amino acids, and the relative stereochemistry is determined by the mechanisms of the subsequent reactions.

In these studies, the syntheses of β-hydroxyphenylalanine derivatives from the corresponding bromides were found to occur with a stereoconvergence which indicated a new type of neighbouring group effect.[67,99] Analogous effects of carboxyl groups on the rate of bromination at the β-position of phenylalanine derivatives have also been observed.[98] These are the first examples of remote neighbouring group effects in atom transfer reactions.

Much of the work in this area is likely to have biochemical implications in the secondary metabolism of amino acid derivatives. The work has also been applied directly to develop a model of peptidylglycine α-amidating monooxygenase[53,114] and to examine the enantioselectivity of the reaction of phenylalanine catalysed by phenylalanine ammonia lyase.[72]

The contributions to the free radical chemistry of amino acid derivatives outlined above have been the subject of two invited reviews,[45,108] while aspects of the associated methods for the stereocontrolled synthesis of amino acid derivatives have been summarised in a report of an invited conference presentation.[109]

Statement of percentage contribution

This work has been carried out with the collaboration of research students and research assistants, who have contributed the bulk of the practical aspects. Their participation is acknowledged on the individual publications. I have contributed to the practical work and been solely responsible for the research supervision and the direction of the work. This is demonstrated by the fact that I am the corresponding author on all but one of the forty seven publications in this area. As required under the regulations for the degree of Doctor of Science at the University of Adelaide, I estimate my contribution to the intellectual aspects of the publications in this area at 70%.

Cyclodextrin Chemistry

Research in this area has involved the synthesis of modified cyclodextrins and the use of natural and modified cyclodextrins as hosts in the formation of host-guest complexes.[115]

The natural cyclodextrins form host-guest complexes with hydrophobic species as guests.[39,41,77] However, it has been shown that through modification to the cyclodextrin hosts, to introduce additional host-guest interactions in the complexes, the thermodynamic stability of the complexes can be tailored to meet specific requirements. For example, the thermodynamic stability of complexes is affected through the introduction of cyclodextrin substituents which form hydrophobic, ion-dipole and ionic interactions with the guests.[57,58,75,82,83,88,116] Cooperative binding by linked cyclodextrins also leads to host-guest complexes of greater stability,[36,105,110,111] as does simultaneous complexation of a cyclodextrin and a guest to a metal centre.[63,64,78,93]

The major focus of the initial work was the development of modified cyclodextrins[36,40,58,110,121] and host-guest complexes of those species for use in the

administration of pharmaceuticals, and the patent applications in this area mainly reflect that activity. Modified cyclodextrins were prepared in order to achieve controlled and sustained release of pharmaceuticals from the corresponding complexes, particularly through oral administration. Host-guest complexes of pharmaceuticals were also designed to protect the pharmaceuticals from degradation, on storage and passage through the gastrointestinal tract, and to protect the gastrointestinal tract from irritation by the pharmaceuticals, and to increase the bioavailability of the pharmaceuticals. Cyclodextrin-guest conjugates were also prepared as prodrugs.[40]

Arising from these applications of modified cyclodextrins and the ability to modify cyclodextrins to introduce new host-guest interactions, procedures for the selective complexation of guests were also developed. Particular attention was paid to chiral discrimination by modified cyclodextrins and the use of modified cyclodextrins in chemical separation technology.[57,58,82] It has been established that modified cyclodextrins show increased chiral discrimination where, through the modification, the degree of asymmetry of the cyclodextrin has been increased and there is the possibility of greater interaction between chiral portions of the cyclodextrin and those of the guest.[106] For these reasons, greater thermodynamic chiral discrimination is evident in cyclodextrin host-guest complexes where both the cyclodextrin and the host are complexed to a metal centre[63,64,78,93] or covalently bonded.[40,69,84,118]

Natural and modified cyclodextrins have also been used to probe reactions of included guests catalysed by cyclodextrin hosts[76] and to increase the efficiency of enzyme catalysed reactions, through selective complexation of the reaction products, thus limiting the extent of product inhibition of the enzymes.[89] Other modified cyclodextrins have been used as templates to reverse the regioselectivity of nitrile oxide cycloaddition reactions.[119]

These contributions to research have resulted in an invited review of chiral discrimination by modified cyclodextrins[106] and an invited book chapter on inclusion complexes of modified cyclodextrins.[117]

Statement of percentage contribution

The early work in this area, which is mostly reflected in the list of patent applications, involved collaboration with Dr. John Coates and Professor Stephen Lincoln of the University of Adelaide. Since the retirement of Dr. Coates, Professor Lincoln and I have continued our collaboration in the area, where we are equally and jointly responsible for the research and for the supervision of the research students and research assistants who have also contributed to the work. In support of my assertion of our equal contributions, I note that I am the corresponding author of fifteen of the twenty nine publications that have been derived from this research. As required under the regulations for the degree of Doctor of Science at the University of Adelaide, I estimate my contribution to the intellectual aspects of the publications in this area at 35%.

Lipid Chemistry

Results in this area have included the development of procedures for the analysis of lipid species,[19] the detection of a new mechanism of fatty acid metabolism,[61] the identification and characterisation of unusual fatty acids,[18] some of which are characteristic of metabolic disorders,[25] and the development of techniques for the synthesis of those compounds.[60] More recent work has involved the synthesis of modified fatty acids[120] and structure-activity correlations of those compounds in immunological assays. The patent applications in this area relate to the latter activity.

Statement of percentage contribution

Most of the research in this area has involved a collaboration with Dr. Alf Poulos of the Adelaide Medical Centre for Women and Children, while the more recent work has also involved Professor Tony Ferrante of the Adelaide Medical Centre for Women and Children and Dr. Debbie Rathjen of Peptide Technology Ltd. We have shared the responsibility for supervising the research in this area, and my contribution is reflected in the fact that I am the corresponding author of three of the six papers arising from the research. As required under the regulations for the degree of Doctor of Science at the University of Adelaide, I estimate my contribution to the intellectual aspects of the publications in this area at 30%.

1,3-Dipolar Cycloaddition Chemistry

This work has involved studies of cycloaddition reactions of nitrile oxides,[73] in order to develop synthetic methods, based on controlling the regioselectivity of cycloaddition,[66,74] limiting side reactions of the nitrile oxides,[112] and finding new procedures for elaboration of the isoxazole and isoxazoline cycloadducts.[68]

Statement of percentage contribution

The research in this area has been carried out in collaboration with Dr. Greg Simpson and Dr. Paul Savage of the CSIRO Division of Molecular Science. Together we have supervised the research and my contribution is reflected in the fact that I am the corresponding author of two of the five papers arising from the work. As required under the regulations for the degree of Doctor of Science at the University of Adelaide, I estimate my contribution to the intellectual aspects of the publications in this area at 30%.

X-Ray Crystallography

Various aspects of the research have been facilitated through the use of X-ray crystallography for structure determination.

Statement of percentage contribution

The research in this area has involved a collaboration with Dr. Edward Tiekink of the University of Adelaide. The compounds used in these studies were available through the research outlined above, while Dr. Tiekink determined the structures and is the corresponding author on all the publications in this area. As required under the regulations for the degree of Doctor of Science at the University of Adelaide, I estimate my contribution to the intellectual aspects of the publications in this area at 10%.

Miscellaneous and Graduate and Postdoctoral Research

Statement of percentage contribution

The publications listed under these categories were derived mostly from research carried out under the supervision of Professor Athel Beckwith and Professor Jeremy Knowles, where I contributed fully to the practical aspects but they were responsible for the overall direction of the work. Four of the publications[1,2,3,6] describe work performed as part of my Ph.D. studies and, as such, that work has already been used towards the award of that degree at the University of Adelaide. Publications in these categories are included in this thesis only for completeness.

Copies of publications arranged in chronological order

[Reprinted from the Journal of the American Chemical Society, 100, 2913 (1978).]

A Stereoelectronic Effect in Hydrogen Atom Abstraction from a Substituted Cyclohexyl Radical

Sir:

Since the pseudoaxial and pseudoequatorial protons of the β positions of cyclohexyl radical in its chair-like conformation are stereochemically nonequivalent with respect to the semi-occupied p orbital, they should exhibit different reactivity toward reagents capable of abstracting hydrogen atoms. Two reports of such reactivity differences have recently appeared. Agosta and Wolff[1] observed preferential intramolecular transfer of the pseudoaxial β-hydrogen atom in biradicals generated photochemically from bicyclo[3.2.1]octan-6-ones. A contrary result was reported by Livant and Lawler[2] who studied the disproportionation of cyclohexyl radicals by the CIDNP technique and obtained evidence for the selective loss of pseudoequatorial β-hydrogen atoms. The discord between these reports prompted the present study of the stereochemical course of reactions of an appropriately substituted, conformationally locked[3] cyclohexyl radical **4**.

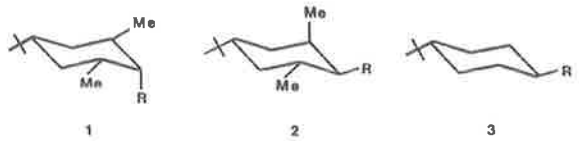
The radical **4** was generated by thermolysis of the *tert*-butyl peroxyglyoxalate **2b**. Catalytic hydrogenation of 4-*tert*-

Table I. Products of Thermolysis of Peroxyglyoxalates **1b** and **2b**

Peroxy ester	Yields of products, %			
	5	**6**	**7**	**8**
1b	22	0	0	69
2b	1.7	14	72	1[a]

[a] Formed from 1% isomeric impurity in starting material.

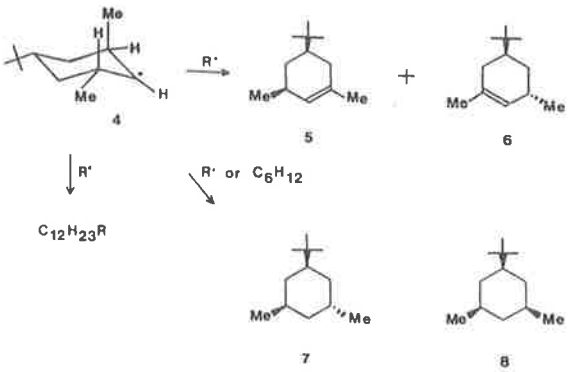
butyl-2,6-dimethylphenol[4] over rhodium on alumina gave the triequatorially substituted cyclohexanol **1a**. Oxidation of **1a**



1 2 3

(a) R = OH
(b) R = $OCOCO_2OBu^t$
(c) R = OTos

gave the related ketone which was converted via the semicarbazone[5] into its stereoisomer containing one pseudoaxial and one pseudoequatorial methyl substituent. The alcohol **2a** obtained by reduction with lithium aluminium hydride was converted into the radical precursor **2b** by successive treatment with oxalyl chloride and *tert*-butyl hydroperoxide.[6] The peroxy ester **2b** was then heated in cyclohexane under nitrogen at 100 °C for 2 h and the mixture was analyzed by GLC. using as reference compounds samples of the cyclohexanes, **7** and **8**, and cyclohexenes, **5** and **6**, synthesized by unambiguous routes.



Stereochemical assignments for all these compounds were consistent with the methods of their preparation and were supported by ^{13}C NMR, ^1H NMR, and infrared spectral data.

Homolysis[6,7] of **2b** initially affords the trisubstituted cyclohexyl radical **4** and *tert*-butoxy radicals, but the latter should be rapidly consumed by reaction with solvent to afford cyclohexyl radicals. Cyclohexene was detected in the reaction mixture in sufficient yield (>0.6 mol/mol of **2b**) to indicate that decomposition of the peroxy ester **2b** induced by direct abstraction of β hydrogen by *tert*-butoxy radicals is unlikely to be a significant reaction pathway.

Disproportionation of **4** affords the olefin **5** by loss of the pseudoequatorial β-hydrogen atom or its isomer **6** by loss of the pseudoaxial hydrogen. Of these two competing processes the former should be favored on steric and thermodynamic grounds, for nonbonded interactions are less severe in the diequatorially substituted cyclohexene **5** than in its isomer **6**. However, the data are not in accord with this simple view, for the high yield of **6** relative to **5** clearly shows that loss of the pseudoaxial hydrogen atom from **4** is the preferred process and occurs approximately eight times more rapidly than loss of the pseudoequatorial hydrogen atom. Our results thus agree nicely with those of Agosta and Wolff.[1] They support the hypothesis that homolysis of the β-C–H bond, like β-C–C bond fission in cycloalkylcarbinyl radicals,[8] is subject to stereoelectronic control: the bond which preferentially undergoes fission is that which lies closest to the plane of the adjacent semioccupied p orbital. Their relatively large ESR hyperfine splittings[9] show that it is the pseudoaxial β protons in cyclohexyl radicals which best satisfy this criterion.

Of the other processes available to **4**, radical coupling is clearly unimportant, for a high yield (~90%) of monomeric products was obtained. The major product from the thermolysis of **2b** is the cyclohexane **7**. Undoubtedly some of it arises by disproportionation between **4** and cyclohexyl radicals. However, in view of the high relative yield of **7**, it seems likely that it is also formed by hydrogen atom abstraction from solvent cyclohexane.

For comparative purposes thermolyses of **3b** and of the triequatorially substituted peroxy ester **1b** were also studied. The former gave *tert*-butylcyclohexane (70%) and 4-*tert*-butylcyclohexene (14%), whilst the latter gave **5** and **8**. Interestingly, the ratio of yields of olefin and cycloalkane, % **5** to % **8**, from decomposition of **1b** is considerably higher than the equivalent ratio, % (**5** + **6**) to % **7** for decomposition of **2b**. This reflects the fact that the intermediate radical **4** has only one pseudoaxial β proton, whereas the radical derived from **1b** has two.

Separate experiments were conducted to test the possible importance of alternative routes to the observed products of decomposition of **2b**. Thus, solvolysis of the tosylate **2c** afforded **5** (53%) and rearranged olefins, but no trans olefin **6**. We conclude that formation of **6** from **2b** cannot involve a cationic intermediate. Also, the acetate and chloroglyoxalate of the alcohol **2a** were each found to be completely stable under the thermolysis reaction conditions: it appears unlikely, therefore, that the peroxy ester **2b** decomposes by intramolecular concerted elimination of *tert*-butyl alcohol and carbon dioxide.

In summary, the results outlined above, and those reported previously, conform to a general pattern: homolysis of a C–H bond adjacent to a semioccupied p orbital, a lone pair,[10] or a π orbital,[11] or of a C–C bond adjacent to a semioccupied p orbital,[8] will occur most readily when the bond undergoing fission and the orbital can assume coplanarity.

Acknowledgment. We thank Dr. R. G. Lawler for helpful discussion and comment.

References and Notes

(1) W. C. Agosta and S. Wolff, *J. Am. Chem. Soc.*, **98**, 4316 (1976); **99**, 3355 (1977).
(2) P. Livant and R. G. Lawler, *J. Am. Chem. Soc.*, **98**, 6044 (1976).
(3) S. Winstein and N. J. Holness, *J. Am. Chem. Soc.*, **77**, 5562 (1955).
(4) K. Hultzsh, *Ber.*, **74**, 1539 (1941).
(5) F. Johnson and L. G. Duquette, *Chem. Commun.*, 1448 (1969).
(6) F. R. Jensen and T. I. Moder, *J. Am. Chem. Soc.*, **97**, 2281 (1975).
(7) P. D. Bartlett and R. E. Pincock, *J. Am. Chem. Soc.*, **82**, 1769 (1960); P. D. Bartlett, B. A. Gontarev, and H. Sakurai, *ibid.*, **84**, 3101 (1962).
(8) S. J. Cristol and R. V. Barbour, *J. Am. Chem. Soc.*, **90**, 2832 (1968); A. L. J. Beckwith and G. Phillipou, *Aust. J. Chem.*, **29**, 123 (1976); E. C. Friedrich, *J. Org. Chem.*, **34**, 528 (1969); W. G. Dauben, L. Schutte, R. E. Wolf, and E. J. Deviny, *ibid.*, **34**, 2512 (1969).
(9) S. Ogawa and R. W. Fessenden, *J. Chem. Phys.*, **41**, 994 (1964).
(10) K. Hayday and R. D. McKelvey, *J. Org. Chem.*, **41**, 2222 (1976); G. Brunton, K. U. Ingold, B. P. Roberts, A. L. J. Beckwith, and P. J. Krusic, *J. Am. Chem. Soc.*, **99**, 3177 (1977).
(11) A. L. J. Beckwith and G. Phillipou, *Aust. J. Chem.*, **29**, 1277 (1976); B. Cross and G. H. Witham, *J. Chem. Soc.*, 1650 (1961).

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Some Guidelines for Radical Reactions

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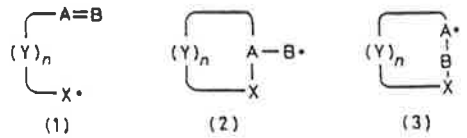
Summary Some generalisations of predictive utility are presented concerning the influence of steric and stereo-electronic effects on radical reactions.

RECENT work in this laboratory and elsewhere[1] has demonstrated that thermochemical criteria, although widely employed for the prediction or rationalisation of the results of free-radical processes, are sometimes misleading because of the influence of steric, polar, or stereo-electronic factors. We believe it may be generally useful, therefore, to adumbrate guidelines which, when used in conjunction with the thermochemical approach, provide a predictive basis for some aspects of free-radical chemistry.

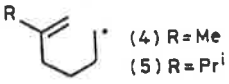
(i) *Intramolecular addition under kinetic control in lower alkenyl and alkynyl radicals and related species occurs preferentially in the* exo-*mode.* This guideline suggests that *exo*-ring closure (**1**) → (**2**) is kinetically favoured over the *endo*-process (**1**) → (**3**) for radicals of type (**1**) where Y represents a chain of atoms ($n \leqslant 5$). It is exemplified by ring closure of butenyl,[2] hexenyl, heptenyl, and octenyl radicals,[3] of various substituted alkenyl radicals,[4–8] of alkynyl radicals,[9] of alkenylaryl radicals,[10] and of O-[11–14] and N-centred[15,16] radicals. Although definitive experimental evidence is not available it possibly applies to S-,[17] P-,[18] and Si-centred[19] radicals. It appears also to apply to ring closure on to C≡N,[20] C=O,[21] and aromatic nuclei.[1,22] It does not apply to systems under thermodynamic control,[23] nor in some instances to radicals bearing a substituent at the ω-1 position, *e.g.* the 5-methylhex-5-enyl radical [see (ii)].



(**1**) (**2**) (**3**)

Exo-ring closure of radicals of type (**1**) is kinetically favoured because the strain generated by the accommodation of the triangular disposition of centres required for homolytic addition[24] within the system leading to *endo*-cyclization outweighs the thermochemical and other factors disfavouring the formation of the thermodynamically less stable product.[25]

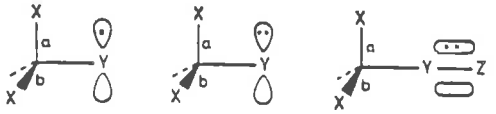
(ii) *Substituents on an olefinic bond disfavour homolytic addition at the substituted position.* The 5-substituted hex-5-enyl radicals (**4**) and (**5**) undergo mainly six-membered ring formation because the rate of 1,5-cyclization is greatly retarded by the presence of the substituent.[4,8] Similar retardations have been noted in intermolecular additions.[26,27] The effect, which is probably mainly steric in origin,[27] accounts more satisfactorily than do thermodynamic criteria for the rates and regioselectivity of homolytic inter- and intra-molecular addition to substituted olefins except when the substituent exerts a strong stabilizing effect (*e.g.* R = Ph).[5]



(**4**) R = Me
(**5**) R = Pr^{i}

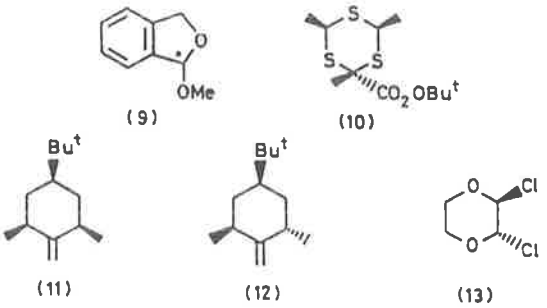
(iii) *Homolytic cleavage is favoured when the bond concerned lies close to the plane of an adjacent semi-occupied orbital or of an adjacent filled non-bonding or π-orbital.* This guideline indicates that in each of the systems (**6**), (**7**), and (**8**) fission of bond (a) will occur more rapidly than fission of bond (b). Since *exo*-radicals of type (**2**) can readily acquire the required overlap whereas *endo*-radicals (**3**) cannot,[25] the former undergo β-fission much more readily than do the latter. Thus cyclopropylmethyl and cyclobutylmethyl radicals undergo rapid ring-opening, but cyclopropyl and cyclobutyl radicals do not.[1,28,29] In rigid systems the direction of ring opening conforms to this guideline.[29,30] and other manifestations include the preferred *exo*-fission of the cyclic ether radical (**9**),[31] the selective loss of pseudoaxial hydrogen atoms in disproportionation

of substituted 4-t-butylcyclohexyl radicals[32] and in hydrogen atom abstraction from cyclic ethers,[33] and the greater rate of homolysis of the axial perester (**10**) as compared with its equatorial isomer.[34] We have now found, as predicted by this guideline that t-butoxyl radicals generated by copper catalysed decomposition of t-butyl perbenzoate react with the conformationally locked olefin (**11**) containing two axial allylic hydrogen atoms twice as rapidly as with the olefin (**12**) containing only one axial hydrogen. Similarly, the *trans*-compound (**13**) which contains two axial chlorine substituents[35] reacts with tributyltin radicals twice as rapidly as does its *cis*-isomer.



(6) (7) (8)

(iv) 1,5-*Ring closures of substituted hex-5-enyl and related radicals are stereoselective:* 1- *or* 3-*substituted systems afford mainly* cis-*disubstituted products, whereas* 2- *or* 4-*substituted systems give mainly* trans-*products.* The preferential formation of the *cis*-product from 1-substituted hexenyl radicals has been ascribed to the effects of orbital symmetry.[6] The guideline may fail when the substituent at C-1 is bulky.[36] The stereoselectivity of ring closure of 2-, 3-, or 4-substituted hexenyl radicals reflects the conformational preference of the transition state[37] and therefore is likely to be more pronounced for systems containing bulky substituents. Substituted 3-butenylperoxyl radicals undergo stereospecific or highly stereoselective ring closure in accord with this guideline.[14,38]



(9) (10) (11) (12) (13)

(*Received*, 31*st December* 1979; *Com.* 1341.)

[1] For a review containing much pertinent material see J. W. Wilt in 'Free Radicals,' ed. J. K. Kochi, Wiley-Interscience, New York, 1973, vol. I, p. 333.
[2] A. Effio, D. Griller, K. U. Ingold, A. L. J. Beckwith, and A. Serelis, *J. Am. Chem. Soc.*, 1980, **102**, in the press.
[3] A. L. J. Beckwith and G. Moad, *J. Chem. Soc., Chem. Commun.*, 1974, 472.
[4] A. L. J. Beckwith, I. A. Blair, and G. Phillipou, *Tetrahedron Lett.*, 1974, 2251.
[5] T. W. Smith and G. B. Butler, *J. Org. Chem.*, 1978, **43**, 6.
[6] A. L. J. Beckwith, I. Blair, and G. Phillipou, *J. Am. Chem. Soc.*, 1974, **96**, 1613.
[7] A. L. J. Beckwith and G. Moad, *J. Chem. Soc., Perkin Trans.* 2, 1975, 1726.
[8] M. Julia, C. Descoins, M. Baillarge, B. Jacquet, D. Uguen, and F. A. Groeger, *Tetrahedron*, 1975, **31**, 1737, and references cited.
[9] S. A. Dodson and R. D. Stepanovic, *J. Chem. Soc., Perkin Trans.* 1, 1975, 410; T. Oknuki, M. Yoshida, and O. Simamura, *Chem. Lett.*, 1972, 797, 999; W. M. Moore, A. Salajegheh, and D. G. Peters, *J. Am. Chem. Soc.*, 1975, **97**, 4954; A. L. J. Beckwith and T. Lawrence, unpublished results.
[10] A. L. J. Beckwith and W. B. Gara, *J. Chem. Soc., Perkin Trans.* 2, 1975, 795.
[11] R. D. Rieke and N. A. Moore, *J. Org. Chem.*, 1972, **37**, 413.
[12] For a review see J.-M. Surzur and M. P. Bertrand, *Bull. Soc. Chim., Fr.*, 1973, 8161.
[13] N. A. Porter, M. O. Funk, D. Gilmore, R. Isaac, and J. Nixon, *J. Am. Chem. Soc.*, 1976, **98**, 6000.
[14] A. L. J. Beckwith and R. S. Wagner, *J. Am. Chem. Soc.*, 1979, **101**, 7099.
[15] For reviews see P. Mackiewicz and R. Furstoss, *Tetrahedron*, 1978, **34**, 3241; Y. L. Chow, W. C. Danen, S. F. Nelson, and D. H. Rosenblatt, *Chem. Rev.*, 1978, **78**, 243.
[16] J.-M. Surzur, L. Stella, and P. Tordo, *Bull. Soc. Chim. Fr.*, 1975, 1425, 1429; M. E. Keuhne and D. A. Horne, *J. Org. Chem.*, 1975, **40**, 1278; R. A. Perry, S. C. Chen, B. C. Menon, K. Hanaya, and Y. L. Chow, *Can. J. Chem.*, 1976, **54**, 2385.
[17] J.-M. Surzur, M.-P. Crozet, and C. Dupuy, *Tetrahedron Lett.*, 1971, 2025.
[18] A. G. Davies, M. J. Parrott, and B. P. Roberts, *J. Chem. Soc., Perkin Trans.* 2, 1976, 1066.
[19] H. Sakurai in 'Free Radicals,' ed. J. K. Kochi, Wiley-Interscience, New York, 1973, vol. II, p. 793.
[20] B. P. Roberts and J. N. Winter, *J. Chem. Soc., Perkin Trans.* 2, 1979, 1353; D. Griller, P. Schmid, and K. U. Ingold, *Can. J. Chem.*, 1979, **57**, 831.
[21] F. Flies, R. Lalande, and B. Maillard, *Tetrahedron Lett.*, 1976, 439.
[22] J. C. Chottard and M. Julia, *Tetrahedron*, 1972, **28**, 5615; J. W. Wilt, W. K. Chwang, C. F. Dockus, and N. M. Tomiuk, *J. Am. Chem. Soc.*, 1978, **100**, 5534.
[23] M. Julia, *Pure Appl. Chem.*, 1967, **15**, 167; *Acc. Chem. Res.*, 1971, **4**, 386.
[24] M. J. S. Dewar and S. Olivella, *J. Am. Chem. Soc.*, 1978, **100**, 5290.
[25] A. L. J. Beckwith, 'Essays in Free-Radical Chemistry,' Special Publication No. 24, The Chemical Society, London, 1970, p. 239.
[26] B. Giese and J. Meixner, *Tetrahedron Lett.*, 1977, 2779.
[27] J. M. Tedder and J. C. Walton, *Adv. Phys. Org. Chem.*, 1978, **16**, 86.
[28] B. Maillard, D. Forrest, and K. U. Ingold, *J. Am. Chem. Soc.*, 1976, **98**, 7024.
[29] A. L. J. Beckwith and G. Moad, *J. Chem. Soc., Perkin Trans.* 2, in the press.
[30] A. L. J. Beckwith and G. Phillipou, *Aust. J. Chem.*, 1976, **29**, 123.
[31] C. M. Rynard, C. Thankachan, and T. T. Tidwell, *J. Am. Chem. Soc.*, 1979, **101**, 1196.
[32] A. L. J. Beckwith and C. Easton, *J. Am. Chem. Soc.*, 1978, **100**, 2913.
[33] K. Hayday and R. D. McKelvey, *J. Org. Chem.*, 1976, **41**, 2222.
[34] K. Arai, H. Iwamura, and O. Michinori, *Chem. Lett.*, 1975, 1181.
[35] C. Y. Chen and R. J. W. Le Fèvre, *J. Chem. Soc.* (*B*), 1966, 544; C. Altona and E. Havinga, *Tetrahedron*, 1966, **22**, 2275.
[36] M. A. M. Bradney, A. D. Forbes, and J. Wood, *J. Chem. Soc., Perkin Trans.* 2, 1973, 1655.
[37] A. L. J. Beckwith, T. Lawrence, and A. K. Serelis, *J. Chem. Soc., Chem. Commun.*, 1980, 484 (following communication).
[38] A. L. J. Beckwith and R. D. Wagner, *J. Chem. Soc., Chem. Commun.*, 1980, 485 (accompanying communication).

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Stereoelectronic Effects in Hydrogen Atom Abstraction from Substituted 1,3-Dioxanes

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Contribution from the Organic Chemistry Department, University of Adelaide, Adelaide, South Australia 5001. *Received March 24, 1980*

Abstract: An EPR technique and a method involving the gas chromatographic determination of the relative rates of consumption of substrates have been employed to measure the relative rates of hydrogen atom abstraction by *tert*-butoxy radicals from various substituted 1,3-dioxanes. The high relative reactivities of dioxanes equatorially substituted at C-2 as compared with their axially substituted epimers are attributed to the favorable stereoelectronic interactions in the former between the axial C–H bonds and adjacent oxygen lone pairs. The effects of methoxy substituents on the relative reactivities of cyclic ethers have been similarly rationalized. The photoinduced reaction between benzophenone and the methoxydioxane (**8**) has been shown to be reversible.

In previous communications[1,2] we have suggested that many bond dissociations and atom-transfer reactions conform to a general pattern; namely, that homolytic fission is favored when the bond undergoing cleavage can assume coplanarity with an adjacent semioccupied p orbital, an occupied nonbonding orbital, or an occupied π orbital. The present work and that in an accompanying paper[3] are directed toward testing this hypothesis with respect to homolysis of C–H bonds adjacent to ethereal oxygen.

Previous work in this area has been conducted by Hayday and McKelvey,[4] who found that the cis isomer (**1**) of 2-methoxy-4-methyltetrahydropyran undergoes hydrogen atom abstraction by triplet benzophenone at ambient temperature 8 times more rapidly that its trans isomer (**2**). Their conclusion that abstraction of axial hydrogen is preferred over abstraction of equatorial hydrogen and that this is due to stereoelectronic effects appears reasonable but is open to objection in that the difference in free energy between the two transition states may reflect the differences between the ground-state energies of the two starting materials[5] and the common free radical generated from them.[10] A further possible ambiguity arises from the fact that the difference in free energy between the more stable form **2** of the trans isomer and its conformer in which the methyl and methoxy groups occupy axial and equatorial positions respectively may not be sufficiently large as to preclude participation of the latter in the hydrogen atom transfer reaction. Finally, there is the possibility that the exo methoxy groups which assume preferred orientations with respect to the reaction center because of anomeric interactions[6] may have different stereoelectronic effects on the reactivities of the isomers **1** and **2**.

In an attempt to define more precisely the magnitude of the stereoelectronic effect of adjacent oxygen on C–H bond homolysis

1 **2**

we have studied reactions of **9** and **10** in which the methyl substituents at the 4- and 6-positions provide an effective conformational lock[12] while the absence of a methoxy substituent precludes difficulties associated with the exoanomeric effect.[13] Also for comparative purposes we have studied the relative rates of hydrogen atom abstraction from the dioxanes **3–8**.

Methods. Relative rates of hydrogen atom abstraction from the cyclic ethers **3–10** by *tert*-butoxy radicals were determined both by an EPR technique and by following the relative rates of consumption of substrates by gas chromatography. The former involves the measurements of the relative stationary concentrations of radicals A· and B· attained when a solution of di-*tert*-butyl peroxide in a mixture of two dioxanes, AH and BH, is subjected to UV irradiation in the cavity of an EPR spectrometer.

$$t\text{-BuOO-}t\text{-Bu} \xrightarrow{h\nu} 2t\text{-BuO}\cdot$$

$$t\text{-BuO}\cdot + \text{AH} \xrightarrow{k_{AH}} t\text{-BuOH} + \text{A}\cdot$$

$$t\text{-BuO}\cdot + \text{BH} \xrightarrow{k_{BH}} t\text{-BuOH} + \text{B}\cdot$$

Malatesta and Ingold[3] have shown that when reasonable assumptions are made concerning the rates of radical decay under these conditions, the ratio of rate constants is given by

$$\frac{k_{AH}}{k_{BH}} = \frac{[\text{A}\cdot]}{[\text{B}\cdot]}\frac{[\text{BH}]}{[\text{AH}]}$$

Consequently, if [A·]/[B·] is determined experimentally k_{AH}/k_{BH} can be readily calculated.

To simplify the integration of EPR signals, we recorded the spectra at relatively low resolution. Under these conditions long-range hyperfine splittings are not resolved and, therefore, the radicals derived from **3** and **6** and the 2-methoxy-substituted radicals derived from **5**, **7**, or **8** show only a broad singlet with $g \approx 2.0030$.[14] Similarly, the radicals derived from **4**, **9**, or **10** appear as broad 1:3:3:1 quartets [$a(H_\alpha) = 14.2$ G in each case]. Signals arising from radicals formed by hydrogen atom abstraction

(1) Beckwith, A. L. J.; Easton, C. *J. Am. Chem. Soc.* **1978**, *100*, 2913.
(2) Beckwith, A. L. J.; Easton, C. J.; Serelis, A. K. *J. Chem. Soc., Chem. Commun.* **1980**, 482.
(3) Malatesta, V.; Ingold, K. U. *J. Am. Chem. Soc.*, preceding paper in this issue.
(4) Hayday, K.; McKelvey, R. D. *J. Org. Chem.* **1976**, *41*, 2222.
(5) The cis isomer **1** is less stable than the trans **2** because of the anomeric effect.[6-8] The difference in free energy between the two isomers of 6-methyl-2-methoxytetrahydropyran has been estimated[9] to be approximately 0.7 kcal mol^{-1}.
(6) For leading references see: Lemieux, R. U. *Pure Appl. Chem.* **1971**, *25*, 527; Lemieux, R. U.; Hendriks, K. B.; Stick, R. V.; James, K. *J. Am. Chem. Soc.* **1975**, *97*, 4056; Szarek, W. A.; Horton, D., Eds. *ACS Symp. Ser.* **1979**, *No. 87*; Anteunis, M. J. O.; Tavernier, D.; Borremans, F. *Heterocycles* **1976**, *4*, 293.
(7) Eliel, E. L. *Angew. Chem., Int. Ed. Engl.* **1972**, *11*, 739.
(8) Deslongchamps, P. *Tetrahedron* **1975**, *31*, 2463.
(9) Eliel, E. L.; Giza, C. A. *J. Org. Chem.* **1968**, *33*, 3754.
(10) The hypothesis, originally based on product studies[4] that hydrogen atom abstraction from either **1** or **2** affords a common radical, has recently been confirmed by EPR measurements.[11]
(11) Malatesta, V.; McKelvey, R. D.; Babcock, B. W.; Ingold, K. U. *J. Org. Chem.* **1979**, *44*, 1872.
(12) (a) Eliel, E. L.; Knoeber, M. C. *J. Am. Chem. Soc.* **1968**, *90*, 3444. (b) Eliel, E. L.; Nader, F. W. *Ibid.* **1970**, *92*, 584.
(13) The exo or generalized anomeric effect[6-8] refers to the stabilization of certain conformations of an exocyclic alkoxy group by interaction of its oxygen lone pairs with endocyclic C–O bonds.
(14) EPR spectral parameters for some of the radicals studied here and related species have been reported previously.[3,15]
(15) Beckwith, A. L. J.; Tindall, P. K. *Aust. J. Chem.* **1971**, *24*, 2099. Dobbs, A. J.; Gilbert, B. C.; Norman, R. O. C. *J. Chem. Soc. A* **1971**, 124. Gage, C.; Gilbert, B. C. *J. Chem. Soc., Perkin Trans 2* **1977**, *116*, 754. Hudson, A.; Root, K. D. J. *Tetrahedron* **1969**, *25*, 5311.

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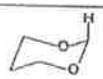
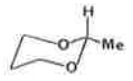
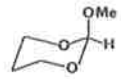
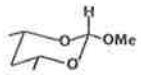
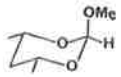
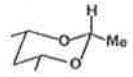
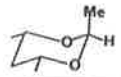
Table I. Relative Reactivities per Equivalent Hydrogen Atom (ρ) toward *t*-BuO· at ca. 20 °C

	DIOXANE	ρ (GC)	ρ (EPR)
3		1·0	1·0
4		1·5	2·4
5		0·6	0·4
6		2·6	1·6
7		3·4	1·4
8		0·3	$0 \cdot 1_6$
9		2·8	2·1
10 [a]		$0 \cdot 2_5$	0·3

[a] Sample contained 8% of epimer 9; an appropriate correction has been made to the value of ρ(EPR).

from positions other than C-2 were also detected, but their intensities were too weak to allow spectral parameters to be determined.

To avoid complications arising from overlapping EPR resonances, we measured the rates of hydrogen atom abstraction from dioxanes **4**, **9**, and **10**, bearing a methyl substituent at C-2, relative to that for 1,3-dioxane (**3**). Likewise, 2-methyl-1,3-dioxane (**4**) was used as a standard for the determination of the hydrogen atom abstraction rates from **3**, **5**, **6**, **7**, and **8**. The consolidated list of relative rate constants obtained by combining the two sets of data is given in Table I which shows the value of ρ, the *reactivity per equivalent hydrogen*, of each dioxane relative to the parent **3**.

If each of the dioxanes, AH and BH, in a suitable mixture of the two undergoes hydrogen atom abstraction by *tert*-butoxy radical or similar species, the ratio of the relevant rate constants can be estimated from the relative rates of consumption of the two substrates.[4,16]

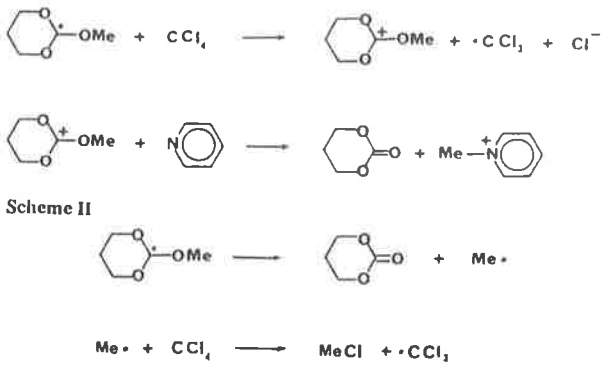
$$k_{AH}/k_{BH} = \ln ([AH]_t/[AH]_0)/\ln ([BH]_t/[BH]_0)$$

However, difficulty was experienced in applying this method to dioxanes because of their propensity to undergo epimerization at C-2. For example, photolysis of benzophenone as described by Hayday and McKelvey[4] in the presence of either of the pure isomers **7** or **8** rapidly affords an equilibrium mixture of the two (see below). Similarly, any radical reaction which results in the formation of traces of acid causes rapid epimerization of 2-substituted 1,3-dioxanes. The system eventually chosen involves photolysis of di-*tert*-butyl peroxide in a solution containing dioxanes and pyridine in carbon tetrachloride. The relative rates of consumption of the two or more substrates were monitored by gas chromatography, and the results were substituted into the rate equation given above to afford values of relative rate constants.

Although the use of carbon tetrachloride and pyridine in the reaction mixture effectively prevented epimerization of 2-substituted 1,3-dioxanes, the eventual fate of the radicals initially generated under these conditions has not been completely clarified.

(16) Walling, C.; Gibion, M. J. *J. Am. Chem. Soc.* **1965**, *87*, 3361.

Scheme I



Scheme II

Table II. Relative Reactivities (ρ_{AH}/ρ_{BH}) of Pairs of Isomeric Dioxanes

		ρ_{AH}/ρ_{BH}		
AH	BH	GC	EPR	ref
1	2	8	4	4, 3
7	8	11	7.5	this work
			9	3
9	10	11	7	this work

For example, when 2-methoxy-1,3-dioxane (**5**) was used as substrate, the only products identified were *N*-methylpyridinium chloride and allyl alcohol. The latter probably arises from 2-keto-1,3-dioxane[17] which was separately shown to be unstable under the reaction conditions, while the former could be formed either directly from a carbonium ion intermediate (Scheme I) or indirectly from methyl chloride generated via homolytic fission (Scheme II).

With 1,3-dioxane (**3**) the reaction afforded polymeric material, while 2-methyl-1,3-dioxane (**4**) gave pyridinium chloride, the formation of which is most easily rationalized in terms of a mechanism involving generation of carbonium ions. Fortunately, any ambiguity concerning the modes of decay of the initially generated radicals does not affect the validity of the method which depends only upon the relative rates of consumption of the substrates in the initial hydrogen atom abstraction step.

Each of the methods employed will be subject to error. The validity of the EPR technique depends on the assumption that the dioxanyl radicals generated have equal rates of decay. Malatesta and Ingold[3] have estimated that minor variations in decay rates could affect the reliability of the results by a factor of about 2 or 3. Also, since photolyses were conducted under nonflow conditions, the concentrations of substrates would decrease during the experiment. It is possible, therefore, that at the time spectra were recorded the proportion of the more reactive component in the mixture was less than estimated. For most of the compounds studied here this effect should result in the range of values of ρ determined by the EPR technique being somewhat decreased. Possible sources of error in the method based on relative rates of consumption of dioxanes include the preferential destruction of one of the suubstrates by nonradical reactions. Also, the consumption of dioxanes by reaction at positions other than C-2 will cause an apparent decrease in the range of values of ρ.

Results and Discussion

Relative Reactivities. The relative rate constants determined by each of the methods outlined above are presented in Table I. Having regard to the possible sources of error in each method, we regard the level of agreement as very satisfactory and certainly sufficient to preclude the possibility of major deviations from the proposed mechanistic schemes. Although the accuracy of the results is not sufficiently high to justify the detailed consideration

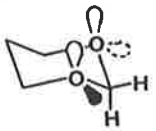
(17) Searles, S.; Hummel, D. G.; Nukina, S.; Throckmorton, P. E. *J. Am. Chem. Soc.* **1960**, *82*, 2928.

of apparent minor variations in relative rates, the major differences recorded here must be regarded as well founded.

The pairs of epimers **7**, **8** and **9**, **10** exhibit just such major differences in their rates of reaction with *tert*-butoxy radicals. The ratios of rate constants are presented in Table II together with relevant data from work carried out elsewhere.[3,4] In general, the results are consistent and show satisfactory agreement. However, it is noteworthy that the EPR technique always affords somewhat lower ratios of rate constants than does the more conventional method presumably because of preferential consumption of the more reactive isomer as outlined above. Nevertheless, it is quite clear that the cis form of each pair of epimers is considerably more reactive than the trans. Since the methyl substituents at C-4 and C-6 effectively lock the conformations of the dioxanes **9** and **10**,[12] the results obtained with these substrates provide compelling evidence that an axial C–H at C-2 is much more readily attacked than an equatorial C–H. The similarity of the reactivity ratio for the pair of monomethyl-substituted tetrahydropyrans, **1** and **2**, to those now recorded for 1,3-dioxanes suggests that they react substantially in the more stable conformers **1** and **2** and do not undergo prior conformational change.

The enhanced reactivity of the cis forms of 2-methoxy-substituted ethers cannot be associated with the fact that they have higher energies than their trans forms, because the pair of 2-methyl-substituted dioxanes, **9** and **10**, of which the cis isomer **9** is the *more* stable[12] shows essentially the same reactivity ratio. Nor for the same reason can the high reactivity of the *cis*-methoxy ethers **1** and **7** be associated with conformational change about the exocyclic C–O bond. We conclude, therefore, that, as predicted earlier,[18] these reactions are under stereoelectronic control and that the enhanced reactivity of axial C–H reflects a favorable interaction in the transition state between the bond undergoing fission and lone pairs on adjacent oxygen atoms.

If, following McKelvey,[4] Deslongchamps,[8] Gorenstein,[19] and others,[20] we assume that ethereal oxygen is sp^3 hybridized with two lone pairs of equal energy, we see that one lone pair on each oxygen of 1,3-dioxane (**3**) is disposed as shown in **11** in an an-



11

tiperiplanar orientation with respect to the axial C–H bond at C-2. There is no such relationship between the equatorial C–H bond and adjacent lone pairs. It is reasonable to conclude, therefore, that an antiperiplanar relationship between a C–H bond and an adjacent lone pair lowers the bond dissociation energy of the former. Stereoelectronic effects in many heterolytic reactions have been similarly rationalized.[20,21]

Recent theoretical[22] and experimental studies[23] suggest that one lone pair of oxygen occupies a p-type orbital of relatively high energy while the other resides in a low-energy s-type orbital. In 1,3-dioxane the axial C–H bond at C-2 lies close to the plane ($\theta = 30°$) of such adjacent p lone pairs, whereas the equatorial C–H is orthogonal to them. In this model the enhanced reactivity of the axial C–H bond is attributable to conjugative delocalization at the transition state between the p lone pairs and the developing radical center.

Of the two models for stereoelectronic interaction of C–H with adjacent oxygen lone pairs we prefer the latter. In any event the

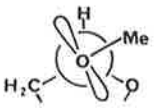


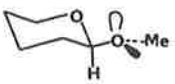
Figure 1. Newman projection of **12** or **13** showing orientation of the exocyclic lone pair.

experiments indicate that the C–H bond in a cyclic ether which lies furthest from the plane of the β,γ-O–C bond will have the weakest dissociation energy. Theoretical studies on methanol lead to a similar conclusion.[24]

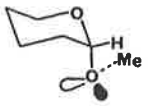
Effects of Exocyclic Methoxy and Methyl Substituents. Since 4,6-dimethyl-1,3-dioxane (**6**) is conformationally locked,[12] its value of ρ may reasonably be attributed mainly to fission of the axial C–H bond at C-2. Each of the 2-methyl-substituted dioxanes **4** and **9** exist preferentially in conformations containing the 2-substituent in an equatorial position. Comparison of the appropriate relative reactivities shows that the axial C–H bonds in these three compounds undergo attack by *tert*-butoxy radicals at similar rates. We conclude that an equatorial 2-methyl substituent has a minor effect on the rate of abstraction of the 2-axial-hydrogen atom from 1,3-dioxanes.

Comparison of the relative reactivities of **7** and **9** and of **8** and **10** leads likewise to the conclusion that a 2-methoxy substituent in either an equatorial or an axial position does not substantially affect the rate of hydrogen atom abstraction from C-2. However, the low reactivity of 2-methoxy-1,3-dioxane (**5**) by comparison with the parent compound **3** appears to strike a discordant note. This can be resolved in the light of the known[9] preference of **5** to exist preferentially, as illustrated in the unreactive conformer containing an equatorial C–H bond at C-2. It is significant that the calculated difference in $\Delta G^{\ddagger}$ (ca. 400 cal mol^{-1}) between reactions of **3** and **5** is of the same order of magnitude as the difference in free energy (ca. 350 cal mol^{-1})[9,12] between **5** and its reactive conformer in which the C–H is axial.

Although methoxy substituents at C-2 exert little effect on the reactivity of 1,3-dioxanes, this appears not to be the case for substituted tetrahydropyrans. The observation by Malatesta and Ingold[3] that the conformationally biased ether **1** reacts with *tert*-butoxy radicals 6 times faster than tetrahydropyran indicates that the exocyclic equatorial methoxy substituent in **1** strongly accelerates the rate of C–H fission. Likewise, the fact that abstraction of the *equatorial* hydrogen at C-2 from the stable conformer **2** occurs at the same rate as abstraction of *axial* hydrogen from tetrahydropyran[3] suggests that an axial methoxy suubstituent is activating. Both of these conclusions appear reasonable because the exoanomeric effect is known[25] to stabilize conformations **12** and **13** in which there is an antiperiplanar



12



13

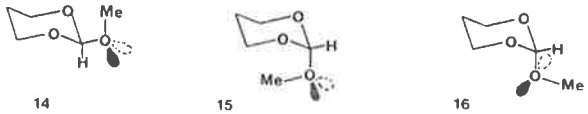
relationship between the C–H bond at C-2 and an sp^3 lone pair on the exocyclic oxygen atom. Alternatively, the same conformations can be regarded as possessing both a favorable anomeric interaction ($\theta = 30°$) between an exocyclic p lone pair and the endocyclic C–O bond and a similarly favorable interaction ($\theta = 30°$) between the same lone pair and the C–H bond at C-2 (see Figure 1).

The failure of axial or equatorial methoxyl substituents to exert a similar activating effect on 1,3-dioxanes suggests that in compounds such as **7** or **8** the substituent adopts conformations in which interactions between lone pairs and the C–H bond are at a minimum. Such a conformation (**14**) in an equatorial meth-

(18) Brunton, G.; Ingold, K. U.; Roberts, B. P.; Beckwith, A. L. J.; Krusic, P. J. *J. Am. Chem. Soc.* **1977**, *99*, 3177.
(19) Gorenstein, D. G.; Luxon, B. A.; Findlay, J. B.; Momii, R. *J. Am. Chem. Soc.* **1977**, *99*, 4170.
(20) For leading references see ref 3, 6–8, and 19.
(21) Meyers, A. I.; Campbell, A. L.; Abatjoglou, A. A.; Eliel, E. L. *Tetrahedron Lett.* **1979**, 4159.
(22) David, S.; Eisenstein, O.; Hehre, W. J.; Salem, L.; Hoffmann, R. *J. Am. Chem. Soc.* **1973**, *95*, 3806.
(23) Sweigart, D. A. *J. Chem. Educ.* **1973**, *50*, 322 and references cited.
(24) Bernardi, F.; Schlegel, H. B.; Wolfe, S. *J. Mol. Struct.* **1976**, *35*, 149.
(25) de Hoog, A. J.; Buys, H. R.; Altona, C.; Havinga, E. *Tetrahedron* **1969**, *25*, 3365. de Hoog, A. J.; Buys, H. R. *Tetrahedron Lett.* **1969**, 4175.

oxy-1,3-dioxane has two favorable anomeric antiperiplanar interactions between exocyclic sp^3 lone pairs and the endocyclic C–O bonds but possesses no lone pair antiperiplanar to the C–H bond. If the lone pair resides in a p-type orbital, the conformation **14** would give two favorable interactions ($\theta = 30°$) with C–O bonds but no such interaction ($\theta = 90°$) with the C–H.

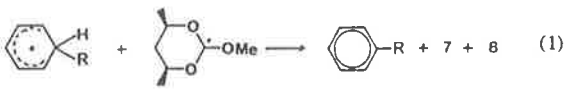
The conformation of an axial methoxydioxane which has the maximum possible anomeric stabilization involving sp^3 lone pairs and the minimum interaction with the C–H at C-2 is that **15** in which there is strong nonbonded repulsion between the *O*-methyl group and axial protons at C-4 and C-6. The concept of a p-type lone pair allows the formulation of a more attractive hypothesis, namely, that the methoxy group adopts a conformation (**16**) in which the magnitude of the two favorable interactions ($\theta = 30°$) of the p-lone pair with C–O bonds, is sufficient to outweigh nonbonded interactions between the eclipsed O–Me and C–H bonds. In this conformation (**16**) there is no interaction ($\theta = 90°$) of the lone pair with C–H.



14 15 16

Interaction of Methoxy-1,3-dioxanes with Triplet Benzophenone. Hayday and McKelvey[4] found that triplet benzophenone, generated by UV irradiation of a solution of benzophenone and the two tetrahydropyrans **1** and **2** in benzene, abstracts hydrogen preferentially from the cis isomer **1**. However, when we applied this procedure to each of the methoxydioxanes **7** and **8**, rapid epimerization occurred, and an equilibrium mixture of the two was formed. In separate experiments it was observed that epimerization proceeded only during irradiation and was not prevented or retarded by addition of pyridine or potassium carbonate to the reaction mixture. We conclude that epimerization is a true photochemical process and is not caused by traces of acid adventitiously generated during the reaction.

Two possible explanations were considered. When free-radical transformations are conducted in benzene, the solvent may undergo homolytic attack to afford a substituted cyclohexadienyl radical (**17**). Interaction of such an intermediate with a dioxanyl radical (**18**) by hydrogen atom transfer could generate both epimers of the substrate (eq 1).



(1)

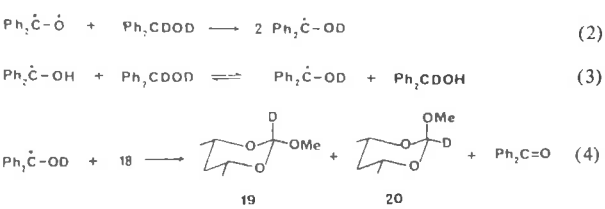
17 18

To test this hypothesis, we conducted the photochemical reaction of benzophenone with the trans isomer **8** in perdeuteriobenzene. Although epimerization occurred, neither of the deuterated species **19** and **20** was formed, a result which precludes the participation of the solvent in the reaction.

An alternative explanation is that methoxydioxanes **7** and **8** can be regenerated from **18** by hydrogen atom abstraction, either outside the solvent cage or within it after intersystem crossing of the triplet geminate pair:

$$18 + Ph_2\dot{C}{-}OH \rightarrow 7 + 8 + Ph_2C{=}O$$

If this hypothesis is valid, the addition of deuterated benzhydrol to the reaction mixture should bring about the formation of deuterated dioxanes by the following hydrogen atom and proton exchange reactions, (2)–(4).



(2)

(3)

(4)

19 20

In the event when the more stable isomer **8** of 2-methoxy-4,6-dimethyl-1,3-dioxane was irradiated with benzophenone and dideuteriobenzhydrol in benzene, epimerization rapidly occurred. Examination of the reaction mixture by ^2H NMR revealed that both of the deuterated isomers, **19** and **20**, were present with the *less* stable isomer **19** in excess [(**19**)/(**20**) ≈ 2].

The preferential formation of **19** provides further evidence that epimerization cannot be due to acid-catalyzed processes, for such reactions should yield predominantly the *more stable* isomer **20**. Thus it appears that the reaction scheme outlined above is substantially correct and that the photoinduced abstraction of hydrogen from **7** or **8** by benzophenone is reversible, a conclusion which is entirely reasonable in view of the known reversibility of related intramolecular processes.[26]

Another interesting mechanistic conclusion arises from the observation that epimerization of **8** in the presence of dideuteriobenzyhydrol occurs more rapidly than formation of the deuterated dioxanes **19** and **20**. The most obvious explanation, namely, that hydrogen atom transfer between **18** and $Ph_2\dot{C}$–OH occurs in the solvent cage, requires that intersystem crossing in the geminate triplet pair be very rapid and also that **18** *either* has a planar radical center *or* undergoes very rapid inversion.[27] The alternative explanation is the benzophenone triplet reacts with deuterated benzhydrol much more slowly than with **8** and that radical decay of $Ph_2\dot{C}$–OH by other routes competes effectively with the proton exchange (reaction 3). Under these circumstances the stationary concentration [PhĊ–OD] would be much less than [PhĊ–OH] and reaction 4 would be a minor reaction pathway.

The fact that the methoxydioxane **7** undergoes hydrogen atom abstraction more readily than its epimer **8** suggests that hydrogen atom donation to the intermediate radical **18** should also be under stereoelectronic control and should take place preferentially from the axial direction. Although further investigation is required, our observation that the *less stable* epimer **19** containing an axial C–D bond is formed more readily than the *more stable* compound **20** provides support for this contention.

Conclusion

The data presented in Tables I and II and discussed above, when considered in the light of results obtained elsewhere, indicate that hydrogen atom abstraction from tetrahydropyran, 1,3-dioxane, and related compounds is under stereoelectronic control. Evidence has also been obtained suggesting that the reverse reactions are subject to the same stereoelectronic influences. These reactions are but one manifestation of the general principle[1,2] that homolytic bond fission (or bond formation) is favored by coplanar interaction of the bond concerned with a neighboring lone pair, π orbital, or semioccupied orbital.

Experimental Section

General Data. EPR spectra were recorded on a Varian E9 EPR spectrometer. Radicals were generated directly in the cavity by irradiation of solutions with an Oriel 1000-W high-pressure mercury lamp. GLC analyses were carried out on a Perkin-Elmer 990 gas chromatograph fitted with an Hitachi Perkin-Elmer 159 recorder with a disk integrator. A SCOT Carbowax 20M, 58 m × 0.5 mm glass column was used with a flow rate of 3 mL min^{-1} of nitrogen carrier gas. At 70 °C the following retention times were observed: **3**, 19.0; **4**, 17.5; **5**, 60.0; **6** 19.7; **7**, 79.0; **8**, 42.0; **9**, 16.7; **10**, 23.2 min. Irradiation of mixtures containing benzophenone or acetone were conducted in a quartz vessel in a Rayonet photochemical reactor equipped with 16 RPR 3500 lamps.

Materials. Di-*tert*-butyl peroxide was purified by passage through alumina.[28] Carbon tetrachloride (reagent grade) was purified by fractional distillation and stored over 4A molecular sieves. It was redistilled from K_2CO_3 before use. Pyridine (reagent grade) was purified by distillation from KOH and stored over 4A molecular sieves. Otherwise, reagent grade chemicals were used without purification.

(26) Wagner, P. J.; Kelso, P. A.; Kemppainen, A. E.; McGrath, J. M.; Schott, H. N.; Zepp, R. G. *J. Am. Chem. Soc.* **1972**, *94*, 7506. Wagner, P. J.; Kochevar, I. E.; Kemppainen, A. E. *Ibid.* **1972**, *94*, 7489.

(27) EPR studies[3,11] of related radicals indicate that **18** probably is not planar but undergoes rapid inversion under the conditions used here.

(28) Offenbach, J. A.; Tobolsky, A. U. *J. Am. Chem. Soc.* **1957**, *79*, 278.

1,3-Dioxane (**3**),[29] 2-methyl-1,3-dioxane (**4**),[30] 2-methoxy-1,3-dioxane (**5**),[9] 4-methyl-*cis*-6-methyl-1,3-dioxane (**6**),[31] 2-methoxy-*cis,cis*-4,6-dimethyl-1,3-dioxane (**7**),[12b] 2-methoxy-*trans,trans*-4,6-dimethyl-1,3-dioxane (**8**),[12b] 2-methyl-*cis,cis*-4,6-dimethyl-1,3-dioxane (**9**),[12a] and 2-methyl-*trans,trans*-4,6-dimethyl-1,3-dioxane (**10**)[12b] were all prepared and purified by literature procedures. They were characterized by ^{1}H NMR, ^{13}C NMR, and IR spectroscopy and had physical constants in agreement with those previously recorded. All were obtained pure with the exception of dioxane **10** which contained approximately 8% of the epimer **9** (as determined by GLC analysis). The dioxanes were stored over K_2CO_3 and distilled before use.

Diphenylmethan-*1-d*-ol-*d* was prepared by treatment of benzophenone (2.0 g, 11 mmol) with lithium aluminum deuteride (0.25 g, 6 mmol) in dry ether (25 mL). After 2 h at reflux the solution was cooled, and a 2.5% solution of NaOD in D_2O (1.0 mL) was added. The usual workup gave the required deuterated alcohol (1.45 g, 72%): mp 69–70 °C; mass spectrum, *m/e* (relative intensity) 186 (M^+, 94%), 185 (4%), 184 (2%).

Methods. (a) EPR Spectrometry. Mixtures of di-*tert*-butyl peroxide and one or two substrates in a peroxide to total substrate ratio of approximately 2:1 (w/w) were degassed by bubbling with nitrogen for 10–15 min and then irradiated in the cavity of the spectrometer. Relative radical concentrations were determined by integration of EPR signals.

(b) Gas Chromatography. Solutions containing mixtures of approximately equal amounts of dioxanes (total dioxane concentration ≈ 0.01 M), di-*tert*-butyl peroxide (0.1–0.5 M), and pyridine (10% v/v) in carbon tetrachloride were degassed and irradiated in the Rayonet reactor. Aliquots were removed at intervals (4–24 h) and, after addition of an internal standard, were analyzed by gas chromatography. Various mixtures of dioxanes were studied including inter alia: **7** and **8**; **9** and **10**; **5**, **7**, and **8**; **4**, **9**, and **10**; **3** and **6**; **3**, **4**, and **5**; **6**, **8**, and **9**. Results of different experiments were consistent as were results obtained from aliquots taken from the same mixture at different times.

Solutions containing dioxanes **7** and/or **8** (total dioxane concentration = 0.01 M) and benzophenone (0.01 M) in benzene were degassed, irradiated in the Rayonet reactor, and analyzed by gas chromatography. When perdeuteriobenzene was used as solvent, the mixture was also examined by ^{2}H NMR; no signal other than that for the solvent was detected, although gas chromatography revealed that epimerization of the substrate had occurred. A solution of dioxane **8** (0.06 M), diphenylmethan-*1-d*-ol-*d* (0.15 M), and benzophenone (0.10 M) in benzene was subjected to irradiation. Gas chromatography after 1 h revealed that an equilibrium mixture of **7** and **8** had been formed. After 12-h irradiation the reaction mixture was concentrated, deuteriochloroform was added, and the solution was filtered. The ^{2}H NMR spectrum showed resonances at δ 4.7 and 5.0 (integral ratio 1:2) assigned to the dioxanes **19** and **20**, respectively, on the basis of comparison with ^{1}H NMR spectra determined under similar conditions.

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(29) Clarke, H. T. *J. Chem. Soc.* **1912**, *101*, 1788.
(30) Rondestvedt, C. S. *J. Org. Chem.* **1961**, *26*, 2247.
(31) Eliel, E. L.; Nader, F. W. *J. Am. Chem. Soc.* **1970**, *92*, 3045.

Biochemistry **1982**, *21*, 2857–2862

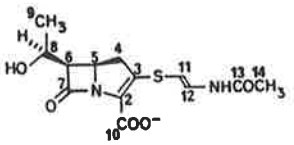
Inhibition of the RTEM β-Lactamase from *Escherichia coli*. Interaction of the Enzyme with Derivatives of Olivanic Acid†

Christopher J. Easton and Jeremy R. Knowles*

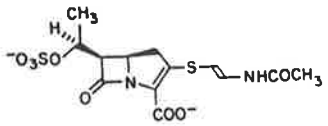
ABSTRACT: From chemical and kinetic studies of the interaction of the RTEM β-lactamase from *Escherichia coli* with three derivatives of olivanic acid, MM22382 (**1**), MM13902 (**2**), and MM4550 (**3**), a mechanism for the inhibition of the enzyme by these compounds is proposed: the interaction proceeds by formation of an acyl-enzyme, the Δ^2-pyrroline, which may either deacylate or undergo tautomerization to the more tightly bound Δ^1-pyrroline. The ability of olivanic acids to inhibit the enzyme thus depends on the partitioning of the acyl-enzyme to the Δ^1-pyrroline (a process that competes with the normal hydrolytic pathway) and on the rate of regeneration of free enzyme from this complex.

Many members of the carbapenem family of β-lactams are effective as antibiotics against β-lactamase-producing bacteria (e.g., Basker et al., 1980). To be effective, these species must be resistant to the enzyme-catalyzed hydrolysis reaction. We report here studies on the interaction of one group of carbapenems, the olivanic acids (Butterworth et al., 1979; Brown et al., 1977; Hood et al., 1979), with the purified plasmid-encoded RTEM[1] β-lactamase, to evaluate how these antibiotics protect themselves against the hydrolytic action of the enzyme.

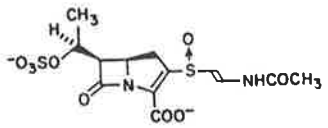
In our earlier report on the interaction of MM22382 (**1**)



MM22382 **1**



MM13902 **2**



MM4550 **3**

and MM13902 (**2**) with the β-lactamase, Schemes I and II (A or B) were shown to be the minimal kinetic schemes necessary to accommodate the behavior of these two olivanic acid derivatives, respectively (Charnas & Knowles, 1981). In the present work we probe the nature of these interactions in more detail and propose a scheme that accommodates the observed behavior. We also report studies on the interaction of the β-lactamase with a third olivanic acid derivative, MM4550 (**3**).

Experimental Procedures

Materials

Olivanic acid derivatives, MM13902, MM4550, and MM22382, were generous gifts of Beecham Pharmaceuticals, Betchworth, Surrey, United Kingdom. MM13902, as the sodium salt, was a pale yellow crystalline solid. MM4550, as the sodium salt, was an off-white powder. MM22382, as the sodium salt, was an orange powder. These materials were stored desiccated at −20 °C. Solutions were prepared by weight by using a Cahn 25 electrobalance. Ultraviolet spectroscopic measurements were made in 0.1 M sodium phosphate buffer, pH 7.0, at 30 °C by using a Perkin-Elmer 554 or 575 spectrophotometer. MM13902 has λ_{max} at 223 and 304 nm (ϵ = 14 100 and 15 200 M^{-1} cm^{-1}, respectively), MM4550 has λ_{max} at 237 and 282 nm (ϵ = 13 500 and 12 000 M^{-1} cm^{-1}, respectively), and MM22382 has λ_{max} at 225 and 304 nm (ϵ = 13 500 and 13 300 M^{-1} cm^{-1}, respectively).

HPLC[1] was carried out on a Waters Associates chromatograph equipped with a differential ultraviolet detector operating at 254 nm, using a μBondapak C_{18} reverse-phase column (0.39 × 30 cm) and eluting with water (0.4 mL min^{-1}). The retention times of MM13902, MM4550, and MM22382 were 35, 29, and 52.5 min, respectively.

TLC was carried out on silica (Analtech) eluted with 1-butanol/methanol/water (4:1:2) and on cellulose (Eastman-Kodak) eluted with 2-propanol/water (7:3). The R_f values of MM13902, MM4550, and MM22382 were 0.6, 0.45, and 0.75, respectively, on silica and 0.85, 0.75, and 0.9, respectively, on cellulose.

Minor decomposition of each of the olivanic acid derivatives occurred on storage, giving rise to two products in each case, as determined by HPLC and TLC. The olivanic acids were purified by HPLC so that, except where noted, they were >95% pure when used.

^1H and ^{13}C NMR spectra were recorded on a Varian CFT20, a Varian XL100, a Jeol FX270, or a Bruker WM300 instrument.

The purification of the TEM-2[1] β-lactamase that was used has been described previously (Charnas & Knowles, 1981).

Methods

The procedures used to investigate the kinetics of the interaction of MM4550 with the β-lactamase have been described previously in the report on the interaction of MM13902 with the enzyme (Charnas & Knowles, 1981). Reactions were studied by following the absorbance change at 282 nm, and calculations were made on the basis of the measured $\Delta\epsilon$ at 282 nm of 10 100 M^{-1} cm^{-1}. The buffer used in experiments in-

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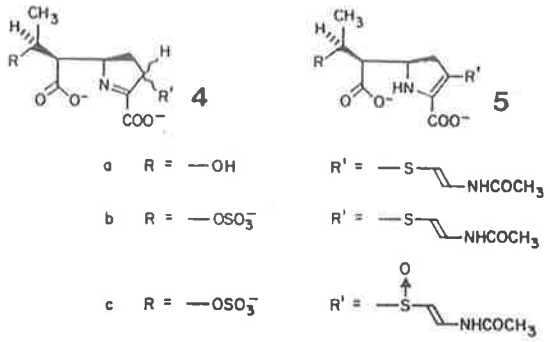
[1] Abbreviations: RTEM specifies the source of the plasmid [see Datta & Kontomichalou (1965)] and TEM-2 specifies the enzyme [see Sutcliffe (1978)]; DEAE, diethylaminoethyl; HPLC, high-performance liquid chromatography.

volving product isolation was 10 mM $Et_3NH{\cdot}HCO_3$, pH 7.0.

Enzyme-Catalyzed Hydrolysis of MM22382 (1). A solution of enzyme (100 μL, 1 μM) in buffer was added to a solution of 1 (0.25 mg, 0.75 μmol) in buffer (20 mL). The mixture was incubated at 30 °C for 0.5 h, then cooled, and concentrated to ~2 mL. For removal of the enzyme, the residue was applied to a Bio-Gel P-2 gel filtration column (1.5 × 20 cm) at 4 °C, eluting with buffer. Fractions eluting between 0.7 and 2.0 column volumes were pooled, aqueous NaOH (15 μL, 0.1 M) was added to prevent acid-catalyzed decomposition of the product, and the mixture was freeze-dried to give 4a as a white powder: UV max 262 nm (ε 10600 M^{-1} cm^{-1}); TLC, R_f values of 0.8 on cellulose and 0.65 on silica; HPLC, retention time 13.5 min.



Enzyme-Catalyzed Hydrolysis of MM13902 (2) *and MM4550* (3). To a solution of enzyme (200 mL, 2.5 μM) in buffer, incubated at 30 °C, were added portions of 2 or 3 (0.3 mg, ~0.7 μmol) at hourly intervals for 10 h. After a further 2 h at 30 °C, the mixtures were worked up as described for the hydrolysis of 1 by the enzyme. Hydrolysis of 2 gave 4b as a white powder: UV max 262 nm (ε 12000 M^{-1} cm^{-1}); TLC, R_f values of 0.7 on cellulose and 0.55 on silica; HPLC, retention time 9 min; ^1H NMR, see Table I. Hydrolysis of 3 gave 4c as a white powder: UV max 248 nm (ε 16300 M^{-1} cm^{-1}); TLC, R_f values of 0.65 on cellulose and 0.4 on silica; HPLC, retention time 8 min; ^1H NMR, see Table I.

Base-Catalyzed Hydrolysis of MM22382 (1), *MM13902* (2), *and MM4550* (3). An aqueous solution of 1, 2, or 3 (0.2–0.3 mg, 0.3 mM) and NaOH (0.6 mM) was incubated at 30 °C for 12 h, and the solution was then freeze-dried. The products were identified as 4a from 1, 4b from 2, and 4c from 3, by comparison with the products obtained by enzyme-catalyzed hydrolysis. So that the product samples for ^{13}C NMR could be obtained, the same procedure was followed, except that 15 mg of 2 or 3 was used. The samples used in this experiment each contained ~15% of the two impurities formed on storage, one of which was shown by HPLC to be the normal hydrolysis product. The freeze-dried reaction products were chromatographed on a column (1 × 20 cm) of DEAE-cellulose (DE-52, Whatman) equilibrated with 10 mM $Et_3NH{\cdot}HCO_3$ at 4 °C, eluting with a linear gradient (10–300 mM) of aqueous $Et_3NH{\cdot}HCO_3$ (200 mL), pH 7.0. Fractions containing 4 (b or c), as determined by HPLC, were pooled, NaOH (2 molar equiv) was added, and the mixtures were then freeze-dried. Analysis by ultraviolet, TLC, HPLC, and ^1H NMR showed that the isolated material was 4 (b or c), and ^{13}C NMR spectra were recorded (see Table II).

Results and Discussion

In our earlier investigation (Charnas & Knowles, 1981) the interaction of the RTEM β-lactamase with 1 and 2 was studied. Compound 1 behaves simply as a good substrate of the enzyme, and the kinetic scheme shown in Scheme I suffices to describe the observed behavior. In contrast, 2 is a poor substrate and an excellent inhibitor of the enzyme. The inhibition derives from a branching of the normal hydrolytic pathway in which the acyl-enzyme intermediate partitions to a transiently stable, inactive form of the enzyme. In the experiments described below we have investigated the interaction of MM4550 (3) with the β-lactamase and extended our studies on the interaction of 1 and 2 with the enzyme.

Scheme I: Minimal Kinetic Pathway for the Interaction of 1 and β-Lactamase[a]

$$e + s \rightleftharpoons e{\cdot}s \longrightarrow a\text{-}e \longrightarrow e + p$$

[a] e, free enzyme; s, 1; e·s, the Michaelis complex of e and 1; a-e, the acyl-enzyme from 1; p, the product of enzymatic hydrolysis of 1.

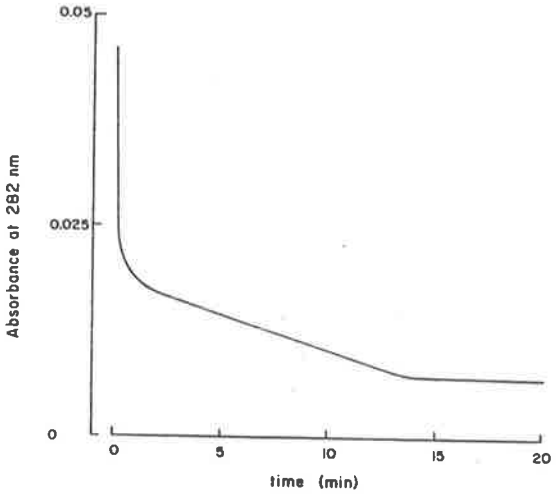
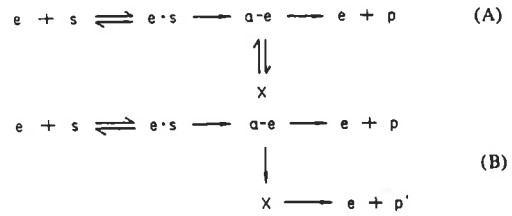


FIGURE 1: Changes in absorbance at 282 nm accompanying the hydrolysis of MM4550 (3) by β-lactamase. MM4550 (3.9 μM) was incubated with β-lactamase (2 μM) in 0.1 M sodium phosphate buffer, pH 7.0, at 30 °C.

Interaction of the β-Lactamase with MM4550 (3). Incubation of β-lactamase with 3 results in the disappearance of the chromophore at 282 nm and the appearance of a new chromophore at 248 nm. The kinetic characteristics of this interaction are complicated by a second-order reaction between 3 and the enzyme that is significant only at concentrations of 3 above about 5 μM. This reaction does not involve the enzyme's active site, since β-lactamase that has been inactivated by clavulanic acid (Fisher et al., 1978) still causes the loss of A_{282nm} with a rate constant of about 25 M^{-1} s^{-1}. At lower concentrations of 3 (<5 μM), however, the dominant reaction is zero order in substrate and first order in enzyme and has a rate constant of (6.5 ± 0.5) × 10^{-4} s^{-1}. All kinetic experiments were done with 3 at <5 μM to avoid complications from the nonspecific reaction of the protein with 3. At these concentrations the first-order reaction predominates, and minor contributions from the nonspecific second-order process are easily subtracted.

The interaction of the sulfoxide 3 with the enzyme is similar to that observed earlier with 2 (Charnas & Knowles, 1981). The time course of the reaction is biphasic: a rapid initial phase gives way over ~2 min to a slower steady-state reaction that proceeds at a constant rate until 3 is exhausted (Figure 1). The rate constant for the rapid phase of the reaction is (8 ± 2) × 10^{-2} s^{-1} and is independent both of the concentration of 3 (above 1 μM) and of the molar excess of 3 over enzyme (from 2- to 20-fold). The steady-state reaction has a rate

Scheme II: Minimal Kinetic Pathways for the Interaction of 2 or 3 and β-Lactamase[a]



[a] e, free enzyme; s, 2 or 3; e·s, the Michaelis complex; a-e, the acyl-enzyme; p and p', products after deacylation; X, the enzyme intermediate responsible for the transient inhibition of the enzyme.

constant of $(6.5 \pm 0.5) \times 10^{-4}$ s^{-1}. When the trace of A_{282nm} vs. time is extrapolated back to time zero, the "burst" size is approximately 1.4 times that of the enzyme concentration on a molar basis. As we have shown earlier for **2** (Charnas & Knowles, 1981), a burst size that is greater than 1.0 cannot be accommodated by the kinetic pathway shown in Scheme I. [As before, the trivial explanation that **3** contains a small amount of a rapidly reacting impurity is eliminated by the facts (a) that the burst size is independent of the concentration of **3** at a fixed enzyme level and (b) that subsequent additions of **3** to an incubation of **3** with the enzyme do not produce a second burst.] The observation of a rapid burst reaction in which more than 1 molar equiv of substrate is consumed can be accommodated by a branched pathway such as is shown in part A or B of Scheme II. In these schemes an intermediate X accumulates, this species deriving from an intermediate on the normal hydrolytic pathway. During the burst phase of the reaction, at least 1.4 hydrolytic turnovers occur before essentially all the enzyme has accumulated as X. The rate of the subsequent steady-state reaction is then governed by the rate of regeneration of free enzyme from X, either via the acyl-enzyme (Scheme IIA) or directly (Scheme IIB).

The proportion of enzyme that, during the steady-state enzyme-catalyzed hydrolysis of **3**, is tied up in the form of covalent intermediates (acyl-enzyme and X) can be estimated from the remaining catalytic activity measured immediately after a sample of an incubation of enzyme with **3** is diluted into an assay solution containing benzylpenicillin. The initial rate of benzylpenicillin hydrolysis provides a measure of the instantaneous concentration of free, active enzyme. Thus when **3** is mixed with the enzyme, there is a rapid decrease in enzyme activity to less than 1% of that present initially. The rate of activity loss has a half-time of about 8 s, corresponding to a first-order rate constant (k_{inact}) of 9×10^{-2} s^{-1}. This is in excellent agreement with the rate constant of 8×10^{-2} s^{-1} derived from the observed changes in A_{282nm} during the burst. It is evident that the rapid phase of the reaction produces complexes between enzyme and **3** that are catalytically inactive, as expected from Scheme II. The value of k_{inact} is constant at concentrations of **3** greater than 0.1 μM, and it is therefore clear that the inactivation process follows the expected saturation behavior.

Analysis of the intial rates of enzyme inactivation at low concentrations of **3** shows that the inactivation rate is half-maximal at a substrate concentration of ~1 nM. This value of $K_{m(inact)}$ is in good agreement with the K_i value of ~1 nM determined in direct competition experiments with benzylpenicillin and indicates that, as with **2**, inactivation of the enzyme occurs at substrate concentrations that may be physiologically relevant. The value of $k_{inact}/K_{m(inact)}$ for **3** is about 10^8 M^{-1} s^{-1}, which, as a second-order rate constant for the inactivation process, shows that **3** is ~100-fold more efficient than **2** in the inactivation of the RTEM β-lactamase at low concentrations.

As with **2**, the inactivation of β-lactamase by **3** is reversible, and after long incubations, full recovery of enzyme catalytic activity is observed. When measured directly after all the **3** has been consumed, or by dilution of an incubation of **3** with enzyme into an assay mixture, the catalytic activity of the enzyme against benzylpenicillin returns in a first-order manner. The rate constant for the recovery of catalytic activity, k_{react}, is $(4.4 \pm 0.3) \times 10^{-4}$ s^{-1}, with the activity rising from <1% to 100% of the original value, within the limits of experimental detection.

The reaction that leads to recovery of enzyme activity (k_{react}) is somewhat slower than the steady-state turnover rate (k_{cat}). This difference in rate constants *requires* a branched pathway: the linear pathway of Scheme I cannot accommodate a rate of substrate turnover that is faster than the rate of recovery of enzyme catalytic activity after all of the substrate has been consumed. In terms of the pathways of parts A and B of Scheme II, the recovery of activity at the end of the reaction represents the decomposition of the intermediate X to regenerate free enzyme, which is slower than the deacylation rate of the acyl-enzyme.

In summary, while Scheme I adequately describes the interaction of **1** with the β-lactamase, the minimal kinetic scheme necessary to accommodate the behavior of compounds **2** and **3** with the enzyme is Scheme II (A or B). Why is there this difference?

Compounds **2** and **3** differ from **1** by the existence of a sulfate ester on C-8. In our earlier study we investigated the possiblity of sulfate elimination from the acyl-enzyme from **2** to give an α,β-unsaturated acyl-enzyme of greater hydrolytic stability than the first-formed saturated ester [see Scheme III of Charnas & Knowles (1981)]. It was found, however, that sulfate ion is *not* released during the interaction of **2** with the enzyme. The most persuasive interpretation of the differences in the observed behavior of **1**, **2**, and **3** is that deacylation is much faster in the case of **1**, the acyl-enzyme from which is so short-lived that formation of the inhibited species X does not compete significantly with deacylation. According to this view, the behavior of **1** is just a special case of the generalized formulation of Scheme II (A or B). Indeed, with **1** at very high substrate-to-enzyme ratios (400 000:1), some biphasic character of the interaction is discernible.

Product Studies. In an attempt to understand the chemical events that are outlined in Scheme II, we undertook product studies of the interaction of **1**, **2**, and **3** with the β-lactamase. The products deriving from the interaction of each of these olivanic acids with the β-lactamase have been isolated, and the characteristics of these products are presented under Experimental Procedures and in Table I. For comparison, the properties of the parent olivanic acids are also included. In each case the enzymic hydrolysis was performed under conditions such that the contribution from nonenzymic hydrolysis was <10%. The products from base-catalyzed hydrolysis were also isolated and characterized.

The products of enzymic and nonenzymic hydrolysis of the carbapenems are identical, as judged by their ultraviolet and ^{1}H NMR spectra and their behavior on HPLC and TLC. The data indicate that the hydrolytic product is in each case a mixture of the Δ^1-pyrrolines (**4**) epimeric at C-3. From their chromatographic behavior it is clear that the products of hydrolysis are more polar than the parent carbapenems. The differences in the ultraviolet absorptions of the olivanic acids

Table I: Chemical Shifts and Coupling Constants in ^1H NMR Spectra[a]

proton	δ from TMS (J in Hz)			
	MM13902 (2)[b]	Δ¹-pyrroline (4b)[c]	MM4550 (3)[b]	Δ¹-pyrroline (4c)[c]
3-CH		4.34 m		~4.8 m[e]
4-CH_2	3.29 dd (8.5, 18), 2.94 dd (9.5, 18)	2.21 m, 2.66 m	3.46 dd (9, 18.5), 2.99 dd (10.5, 18.5)	2.18 m, 2.57 m
5-CH	4.25 ddd (5.5, 8.5, 9.5)	4.22 m	4.37 ddd (6, 9, 10.5)	4.50 m [4.40 m]
6-CH	3.78 dd (5.5, 9)	2.84 dd (7, 7) [2.89 dd (7, 7)]	3.88 dd (6, 9)	2.96 dd (7, 7) [2.88 dd (7, 7)]
8-CH	4.75 dq (9, 6)	~4.8 m[e]	4.97 dq (9, 6.5)	~4.8 m[e]
9-CH_3	1.47 d (6)	1.44 d (6) [1.46 d (6)]	1.45 d (6.5)	1.46 d (6) [1.48 d (6)]
11-CH	7.07 d (14)	7.09 d (14) [7.06 d (14)]	7.18 d (14)	7.48 d (14) [7.60 d (14)]
12-CH	5.98 d (14)	5.72 d (14) [5.70 d (14)]	6.24 d (14)	5.97 d (14) [6.14 d (14)]
14-CH_3 NH[d]	2.00 s	2.04 s [2.06 s]	2.05 s	2.10 s [2.14 s]

[a] Spectra were recorded in D_2O by using sodium 3-(trimethylsilyl)-1-propanesulfonate (TMS) as an internal reference. [b] Values reported by Brown et al. (1977). [c] Where the chemical shifts of the epimers, for any particular proton, are different, that of the minor component is listed in brackets. [d] Amide proton exchanges (Brown et al., 1977). [e] Signal partially obscured by HDO resonance.

and their respective hydrolysis products are expected. The higher wavelength absorptions of the β-lactams are characteristic of the unsaturated sulfides (**1** and **2**) and sulfoxide (**3**), and the changes in the λ_{max} values upon hydrolysis are consistent with the Δ^1–Δ^2 conversion. Further evidence for the epimeric Δ^1-pyrrolines (**4**) is obtained from NMR spectra of the hydrolysis products from **2** and **3**.[2] The ^1H NMR spectra (Table I) show that in each case the product is a mixture of two similar components, with each component having new signals from 3-CH and major shifts of the signals from 4-CH_2, 6-CH, and 11-CH. The ^{13}C NMR spectra of the base-catalyzed hydrolysis products from **2** and **3** (Table II) are consistent with the proposed structures, and although it was impractical to obtain ^{13}C NMR spectra of the enzymic hydrolysis products, the enzymic and nonenzymic hydrolysis products are identical by all other criteria.

The results described above are in good agreement with the report by Maeda et al. (1977) of the products from the nonenzymic hydrolysis of a carbapenem (MC696-SY2-A) (**6**) which is believed to have the same structure as **3**. These workers established that the two epimeric Δ^1-pyrrolines were the main products from hydrolysis of **6**. The ^{13}C and ^1H NMR spectra of **6** and of its hydrolysis products show almost identical relative chemical shifts as we have observed for **3** and the products of its hydrolysis, though the absolute shifts of the ^1H NMR spectra differ by approximately 0.5 ppm. We may therefore conclude that with each of the olivanic acids, enzymic hydrolysis results in the formation simply of a mixture of the two Δ^1-pyrrolines (**4**) epimeric at C-3.

The Δ^1-pyrrolines obtained from the enzymic hydrolysis are also an equilibrium mixture of the C-3 epimers. Whereas base-catalyzed hydrolysis of the β-lactam gives a nonequilibrium ratio of C-3 epimers of **4**, neutralization rapidly effects equilibrium. Hydrolysis in D_2O under basic conditions results in deuterium incorporation at C-3, and this deuterium washes out in H_2O at neutral pH. Conversely, deuterium incorporation at C-3 occurs rapidly on incubation of unlabeled **4** at neutral pH in D_2O. This suggests that the Δ^1–Δ^2-pyrroline tautomerization is rapid near neutrality, but relatively slow at high pH. The Δ^1-pyrroline (**4**) is evidently strongly favored thermodynamically over the Δ^2 isomer (**5**), since no **5** can be detected by NMR either at neutral pH or under basic conditions. A preference for the Δ^1-pyrroline in analogous compounds has previously been reported (e.g., Hausler & Schmidt, 1979).

Table II: Chemical Shifts in ^{13}C NMR Spectra[a]

carbon	δ from TMS			
	MM13902 (2)[b]	Δ¹-pyrrolines (4b)[c]	MM4550 (3)[b]	Δ¹-pyrrolines (4c)[c]
2	144	170.4 (171.2)[d]	140	169.1 (169.3)[d]
3	128	52.7 (52.2)	139	72.9 (71.2)
4	37	33.6 (33.0)	29	25.5
5	58	70.9 (72.0)	59	70.6 (71.2)
6	54	59.9 (59.6)	55	59.9 (59.7)
7	169	173.3 (174.5)[d]	166	168.6[d]
8	74	77.3	74	77.2 (77.1)
9	19	18.9 (18.1)	19	19.1 (18.1)
10	178	178.6 (178.4)[d]	177	178.2[d]
11	103	101.8 (102.7)	112	111.0 (108.1)
12	131	133.0 (131.7)	135	135.8
13	172	172.2[d]	173	173.5[d]
14	23	22.8	23	23.2

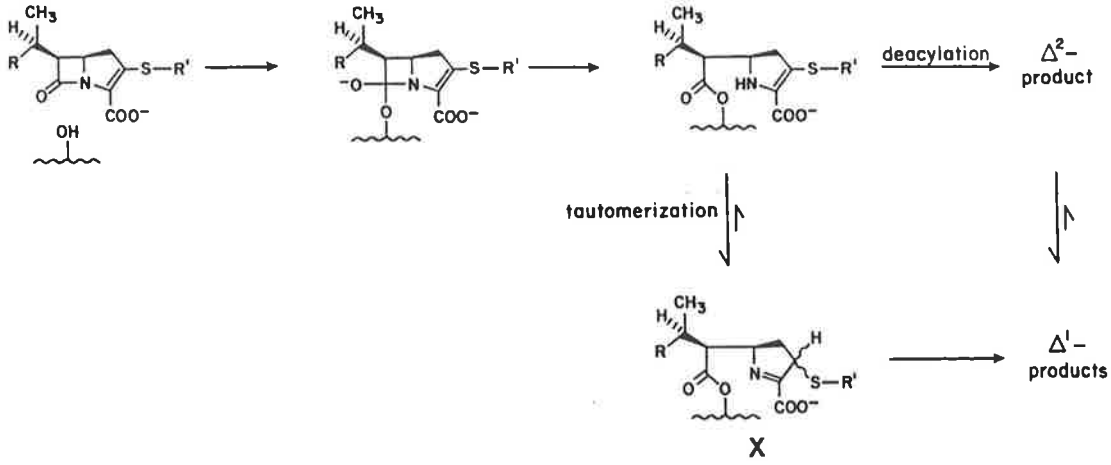
[a] Spectra were recorded in D_2O by using dioxane as an internal standard. [b] Values reported by Brown et al. (1977). [c] Where the chemical shifts of the epimers, for any particular carbon, are different, that of the minor component is listed in parentheses. [d] Assignments within any vertical column may be reversed.

Since the enzyme-catalyzed reactions are conducted near neutrality where there is rapid epimerization at C-3, we cannot say what species is released by the enzyme. It is clear, however, that the primary product is one of the epimeric Δ^1-pyrrolines (**4**), the Δ^2-pyrroline (**5**), or a mixture of these species.

Nature of the Inactive Complex. Given that enzymic hydrolysis of the olivanic acids yields the epimeric Δ^1 pyrrolines from the corresponding Δ^2 parent species, what is the nature of the transiently inhibited enzyme intermediate? Both the

[2] Lack of material prevented an NMR analysis of the product from **1**.

Scheme III: Tentative Scheme That Accommodates the Interaction of Olivanic Acids with β-Lactamase



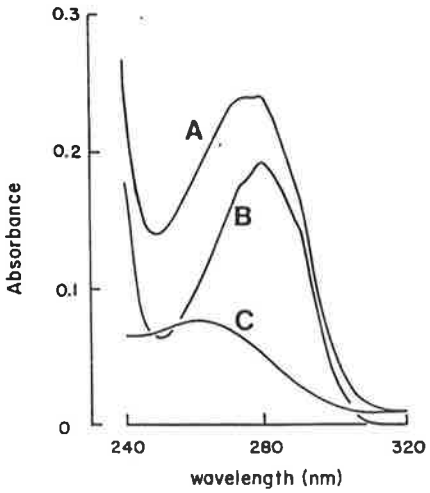


FIGURE 2: Ultraviolet spectra (A) of the inhibited enzyme species X (see Scheme II) (6 μM) obtained by gel filtration at 4 °C (Bio-Gel P-2, 1.5 × 17 cm) in 10 mM *N*-ethylmorpholinium acetate buffer, pH 7.0, of a mixture of enzyme (10 μM) and MM13902 (**2**) (100 μM) in buffer (1 mL) that had been incubated at 30 °C for several minutes, (B) of free enzyme (6 μM) in buffer, and (C) of the difference between (A) and (B). All spectra recorded during the reactivation of X (from <20% to >95% activity, assayed by measuring the rate of hydrolysis of benzylpenicillin and based on the total activity of free enzyme added to the incubation mixture) are superimposable on (A).

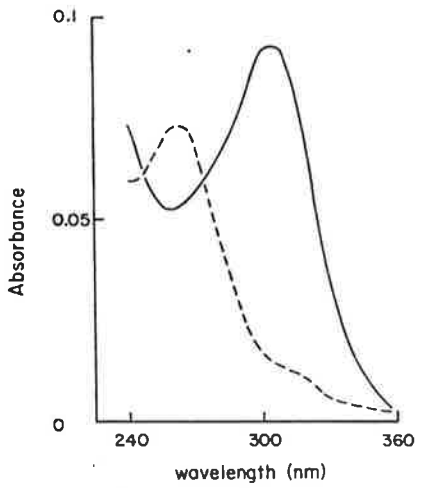


FIGURE 3: Ultraviolet spectra of MM13902 (**2**) (6 μM) (—) and of its hydrolysis product, **4b** (6 μM) (---), each in 10 mM *N*-ethylmorpholinium acetate buffer, pH 7.0, at 30 °C.

acyl-enzyme and the intermediate X must be the Δ^1- or Δ^2-pyrroline, or a species that can readily rearrange to one or other of them. The simplest formulation consistent with all the results appears to be that illustrated in Scheme III, where collapse of the first-formed tetrahedral intermediate produces the acyl-enzyme as a Δ^2-pyrroline. This intermediate may undergo deacylation or in a competing process tautomerize to the more stable Δ^1-pyrroline. This Δ^1-pyrroline may deacylate directly (Scheme IIB) or tautomerize back to the bound Δ^2-pyrroline, which then deacylates via the normal hydrolytic path (Scheme IIA).

The inhibited enzyme species X (obtained from an incubation of enzyme with **2**) was isolated by gel filtration according to the procedure of Charnas & Knowles (1981) to obtain evidence in support of this postulate. Upon deacylation of this species, and repeated gel filtration to remove enzyme, the recovered product was the Δ^1-pyrroline (**4b**) as deduced from its spectroscopic and chromatographic properties. The ultraviolet spectrum of the inhibited enzyme before and during reactivation was also studied (Figure 2), and it was found that *no changes* in the spectrum occurred during the reactivation process. Indeed, it is clear that the difference in the ultraviolet spectra of free enzyme and inhibited enzyme X (Figure 2) closely resembles the spectrum of the Δ^1-pyrroline (**4b**) (Figure 3) and is quite different from the spectrum of the Δ^2 substrate (**2**) itself (Figure 3). It is thus evident that the inhibited enzyme species X contains the carbapenem moiety bound as a Δ^1-pyrroline, as shown in Scheme III.

In qualitative terms, the observed behavior of the carbapenems **2** and **3** could be accommodated by postulating a slow substrate-induced enzyme conformational change of the kind proposed by Citri and co-workers (Samuni & Citri, 1975; Citri et al., 1976). Thus the nonstoichiometric burst would represent the transition of the enzyme into a form appropriate for the hydrolysis of those carbapenems, and the recovery of enzyme activity when an incubation with carbapenem is diluted into a solution containing benzylpenicillin would derive from the isomerization of the enzyme back to the more active state appropriate for benzylpenicillin hydrolysis. However, aside from the sharp difference in the behavior of **1** from that of **2** and **3**, the recovery reaction (of **2**) is accelerated by (and is first order with respect to) hydroxylamine and has a rate

constant of 5.6×10^{-2} M^{-1} s^{-1} (Charnas & Knowles, 1981). Analogously, the recovery of activity from incubations of 3 with enzyme is accelerated by hydroxylamine, this reaction having a second-order rate constant of 3.3×10^{-2} M^{-1} s^{-1}. These results are not readily accommodated by a scheme involving substrate-induced conformational changes but are entirely consistent with the picture presented in Scheme III.

The chemical identity of the acyl-enzyme (Scheme III), while most logically the Δ^2-pyrroline, is unproven. Indeed, there will be little difference in the inherent chemical stabilities of the ester linkages of the acyl-enzyme (the Δ^2-pyrroline) and of X (the Δ^1-pyrroline), and it is possible that the acyl-enzyme is the C-3 epimer of X. Moreover, we cannot distinguish between parts A and B of Scheme II. These points are not particularly important, however, since the ability of the carbapenems to inhibit the enzyme clearly depends on the rate of formation of the Δ^1-pyrroline, X, and on the rate of release of free enzyme from this complex.

From the mechanism outlined here, it is evident that carbapenems will be β-lactamase inhibitors if the formation of the Δ^1-pyrroline species competes effectively with the deacylation process. The olivanic acids are one class of the larger group of carbapenem antibiotics, all of which may well follow the path of Scheme III. These include the thienamycins (Leanza et al., 1979; Kahan et al., 1979; Tally et al., 1978; Kropp et al., 1980), PS-5, PS-6, and PS-7 (Okamura et al., 1978–1980; Sakamoto et al., 1980), the carpetimycins (Nakayama et al., 1980), the epithienamycins (Stapley et al., 1981; Cassidy et al., 1981), C-19393 S_2 and H_2 (Nozaki et al., 1981; Okonogi et al., 1981; Imada et al., 1980; Harada et al., 1980), and asparenomycin A (Tanaka et al., 1981). The results of our preliminary studies on the interaction of asparenomycin A, thienamycin, *N*-acetylthienamycin, and *N*-formimidoylthienamycin with β-lactamase are consistent with the branched pathway shown in Scheme III (C. Easton, J. Fisher, E. Jaffe, and C. Kemal, unpublished results). Elucidation of the mode of lactamase inhibition exhibited by these compounds may aid the search for new antibiotics suitable for treating infections of β-lactamase-producing bacteria.

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References

Basker, M. J., Boon, R. J., & Hunter, P. A. (1980) *J. Antibiot. 33*, 878–884.

Brown, A. G., Corbett, D. F., Eglington, A. J., & Howarth, T. T. (1977) *J. Chem. Soc., Chem. Commun.*, 523–525.

Butterworth, D., Cole, M., Hanscomb, G., & Rolinson, G. N. (1979) *J. Antibiot. 32*, 287–294.

Cassidy, P. J., Albers-Schonberg, G., Goegelman, R. T., Miller, T., Arison, B., Stapley, E. O., & Birnbaum, J. (1981) *J. Antibiot. 34*, 637–648.

Charnas, R. L., & Knowles, J. R. (1981) *Biochemistry 20*, 2732–2737.

Citri, N., Samuni, A., & Zyk, N. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 1048–1052.

Datta, N., & Kontomichalou, P. (1965) *Nature (London) 208*, 239.

Fisher, J., Charnas, R. L., & Knowles, J. R. (1978) *Biochemistry 17*, 2180–2184.

Harada, S., Shinagawa, S., Nozaki, Y., Asai, M., & Kishi, T. (1980) *J. Antibiot. 33*, 1425–1430.

Hausler, J., & Schmidt, U. (1979) *Liebigs Ann. Chem.*, 1881–1889.

Hood, J. D., Box, S. J., & Verral, M. S. (1979) *J. Antibiot. 32*, 295–304.

Imada, A., Nozaki, Y., Kintaka, K., Okonogi, K., Kitano, K., & Harada, S. (1980) *J. Antibiot. 33*, 1417–1424.

Kahan, J. S., Kahan, F. M., Goegelman, R., Currie, S. A., Jackson, M., Stapley, E. O., Miller, T. W., Miller, A. K., Hendlin, D., Mochales, S., Hernandez, S., Woodruff, H. B., & Birnbaum, J. (1979) *J. Antibiot. 32*, 1–12.

Kropp, H., Sundelof, J. G., Kahan, J. S., Kahan, F. M., & Birnbaum, J. (1980) *Antimicrob. Agents Chemother. 17*, 993–1000.

Leanza, W. J., Wildonger, K. J., Miller, T. W., & Christensen, B. G. (1979) *J. Med. Chem. 22*, 1435–1436.

Maeda, K., Takahashi, S., Sezaki, M., Iinuma, K., Naganawa, H., Kondo, S., Ohno, M., & Umezawa, H. (1977) *J. Antibiot. 30*, 770–772.

Nakayama, M. Iwasaki, A., Kimura, S., Mizoguchi, T., Tanabe, S., Murakami, A., Watanabe, I., Okuchi, M., Itoh, H., Saino, Y., Kobayashi, F., & Mori, T. (1980) *J. Antibiot. 33*, 1388–1390.

Nozaki, Y., Kawashima, F., & Imada, A. (1981) *J. Antibiot. 34*, 206–211.

Okamura, K., Hirata, S., Okumura, Y., Fukagawa, Y., Shimauchi, Y., Kouno, K., & Ishikura, T. (1978) *J. Antibiot. 31*, 480–482.

Okamura, K., Hirata, S., Koki, A., Hori, K., Shibamoto, N., Okumura, Y., Okabe, M., Okamoto, R., Kouno, K., Fukagawa, Y., Shimauchi, Y., & Ishikura, T. (1979) *J. Antibiot. 32*, 262–271.

Okamura, K., Sakamoto, M., & Ishikura, T. (1980) *J. Antibiot. 33*, 293–302.

Okonogi, K., Nozaki, Y., Imada, A., & Kuno, M. (1981) *J. Antibiot. 34*, 212–217.

Sakamoto, M., Shibamoto, N., Iguchi, H., Okamura, K., Hori, S., Fukagawa, Y., & Ishikura, T. (1980) *J. Antibiot. 33*, 1138–1145.

Samuni, A., & Citri, N. (1975) *Biochem. Biophys. Res. Commun. 62*, 7–11.

Stapley, E. O., Cassidy, P. J., Tunac, J., Monaghan, R. L., Jackson, M., Hernandez, S., Zimmerman, S. B., Mata, J. M., Currie, S. A., Daoust, D., & Hendlin, D. (1981) *J. Antibiot. 34*, 628–636.

Sutcliffe, J. G. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3737–3741.

Tally, F. P., Jacobus, N. V., & Gorbach, S. L. (1978) *Antimicrob. Agents Chemother. 14*, 436–438.

Tanaka, K., Shoji, J., Terui, Y., Tsuji, N., Kondo, E., Mayama, M., Kawamura, Y., Hattori, T., Matsumoto, K., & Yoshida, T. (1981) *J. Antibiot. 34*, 909–911.

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Reactions of Methyl-Substituted Hex-5-enyl and Pent-4-enyl Radicals

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Abstract

Relative and absolute kinetic data have been determined for ring closure of methyl-substituted hex-5-enyl radicals: 2-methyl- (10a), 3-methyl- (4a), 4-methyl- (5a), 2,2-dimethyl- (10c), 3,3-dimethyl- (4c) and 4,4-dimethyl-hex-5-enyl (5c) radicals, generated by interaction of tributylstannane with the corresponding bromides (1a)–(3a) and (1c)–(3c). Each radical undergoes regiospecific or highly regioselective 1,5-cyclization more rapidly than does the unsubstituted radical (4d). The rate enhancements, which arise mainly from lowering of the activation energy, can be rationalized in terms of the *gem*-dimethyl effect. 1,5-Ring closures of monosubstituted species are stereoselective: 2-methyl- and 4-methyl-hex-5-enyl radicals (10a) and (5a) give mainly *trans* products, whereas 3-methylhex-4-enyl radical gives mainly the *cis*. This behaviour reflects the effect of the substituent on the stabilities of cyclic transition complexes in chair-like conformations. Ring closure of 2,2-dimethylpent-4-enyl radical or of 3,3-dimethylpent-4-enyl radical (19) could not be detected.

Introduction

Of the available free-radical 'clock' reactions,[1] the most intensively studied and the most extensively used is the ring closure of hex-5-enyl radical. Values of its rate constant (k $2{\cdot}35\times10^5$ s^{-1} at 25°) and Arrhenius parameters have been accurately determined[2,3] and can now be used as reliable standards for the kinetic investigation of reactions having comparable rates. Furthermore, since neither the corresponding cation nor anion undergoes fast ring closure in the '*exo*-mode', the hex-5-enyl radical is an excellent probe for elucidating reaction mechanisms.[1,4]

The clock reaction mainly used for kinetic and mechanistic scrutiny of processes too fast to be studied conveniently with the hex-5-enyl system is the ring opening of cyclopropylmethyl radical.[5] It suffers, however, from the disadvantage that both the corresponding anion and cation also undergo fast ring opening. Consequently, the use of the cyclopropylmethyl system as a mechanistic probe may lead to ambiguous conclusions.

[1] Griller, D., and Ingold, K. U., *Acc. Chem. Res.*, 1980, **13**, 317.
[2] Chatgilialoglu, C., Ingold, K. U., and Scaiano, J. C., *J. Am. Chem. Soc.*, 1981, **103**, 7739.
[3] Beckwith, A. L. J., and Lawrence, T., unpublished data.
[4] Beckwith, A. L. J., *Tetrahedron*, 1981, **37**, 3073.
[5] Maillard, B., Forrest, D., and Ingold, K. U., *J. Am. Chem. Soc.*, 1976, **98**, 7024.

0004-9425/83/030545$02.00

There is, therefore, a clear need for free-radical clock reactions which are as reliable as ring closure of hex-5-enyl for mechanistic studies but which proceed at faster rates. Alkyl-substituted hexenyl radicals should fulfil these criteria since they are expected to undergo '*exo*-ring closure' at rates which are enhanced by the '*gem*-dimethyl effect'. Indeed, we have previously shown that cyclization of the 2,2-dimethylhex-5-enyl radical (10c) affords solely 3,3-dimethylcyclopentylmethyl radical (14c) and proceeds some 10 times more rapidly than that of the parent at 80°.[6] 2,2-Dimethylhex-5-enyl radical has already been used as a mechanistic probe but not as an accurate kinetic standard.[7,8]

Hex-5-enyl radicals bearing one methyl substituent are expected to undergo ring closure at rates intermediate in value between those for the parent radical and for dimethyl-substituted systems. The methylcyclopentylmethyl radicals so formed exist as geometrical isomers of which the relative amounts formed may afford information concerning the nature of the cyclization transition complex.

Unlike its higher and lower homologues the unsubstituted pent-4-enyl radical does not undergo ring closure at a measurable rate.[4] However, it is possible that ring closure of the 3,3-dimethylpent-4-enyl radical (19) will be sufficiently enhanced by the *gem*-dimethyl effect to be detectable under the usual experimental conditions. Since the expected primary product of such a process, the 2,2-dimethylcyclobutylmethyl radical (20), itself undergoes facile ring opening to afford 1,1-dimethylpent-4-enyl radical (21),[9] the isolation of 5-methylhex-1-ene would indicate that the radical (19) has undergone ring closure.

The aims of the present work, therefore, were: (i) to determine the absolute rate constants for ring closure of a series of methyl-substituted hex-5-enyl radicals and so create an 'horlogerie'[1] of reactions suitable for mechanistic investigation of relatively fast competing processes; (ii) to examine the stereochemical outcome of cyclization of monomethyl-substituted hex-5-enyl radicals; (iii) to determine whether dimethyl-substituted pent-4-enyl radicals undergo ring closure and/or rearrangement.

Results and Discussion

The method employed for the determination of the relative rate constants for ring closure of hex-5-enyl radical and related species has been described in detail elsewhere.[6] In the present study each of the bromides (1a)–(3a) and (1c)–(3c) was heated with tributylstannane and azobisisobutyronitrile as initiator in deaerated benzene, and the mixture was then analysed by gas chromatography. The mechanistic steps are depicted in Scheme 1. Full details of the results are given in the Experimental section; relative and total yields of products from some typical experiments are recorded in Table 1. Relative rate constants $\Sigma k_c/k_H$, where Σk_c is the sum of rate constants for various modes of ring closure and k_H is the rate constant for transfer of a hydrogen atom from tributylstannane to a primary alkyl radical,[2] were obtained in the usual way by fitting the experimental data to the appropriate integrated rate equation.[10] Values of $k_{1,5}/k_H$ for 1,5-ring closure were then calculated by simple proportion. They are given in Table 2 together with values of relative rate constants for formation

[6] Beckwith, A. L. J., and Lawrence, T., *J. Chem. Soc., Perkin Trans. 2*, 1979, 1535.
[7] Ashby, E. C., DePriest, R. N., and Goel, A. B., *Tetrahedron Lett.*, 1981, **22**, 1763.
[8] House, H. O., and Weeks, P. D., *J. Am. Chem. Soc.*, 1975, **97**, 2778.
[9] Beckwith, A. L. J., and Moad, G., *J. Chem. Soc., Perkin Trans. 2*, 1980, 1083.
[10] Beckwith, A. L. J., and Moad, G., *J. Chem. Soc., Chem. Commun.*, 1974, 472.

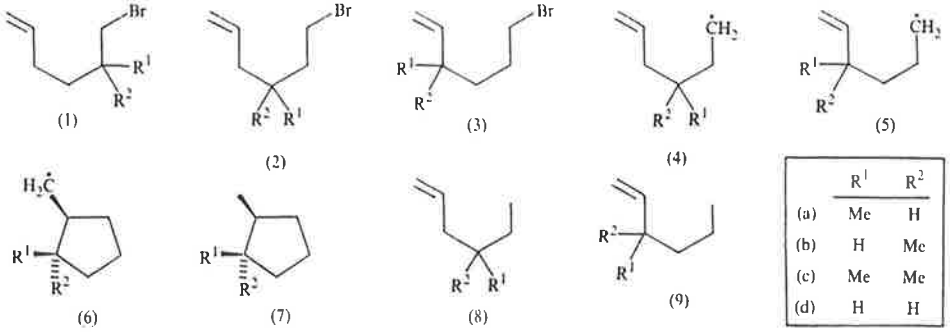
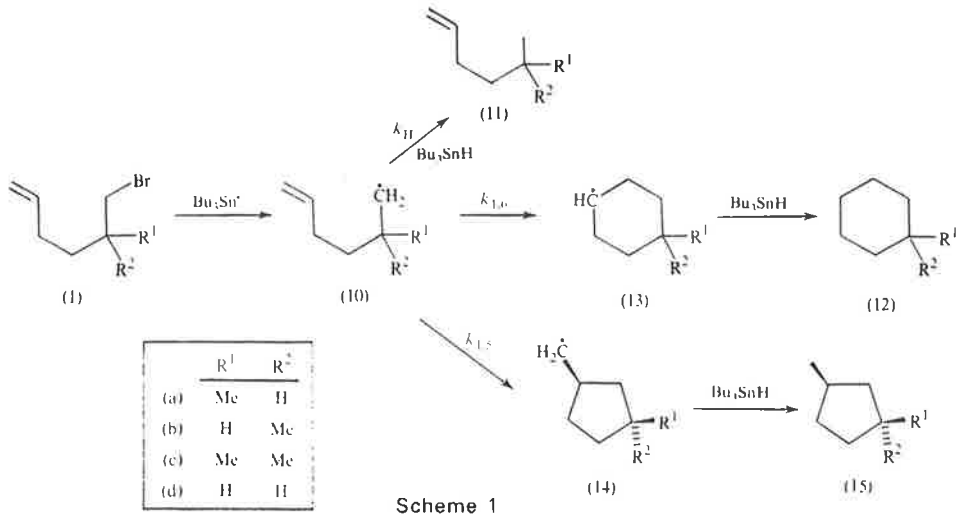


Table 1. Representative products and yields from reactions of substituted hex-5-enyl bromides with tributylstannane in benzene

Reactant	$[Bu_3SnH]_0$ (M)	Temp. (°C)	Products, and relative yields (%) Acyclic	Cyclopentanes	Cyclohexanes	Total yield (%)
(1a)	0·206	45	(11a), 20·0	(15a), 28·2; (15b), 50·4	(12a), 1·4	88
(1a)	0·461	80	(11a), 26·3	(15a), 26·8; (15b), 45·6	(12a), 1·3	95
(2a)	0·511	45	(8a), 30·8	(15a), 50·2; (15b), 18·1	(12a), 0·9	92
(2a)	0·825	80	(8a), 32·5	(15a), 48·1; (15b), 18·5	(12a), 0·9	96
(3a)	0·175	60	(9a), 20·4	(7a), 16·0; (7b), 63·6	(12a), *c.* 2	90
(3a)	0·556	100	(9a), 31·5	(7a), 15·9; (7b), 52·6	(12a), *c.* 2	96
(1c)	1·395	40	(11c), 26·7	(15c), 73·3		85
(1c)	1·00	80	(11c), 16·9	(15c), 83·1		90
(2c)	0·835	40	(8c), 14·2	(15c), 85·8		88
(2c)	1·464	80	(8c), 17·5	(15c), 82·5		93
(3c)	0·947	40	(9c), 22·3	(7c), 76·7	(12c), *c.* 1	
(3c)	1·295	80	(9c), 22·1	(7c), 76·9	(12c), *c.* 1	



Scheme 1

of the *cis* and *trans* isomers of methyl-substituted cyclopentylmethyl radicals where this is appropriate. The results were highly reproducible. The standard deviation was less than $\pm 1\%$ for methylhex-5-enyl radicals, and in the range from ± 1 to $\pm 4\%$ for dimethylhex-5-enyl radicals.

Table 2. Relative rate constants for 1,5-ring closure of substituted hex-5-enyl radicals in benzene
Stereoselectivity is defined as the ratio of rate constants for formation of major and minor stereoisomers

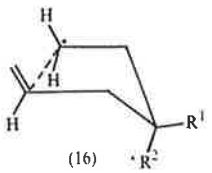
Temp. (°C)	$k_{1,5}/k_H$ (mol l^{-1})	k_{trans}/k_H (mol l^{-1})	k_{cis}/k_H (mol l^{-1})	Stereo-selectivity	Temp. (°C)	$k_{1,5}/k_H$ (mol l^{-1})
	(10a) → (14a)+(14b)				(10c) → (14c)	
45	$0{\cdot}37_9$	$0{\cdot}24_3$	$0{\cdot}13_6$	$1{\cdot}7_9$	30	$1{\cdot}5_2$
60	$0{\cdot}45_6$	$0{\cdot}29_2$	$0{\cdot}16_5$	$1{\cdot}7_7$	40	$1{\cdot}7_4$
80	$0{\cdot}57_4$	$0{\cdot}36_1$	$0{\cdot}21_3$	$1{\cdot}6_9$	55	$1{\cdot}9_8$
100	$0{\cdot}69_2$	$0{\cdot}42_6$	$0{\cdot}26_6$	$1{\cdot}6_0$	80	$2{\cdot}2_9$
	(4a) → (14a)+(14b)				(4c) → (14c)	
45	$0{\cdot}50_1$	$0{\cdot}13_4$	$0{\cdot}36_7$	$2{\cdot}7_4$	40	$2{\cdot}3_8$
60	$0{\cdot}60_2$	$0{\cdot}16_2$	$0{\cdot}44_0$	$2{\cdot}7_1$	60	$2{\cdot}7_6$
80	$0{\cdot}74_1$	$0{\cdot}20_6$	$0{\cdot}53_5$	$2{\cdot}5_9$	80	$3{\cdot}1_2$
100	$0{\cdot}89_2$	$0{\cdot}25_6$	$0{\cdot}63_6$	$2{\cdot}4_8$	100	$3{\cdot}4_7$
	(5a) → (6a)+(6b)				(5c) → (6c)	
60	$0{\cdot}30_9$	$0{\cdot}24_7$	0·063	$3{\cdot}8_6$	40	$1{\cdot}4_9$
80	$0{\cdot}40_7$	$0{\cdot}31_9$	0·087	$3{\cdot}6_7$	60	$1{\cdot}8_6$
100	$0{\cdot}51_7$	$0{\cdot}40_0$	$0{\cdot}11_8$	$3{\cdot}3_9$	80	$2{\cdot}0_8$

A significant feature revealed by the data in Table 1 is that all of these reactions are highly regioselective. In each case the cyclic product formed is composed either exclusively or predominantly (>97%) of the appropriately substituted cyclopentane. It is clear, therefore, that the radicals (4a,c), (5a,c) and (10a,c) conform to the guideline[11] that hex-5-enyl radicals and related systems undergo ring closure preferentially in the *exo*-mode. The stereoelectronic basis of this behaviour has been discussed elsewhere.[4]

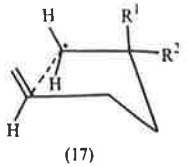
Essential to our conception of stereoelectronic control of radical ring closures is the hypothesis[4,12,13] that the structure of the transition complex for homolytic addition incorporates the three participating atoms at the vertices of an obtuse triangle orthogonal to the nodal plane of the π system. Theoretical treatments[14,15] support this model and indicate that the transition complex is reactant-like. In particular, the C–C bond being formed is very long (*c.* 2·3 Å). Since this is not much less than the normal distance (*c.* 2·5 Å) between C1 and C3 in cyclohexane, it seems likely that the intimate transition complex for 1,5-intramolecular addition in hex-5-enyl radical will comprise a structure (16d) somewhat similar in dimensions to cyclohexane and existing preferentially in a chair-like conformation. Consequently, for 1,5-ring

[11] Beckwith, A. L. J., Easton, C. J., and Serelis, A. K., *J. Chem. Soc., Chem. Commun.*, 1980, 482.
[12] Struble, D. L., Beckwith, A. L. J., and Gream, G. E., *Tetrahedron Lett.*, 1968, 3701; Beckwith, A. L. J., Gream, G. E., and Struble, D. L., *Aust. J. Chem.*, 1972, **25**, 1081.
[13] Beckwith, A. L. J., in 'Essays on Free-Radical Chemistry' Chem. Soc. Spec. Publ. No. 24, 1970, 239.
[14] Dewar, M. J. S., and Olivella, S., *J. Am. Chem. Soc.*, 1978, **100**, 5290.
[15] Fujimoto, H., Yamabe, S., Minato, T., and Fukui, K., *J. Am. Chem. Soc.*, 1972, **94**, 9205.

closure of a monosubstituted hex-5-enyl radical there will be two such transition complexes in which the substituent occupies axial and equatorial positions respectively. That transition complex, e.g. (16a), which is equatorially substituted should be of lower free energy than the axially substituted complex, e.g. (16b), and will therefore lie on the pathway to the more rapidly formed product.



(16)



(17)

	R^1	R^2
(a)	Me	H
(b)	H	Me
(c)	Me	Me
(d)	H	H

Our results are consistent with this hypothesis. For example, 3-methylhex-5-enyl radical (4a) can undergo ring closure to *cis*-3-methylcyclopentylmethyl radical (14a) through an equatorially substituted chair-like transition complex (16a), or to the *trans* radical (14b) through an axially substituted complex (16b). As predicted the former pathway is preferred and treatment of 6-bromo-4-methylhex-1-ene (2a) with tributylstannane affords preferentially the *cis* product (15a). Ring closure of 2-methylhex-5-enyl radical (10a), generated from the bromide (1a), affords the same products (15a,b) except that *trans* (15b) is preferred to *cis* (15a), an observation which precludes any explanation of stereoselectivity based on the relative stabilities of the intermediate cyclic radicals (14a,b). Preferential formation of the *trans* product from 2-methylhex-5-enyl radical (10a) occurs because the appropriate transition complex (17b) is equatorially substituted whereas that (17a) leading to *cis* product is axially substituted. The observed stereoselectivity (*trans* > *cis*) of ring closure of 4-methylhex-5-enyl radical (5a) may be similarly rationalized.

If the transition complex for 1,5-ring closure of hex-5-enyl radical were closely cyclohexane-like we should expect the three monomethyl-substituted radicals to exhibit similar stereoselectivity, and the ratios of stereoisomers to be related to the conformational preference of methylcyclohexane. However, this is not the case. Because of the presence of the ethylenic moiety and the trigonal radical centre, the energies of the monosubstituted transition complexes are influenced by different non-bonded interactions. Thus, inspection of a model of the axially substituted transition complex (17a) derived from 2-methylhex-5-enyl radical (10a) reveals *one* unfavourable interaction between the substituent and the axial proton at C 4. In the axially substituted transition complex (16b) from 3-methylhex-5-enyl radical (4a) there are *two* similar but slightly less severe interactions between the substituent and protons at C 1 and C 5 respectively. However, the relative stability of the axially substituted transition complex derived from 4-methylhex-5-enyl radical (5a) is adversely affected both by an interaction between the substituent and the axial proton at C 2, and by a much more severe interaction between the substituent and the *syn*-proton at C 6. It is not surprising, therefore, that the stereoselectivity of ring closure is most pronounced for the 4-substituted radical (5a) and least for its 2-substituted isomer (10a).

Further insight into the factors affecting the rates and stereoselectivity of ring closure of monosubstituted radicals can be deduced from the data obtained

by the usual Arrhenius treatment and presented in Table 3. First, in accord with the above analysis of transition complex stability, the stereoselectivity of ring closure for each radical is due primarily to the difference in activation energy between the reactions leading to the two stereoisomers. The entropy of activation slightly favours formation of the *cis* product in each reaction. Consequently, the stereoselectivity of ring closure increases with decrease in temperature (see Table 2).

Table 3. Relative kinetic data for formation of *cis* and *trans* cyclic radicals from monosubstituted hex-5-enyl systems

$\Delta \log A = \log A$(1,5-ring closure) $- \log A$(H transfer) $\equiv \log(A_{1,5}/A_H)$
$\Delta E = E$(1,5-ring closure) $- E$(H transfer)

Reaction	$\Delta \log A$ ($A_{1,5}/A_H$ in mol l^{-1})	ΔE (kJ mol^{-1})	$k_{1,5}/k_H$ [A] (mol l^{-1}) At 25°C	At 60°C
(10a) → (14a)	1·11±0·03	12·05±0·13	0·100±0·001	0·166±0·002
(10a) → (14b)	1·05±0·02	10·17±0·13	0·188±0·002	0·290±0·003
(4a) → (14a)	1·18±0·02	9·83±0·13	0·287±0·002	0·436±0·004
(4a) → (14b)	1·03±0·01	11·63±0·08	0·0996±0·0006	0·163±0·001
(5a) → (6a)	1·42±0·04	16·74±0·25	0·0304±0·0004	0·0619±0·0009
(5a) → (6b)	1·34±0·02	12·43±0·08	0·146±0·002	0·247±0·002

[A] Calculated from the Arrhenius parameters given in this table.

If we make the very reasonable assumption that k_H has the same value for all primary radicals,[2] the rates of ring closure of the methyl-substituted hex-5-enyl radicals can be compared with each other (Table 3) and with the rate for the parent hex-5-enyl radical [see reaction (10d) → (14d) of Table 4]. Perhaps the most significant feature of such a comparison is that *cis* ring closure of the 4-methylhex-5-enyl radical (5a) is much slower than any other cyclization reaction of its isomers or of the hex-5-enyl radical. This is due to the exceptionally large activation energy which reflects, presumably, the unfavourable steric interactions in the transition complex discussed above. The preexponential term, for reasons which are not apparent, is unusually favourable. The rates of ring closure of the minor products from the other methyl-substituted hex-5-enyl radicals are about the same as that for the parent hex-5-enyl system, while the rates for formation of the major products are faster and have smaller activation energies. The order of rate constants is k_{cis} for 3-methylhex-5-enyl (4a) > k_{trans} for 2-methylhex-5-enyl (10a) > k_{trans} for 4-methylhex-5-enyl (5a) > k_c for hex-5-enyl radical (10d). This is in accord with expectation based on consideration of the *gem*-dimethyl effect.

In order to avoid ambiguities arising from the effect of statistical factors on the magnitude of $\log A$, the *gem*-dimethyl effect for monomethyl-substituted hex-5-enyl radicals is best considered in the light of the apparent Arrhenius parameters and total rate constants for *cis* and *trans* 1,5-ring closure. These data are presented in Table 4 together with kinetic data for 1,5-ring closure of the disubstituted analogues and of hex-5-enyl radical. They reveal that all of the methyl-substituted radicals undergo ring closure more rapidly than does the parent, particularly at lower temperatures, while the rates of ring closure for dimethyl-substituted radicals are further enhanced. Within both groups of radicals the rate enhancement is most pronounced for 3-substituted and least for 4-substituted systems. The trends conform to the proposal

first made by Allinger and Zalkow[16] that the enhancement of the rate of ring closure by substituents arises from an increase in the ground-state free energy of the acyclic reactant associated with the increased number of *gauche* interactions involving the substituents. The number of such interactions is greatest for the 3,3-dimethylhex-5-enyl radical (4c) in which the two methyl substituents interact with substituents at C2 and C4.

Table 4. Kinetic data for 1,5-ring closure of substituted hexenyl radicals in benzene

k_{rel}, rate constant for 1,5-ring closure compared with that for hex-5-enyl radical. $\Delta \log A$ and ΔE are defined in the subtitle to Table 3

Reaction	Temp. (°C)	$k_{1,5}/k_H$ (mol l^{-1})	k_{rel}	$\Delta \log A$ ($A_{1,5}/A_H$ in mol l^{-1})	ΔE (kJ mol^{-1})
(10d) → (14d)	25	0·097	1·0	1·28	13·10
	60	0·169	1·0		
(10a) → (14a)+(14b)	25	0·288	2·97	1·36±0·01	10·84±0·08
	60	0·456	2·70		
(4a) → (14a)+(14b)	25	0·387	3·99	1·40±0·01	10·34±0·08
	60	0·599	3·54		
(5a) → (6a)+(6b)	25	0·176	1·81	1·58±0·02	13·35±0·17
	60	0·309	1·82		
(10c) → (14c)	25	1·47	15·1	1·43±0·08	7·24±0·54
	60	2·00	11·83		
(4c) → (14c)	25	2·12	21·86	1·39±0·03	6·11±0·25
	60	2·75	16·27		
(5c) → (6c)	25	1·33	13·71	1·45±0·01	7·57±0·71
	60	1·83	10·82		

In 2,2-dimethylhex-5-enyl radical (10c) *gauche* interactions involving the methyl groups occur only with the substituent at C3. The 4,4-dimethylhex-5-enyl radical (5c) is expected to be very similar in ground-state energy since the only major *gauche* interactions are also with the substituent at C3. Interactions between the methyl substituents and the vinyl group are small. The difference in rate constants for 1,5-ring closure of 2,2-dimethylhex-5-enyl (10c) and 4,4-dimethylhex-5-enyl radical (5c) probably reflects unfavourable interactions in the transition complex for the latter rather than in the ground state of the former. The variation in rates of ring closure of the monomethyl-substituted species can be similarly rationalized.

The earlier theoretical treatment[16] suggested that the *gem*-dimethyl effect should influence the magnitudes of both Arrhenius parameters for ring closure. However, the data presented in Table 4 show that the differences in rates of ring closure of substituted hexenyl radicals arise almost solely from differences in the magnitude of the activation energies. There is very little variation in $\Delta \log A$.

We turn now to the possible use of methyl-substituted hex-5-enyl systems as free-radical clocks. Absolute rate constants and Arrhenius parameters can be obtained by combining our relative data with results recently reported by Ingold's group[2] for

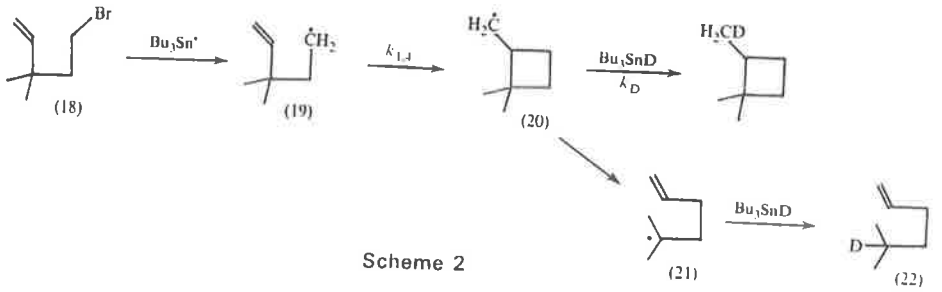
[16] Allinger, N. L., and Zalkow, V., *J. Org. Chem.*, 1960, **25**, 701.

the reaction of primary alkyl radicals with tributylstannane. The values so obtained are presented in Table 5. The only assumption involved in this treatment is that k_H has the same value for all primary radicals. Since there is considerable evidence available[2] to support the validity of this assumption we believe the data given in Table 5 to be reliable. They show that ring closure of dimethylhex-5-enyl systems and particularly of 3,3-dimethylhex-5-enyl radical (4c) occurs sufficiently rapidly to serve as a useful kinetic standard for fast radical–molecule processes.

Table 5. Arrhenius parameters and absolute rate constants for 1,5-ring closure of substituted hex-5-enyl radicals in benzene

Reaction	log A (A in s^{-1})	E (kJ mol^{-1})	$k_{1,5}$ (s^{-1}) at 25°
(10d) → (14d)	10·35	28·3	$2·4 \times 10^5$
(10a) → (14a)	10·2	27·2	$2·4 \times 10^5$
(10a) → (14b)	10·1	25·5	$4·5 \times 10^5$
(4a) → (14a)	10·2	25·1	$7·0 \times 10^5$
(4a) → (14b)	10·1	26·8	$2·4 \times 10^5$
(5a) → (6a)	10·5	32·2	$7·5 \times 10^4$
(5a) → (6b)	10·4	27·6	$3·6 \times 10^5$
(10c) → (14c)	10·5	22·6	$3·6 \times 10^6$
(4c) → (14c)	10·5	21·3	$5·2 \times 10^6$
(5c) → (6c)	10·5	23·0	$3·2 \times 10^6$

Our experimental data for the yields of cyclohexane derivatives are not sufficiently accurate to allow full kinetic analysis of the rates of 1,6-ring closure of substituted hex-5-enyl radicals. *Approximate* relative and absolute values of rate constants are: (i) for 2-methylhex-5-enyl radical (10a), $k_{1,6}/k_H$ 0·006 mol l^{-1} and $k_{1,6}$ $4·6 \times 10^3$ s^{-1} at 60°; (ii) for 3-methylhex-5-enyl radical (4a), $k_{1,6}/k_H$ 0·009 mol l^{-1} and $k_{1,6}$ 7×10^3 s^{-1} at 60°; (iii) for 4-methylhex-5-enyl radical (5a), $k_{1,6}/k_H$ 0·007 mol l^{-1} and $k_{1,6}$ $5·4 \times 10^3$ s^{-1}; (iv) for 4,4-dimethylhex-5-enyl radical (5c), $k_{1,6}/k_H$ 0·024 mol l^{-1} and $k_{1,6}$ 2×10^4 s^{-1}.



Scheme 2

Finally, we turn to the question of whether the presence of *gem*-dimethyl substituents enhances the rate of ring closure of pent-4-enyl radical sufficiently to allow it to be detected under our experimental conditions. When 5-bromo-4,4-dimethylpent-1-ene or 5-bromo-3,3-dimethylpent-1-ene (18) was heated with tributyltin deuteride at low concentration (0·0025 M) in benzene at temperatures in the range 60–160° no four- or five-membered acyclic products were formed. Nor in reactions of (18) could we detect any of the rearrangement product (22) expected to arise by ring opening

of an intermediate cyclobutylmethyl radical (20) as shown in Scheme 2. It is possible to estimate therefore that $k_{1,4}/k_D < 4 \times 10^{-4}$ mol l^{-1} at either temperature, and consequently that $k_c < 1{\cdot}3 \times 10^2$ s^{-1} at 60°. It we assume that the *gem*-dimethyl effect in this system[17] is at least as large as it is for the hex-5-enyl radical, we conclude that the pent-4-enyl radical has $k_c < 10$ s^{-1} at 60°. This is in accord with earlier estimates.[9]

Experimental

General experimental details have been given previously.[18] G.l.c. was carried out on a Perkin–Elmer 990 instrument fitted with a flame ionization detector. The following columns were used: (A) 70 m by 0·5 mm, FFAP glass SCOT column; (B) 70 m by 0·5 mm, squalane glass SCOT column; (C) 4·6 m by 2·1 mm, 5% Apiezon M on Varaport 30 stainless steel column; (D) 100 m by 0·5 mm, Apiezon L stainless steel capillary column; (E) 6·7 m by 7·0 mm, 20% Carbowax 20M-TPA on Chromosorb A glass column; (F) 3·0 m by 7·0 mm, 10% SE30 on Varaport 31 glass column; (G) 2·0 m by 2·5 mm, 3% SE30 on Chromosorb W glass column.

Tributylstannane, b.p. 80°/3 mm, d_{20} 1·0996 g ml^{-1}, and tributyltin deuteride, b.p. 90°/7 mm, d_{20} 1·1039 g ml^{-1}, were prepared by reduction of tributyltin chloride with lithium aluminium hydride and lithium aluminium deuteride respectively.[19] 6-Bromo-5,5-dimethylhex-1-ene (1c), 5-methylhex-1-ene (11a), 5,5-dimethylhex-1-ene (11c), 1,1-dimethylcyclohexane (12c), 1,1,2-trimethylcyclopentane (7c), *cis* and *trans*-1,2-dimethylcyclopentane (7a,b), 3,3-dimethylpent-1-ene, 4,4-dimethylpent-1-ene and 1,1,3-trimethylcyclopentane (15c) were available from earlier work.[6,9,20]

Ethyl 2-Methylhex-5-enoate

Alkylation of methylmalonic ester with 4-bromobut-1-ene in ethanolic sodium ethoxide by the usual procedure afforded *diethyl 2-(but-3-enyl)-2-methylpropanedioate* (77%), b.p. 90°/1·5 mm (Found: C, 62·7; H, 8·5. $C_{12}H_{20}O_4$ requires C, 63·1; H, 8·8%). ^{1}H n.m.r.: δ 1·2, t, J 7 Hz, 6H; 1·4, s, 3H; 2·0, m, 4H; 4·2, q, J 7 Hz, 4H; 4·8–5·3, m, 2H; 5·4–6·2, m, 1H.

A sample (11·4 g) of this ester was added to a solution of lithium chloride (4·2 g) and water (0·9 ml) in dimethyl sulfoxide (100 ml), and the mixture was then heated under reflux for 14 h, cooled, and poured into brine. Extraction of the mixture with pentane, and fractional distillation of the crude product, gave the required *ester* (4·5 g, 58%), b.p. 103°/90 mm (Found: C, 69·6; H, 10·1. $C_9H_{16}O_2$ requires C, 69·2; H, 10·3%). ^{1}H n.m.r.: δ 1·2, t, J 8 Hz, 3H; 1·25, d, J 7 Hz, 3H; 1·45–2·5, m, 5H; 4·2, q, J 8 Hz, 2H; 4·8–5·2, m, 2H; 5·4–6·1, m, 1H.

6-Bromo-5-methylhex-1-ene (1a)

The foregoing ester was reduced with lithium aluminium hydride in ether in the usual way to afford 2-methylhex-5-en-1-ol[21] (90%), a sample (2·0 g) of which was mixed with triphenylphosphine (4·7 g) in dichloromethane (10 ml) under nitrogen. The solution was then cooled and stirred while carbon tetrabromide (6·6 g) was added in small portions. After being stirred for 12 h the mixture was distilled under reduced pressure. Fractional distillation of the distillate gave the required *bromide* (1a) (2·7 g, 86%), b.p. 62°/25 mm (Found: C, 47·4; H, 7·2; Br, 44·8. $C_7H_{13}Br$ requires C, 47·5; H, 7·4; Br, 45·1%). ^{1}H n.m.r.: δ 1·0, d, J 7·5 Hz, 3H; 1·3–2·3, m, 5H; 3·4, d, J 6 Hz, 2H; 4·8–5·3, m, 2H; 5·5–6·2, m, 1H. The sample used for kinetic work was purified further by preparative g.l.c. [column (E)].

Ethyl 3-Methylhex-5-enoate

Sodium hydride (2·4 g) was added slowly to diethyl malonate (16·0 g) in dimethylformamide (100 ml) under nitrogen, and the mixture was then stirred whilst 4-bromopent-1-ene (14·9 g; pre-

[17] Hill, E. A., Link, D. C., and Donndelinger, P., *J. Org. Chem.*, 1981, **46**, 1177.
[18] Beckwith, A. L. J., Rodgers, J. R., and Wagner, R. D., *Aust. J. Chem.*, 1982, **35**, 989.
[19] Kuivila, H. G., and Beumel, O. F., *J. Am. Chem. Soc.*, 1961, **83**, 1246.
[20] Beckwith, A. L. J., Blair, I. A., and Phillipou, G., *Tetrahedron Lett.*, 1974, 2251.
[21] Closson, W. D., and Gray, D., *J. Org. Chem.*, 1970, **35**, 3737.

pared by treatment of the corresponding alcohol with phosphorus tribromide) was added during 15 min. After being stirred for 3 h the resultant mixture was diluted with water (200 ml) and extracted with ether to afford *diethyl 2-(1-methylbut-3-enyl)propanedioate* (16·6 g, 73%), b.p. 76°/4 mm (Found: C, 63·2; H, 8·8. $C_{12}H_{20}O_4$ requires C, 63·1; H, 8·8%). ^{1}H n.m.r.: δ 1·0, t, J 7 Hz, 6H; 1·3, d, J 8 Hz, 3H; 1·9–2·5, m, 3H; 3·3, d, J 8 Hz, 1H; 4·2, q, J 7 Hz, 4H; 4·8–5·3, m, 2H; 5·4–6·2, m, 1H. Treatment of this diester with lithium chloride in dimethyl sulfoxide, as described above, gave the required *ester* (84%), b.p. 100°/78 mm (Found: C, 69·0; H, 10·0. $C_9H_{16}O_2$ requires C, 69·2; H, 10·3%). ^{1}H n.m.r.: δ 1·0, m, 3H; 1·2, t, J 7 Hz, 3H; 2·0–2·4, m, 5H; 4·1, q, J 7 Hz, 2H; 4·8–5·3, m, 2H; 5·4–6·2, m, 1H.

6-Bromo-4-methylhex-1-ene (*2a*)

The foregoing ester was reduced with lithium aluminium hydride to afford 3-methylhex-5-en-1-ol[21] (88%), b.p. 77°/18 mm. ^{1}H n.m.r.: δ 1·0, d, J 6 Hz, 3H; 1·2–2·2, m, 5H; 1·6, s, 1H (OH, D_2O exchange); 3·7, t, J 6 Hz, 2H; 4·8–5·3, m, 2H; 5·4–6·2, m, 1H.

Treatment of this alcohol with carbon tetrabromide and triphenylphosphine, as described above, gave *6-bromo-4-methylhex-1-ene* (89%), isolated by preparative g.l.c. on column (E) (Found: C, 47·4; H, 7·3; Br, 45·1. $C_7H_{13}Br$ requires C, 47·5; H, 7·4; Br, 45·1%). ^{1}H n.m.r.: δ 0·9, d, J 7 Hz, 3H; 1·3–2·3, m, 5H; 3·5, t, J 8 Hz, 2H; 4·8–5·3, m, 2H; 5·4–6·2, m, 1H.

6-Bromo-3-methylhex-1-ene (*3a*)

Oxetan (11·6 g) was added to the Grignard reagent prepared from 1-bromobut-2-ene (13·5 g) and magnesium (7·2 g) in ether (80 ml), and the mixture was then stirred at 0° for 4 h, and at ambient temperature for 19 h. The usual workup gave 4-methylhex-5-en-1-ol[21] (7·4 g, 65%), b.p. 75°/15 mm, which was further purified by fractional distillation through a spinning-band column. ^{1}H n.m.r.: δ 1·0, d, J 7 Hz, 3H; 1·2–1·8, m, 4H; 1·9–2·4, m, 1H; 2·3, s, 1H (OH, D_2O exchange); 3·6, t, J 7 Hz, 2H; 4·7–5·2, m, 2H; 5·4–6·1, m, 1H.

Treatment of this alcohol with carbon tetrabromide and triphenylphosphine, as described above, gave 6-bromo-3-methylhex-1-ene[22] (83%), b.p. 62–63°/15 mm, which was purified further by preparative g.l.c. on column (E) (Found: C, 47·8; H, 7·1; Br, 45·2. Calc. for $C_7H_{13}Br$: C, 47·5; H, 7·4; Br, 45·1%). ^{1}H n.m.r.: δ 1·0, d, J 7 Hz, 3H; 1·2–2·3, m, 5H; 3·4, t, J 7 Hz, 2H; 4·7–5·1, m, 2H; 5·4–6·1, m, 1H.

cis- *and* trans-*1,3-Dimethylcyclopentane* (*15a,b*)

A sample (2·52 g) of the mixture of diastereoisomers of 1,3-dimethylcyclopentanol, prepared by reaction of 3-methylcyclopentanone with methylmagnesium iodide,[23] was heated with anhydrous oxalic acid (3·6 g) at 130–160°. The product[23] (1·93 g, 91%), b.p. 91–93°, isolated by distillation directly from the reaction mixture, was hydrogenated over platinum oxide in acetic acid to give a mixture of the *cis* and *trans* isomers of 1,3-dimethylcyclopentane (*cis*/*trans* 7·6 : 1), b.p. 91° (lit.[24] 91°). ^{1}H n.m.r.: δ 1·0, d, J 6 Hz, 6H; 1·2–2·2, m, 8H. ^{13}C n.m.r.[25] (*cis*): δ 21·26, CH_3; 34·13, C4; 35·22, C1; 44·82, C2. ^{13}C n.m.r.[25] (*trans*): 21·62, CH_3; 33·40, C1; 35·22, C4; 43·00, C2. The isomers could be separated by g.l.c. [column (A)].

4-Methylhex-1-ene (*8a*)

Coupling of s-butylmagnesium bromide with allyl bromide, as described above, gave the required olefin (8a) (74%), b.p. 86° (lit.[24] 86·7°). ^{1}H n.m.r.: δ 0·7–2·2, complex m, 11H; 4·8–5·2, m, 2H; 5·4–6·2, m, 1H.

3-Methylhex-1-ene (*9a*)

Treatment of 2-bromopentane with 1 mol. equiv. of vinyllithium in tetrahydrofuran gave the required olefin (9a) (43%), b.p. 84° (lit.[24] 83·9°), which was further purified by preparative g.l.c. [column (F)]. ^{1}H n.m.r.: δ 0·9, m, 6H; 1·1–1·2, m, 5H; 4·7–5·1, m, 2H; 5·4–6·1, m, 1H.

[22] Mori, K., Masuda, S., and Suguro, T., *Tetrahedron*, 1981, **37**, 1329.
[23] Doering, W. von E., and Sachdev, K., *J. Am. Chem. Soc.*, 1975, **97**, 5512.
[24] Rossini, F. D., 'Selected Values of Physical and Thermodynamic Properties of Hydrocarbons and Related Compounds' (Carnegie Press: Pittsburgh 1953).
[25] Christl, M., Reich, H. J., and Roberts, J. D., *J. Am. Chem. Soc.*, 1971, **93**, 3463.

Ethyl 3,3-Dimethylhex-5-enoate

Cuprous chloride (8·0 g) was added to allylmagnesium bromide (prepared from 9·7 g of allyl bromide in 150 ml of ether) at −45°, and the mixture was stirred for 10 min. Diethyl isopropylidenemalonate[26] (16·0 g) in ether (50 ml) was then added during 30 min, and the mixture was stirred, first for 1 h at −35°, then at ambient temperature for 1 h. Workup with saturated ammonium chloride solution and distillation of the ethereal solution gave *diethyl 2-(1,1-dimethylbut-3-enyl)-propanedioate* (14·8 g, 76%), b.p. 90°/0·5 mm (Found: C, 64·7; H, 8·9. $C_{13}H_{22}O_4$ requires C, 64·4; H, 9·1%). ^{1}H n.m.r.: δ 1·2, s, 6H; 1·3, t, *J* 8 Hz, 6H; 2·3, m, 2H; 3·3, s, 1H; 4·2, q, *J* 8 Hz, 4H; 4·8–5·2, m, 2H; 5·5–6·2, m, 1H.

Treatment of this malonate with lithium chloride, as described above, gave *ethyl 3,3-dimethylhex-5-enoate* (86%), b.p. 87°/20 mm (Found: C, 70·3; H, 10·8. $C_{10}H_{18}O_2$ requires C, 70·6; H, 10·7%). ^{1}H n.m.r.: δ 1·0, s, 6H; 1·2, t, *J* 8 Hz, 3H; 2·1, d, *J* 8 Hz, 2H; 2·3, s, 2H; 4·1, q, *J* 8 Hz, 2H; 4·8–5·2, m, 2H; 5·6–6·2, m, 1H.

6-Bromo-4,4-dimethylhex-1-ene (*2c*)

Reduction of the foregoing ester with lithium aluminium hydride gave *3,3-dimethylhex-5-en-1-ol* (88%), b.p. 81°/13 mm (Found: C, 74·9; H, 12·8. $C_8H_{16}O$ requires C, 74·9; H, 12·6%). ^{1}H n.m.r.: δ 0·9, s, 6H; 1·5, t, *J* 8 Hz, 2H; 2·0, d, *J* 8 Hz, 2H; 2·5, s, 1H (OH, D_2O exchange); 3·7, t, *J* 8 Hz, 2H; 4·7–5·2, m, 2H; 5·5–6·3, m, 1H.

Treatment of this alcohol with carbon tetrabromide/triphenylphosphine gave *6-bromo-4,4-dimethylhex-1-ene* (75%), b.p. 88°/35 mm, which was further purified by preparative g.l.c. on column (F) (Found: C, 50·1; H, 7·9; Br, 41·9. $C_8H_{15}Br$ requires C, 50·3; H, 7·9; Br, 41·8%). ^{1}H n.m.r.: δ 0·9, s, 6H; 1·7, t, *J* 8 Hz, 2H; 2·0, d, *J* 7 Hz, 2H; 3·5, t, *J* 8 Hz, 2H; 4·8–5·2, m, 2H; 5·3–5·5, m, 1H.

4,4-Dimethylhex-1-ene (*8c*)

3,3-Dimethylhex-5-en-1-ol was treated in the usual way with *p*-toluenesulfonyl chloride in pyridine, and the resultant *p*-toluenesulfonate (5·5 g) was stirred with lithium aluminium hydride (0·76 g) in ether (100 ml) at 5° for 20 h. Aqueous sodium hydroxide (50 ml, 10% w/v) was then added, and the organic layer was separated, washed, dried, and distilled to afford 4,4-dimethylhex-1-ene (1·2 g, 54%), b.p. 107° (lit.[24] 107·2°). ^{1}H n.m.r.: δ 0·9, s, 6H; 1·0–2·1, m, 7H; 4·8–5·2, m, 2H; 5·5–6·3, m, 1H.

6-Bromo-3,3-dimethylhex-1-ene (*3c*)

Treatment of oxetan with 3-methylbut-2-enylmagnesium chloride, as described above, afforded *4,4-dimethylhex-5-en-1-ol* (73%), b.p. 82–86°/18 mm (Found: C, 75·0; H, 12·7. $C_8H_{16}O$ requires C, 74·9; H, 12·6%). ^{1}H n.m.r.: δ 0·96, s, 6H; 1·2–1·9, m, 4H; 2·2, s, 1H (OH, D_2O exchange); 3·6, t, *J* 7 Hz, 2H; 4·7–5·1, m, 2H; 5·5–6·1, m, 1H. Treatment of this alcohol with carbon tetrabromide and triphenylphosphine in the usual way gave *6-bromo-3,3-dimethylhex-1-ene* (86%), b.p. 74–77°/19 mm (Found: C, 50·4; H, 7·9. $C_8H_{15}Br$ requires C, 50·3; H, 7·9%). ^{1}H n.m.r.: δ 1·0, s, 6H; 1·1–2·1, m, 4H; 3·4, t, *J* 7 Hz, 2H; 4·7–5·1, m, 2H; 5·5–6·1, m, 1H.

3,3-Dimethylhex-1-ene (*9c*)

Reduction of the *p*-toluenesulfonate of 4,4-dimethylhex-5-en-1-ol with lithium aluminium hydride, as described above, gave 3,3-dimethylhex-1-ene (39%), b.p. 98–101°, and spectral properties identical with those previously reported.[27]

5-Bromo-4,4-dimethylpent-1-ene

Alkylation of ethyl isobutyrate with allyl bromide in the usual way[6] gave ethyl 2,2-dimethylpent-4-enoate (73%), b.p. 61°/17 mm, which was reduced with lithium aluminium hydride to afford 2,2-dimethylpent-4-en-1-ol[28] (80%), b.p. 62°/15 mm. Treatment of this alcohol with triphenyl-

[26] Eliel, E. L., Hutchins, R. O., and Knoeber, Sr. R., *Org. Synth.*, 1970, **50**, 38.
[27] Derek, A., Clague, H., and Masters, C., *J. Chem. Soc., Dalton Trans.*, 1975, 858.
[28] McConnell, W. V., and Moore, W. H., *J. Org. Chem.*, 1965, **30**, 3480.

phosphine and carbon tetrabromide, as described above, gave 5-bromo-4,4-dimethylpent-1-ene[17] (68%), which was purified further by preparative g.l.c. on column (F) (Found: C, 47·4; H, 7·5; Br, 45·2. Calc. for $C_7H_{13}Br$: C, 47·5; H, 7·4; Br, 45·1%). ^{1}H n.m.r.: δ 1·0, s, 6H; 2·1, d, J 8 Hz, 2H; 3·3, s, 2H; 4·8–5·2, m, 2H; 5·4–6·1, m, 1H.

5-Bromo-3,3-dimethylpent-1-ene (18)

A mixture of 3-methylbut-2-en-1-ol (8·5 g), trimethyl orthoacetate (12 g) and propionic acid (4·5 g) was slowly heated in a distillation apparatus to 145° over 2 h, during which time methanol (7·5 ml) was collected by distillation. The cooled mixture was then diluted with ether, washed with aqueous sodium bicarbonate, dried, and distilled to afford *methyl 3,3-dimethylpent-4-enoate* (81%), b.p. 59°/33 mm (Found: C, 67·6; H, 9·8. $C_8H_{14}O_2$ requires C, 67·6; H, 9·9%). ^{1}H n.m.r.: δ 1·1, s, 6H; 2·3, s, 2H; 3·7, s, 3H; 4·8–5·2, m, 2H; 5·7–6·3, m, 1H.

Reduction of this ester with lithium aluminium hydride gave 3,3-dimethylpent-4-en-1-ol[29] (82%), b.p. 72°/19 mm, a sample (3·42 g) of which was slowly added to a mixture of tributylphosphine (6·1 g) and bromine (4·8 g) in dimethylformamide at 0–5°. After the addition, the mixture was stirred at room temperature for 2 h, and the crude product was then isolated by distillation. After being washed with water and dried, it was redistilled to afford the required *bromide* (18) (4·2 g, 79%), b.p. 56–57°/18 mm (Found: C, 47·3; H, 7·5; Br, 45·1. $C_7H_{13}Br$ requires C, 47·5; H, 7·4; Br, 45·1%). ^{1}H n.m.r.: δ 1·0, s, 6H; 2·9, t, J 8 Hz, 2H; 3·4, t, J 8 Hz, 2H; 4·8–5·1, m, 2H; 5·6–6·1, m, 1H.

Reactions of Bromo Compounds with Tributylstannane

The bromo compounds (1a)–(3a), (1c–3c) and (18) were purified by preparative g.l.c. [columns (E) and (F)] until no impurity could be detected (< 1%). Benzene was purified by repeated fractional freezing until it contained no impurity detectable by g.l.c. under the conditions subsequently used for analysis of reaction mixtures. The purified benzene was degassed by repeated freezing and thawing in vacuum, and was then stored under a positive pressure of purified nitrogen.

Reaction mixtures were made up by placing the required amounts of tributylstannane, bromo compound (*c.* 1·2 moles per mole of stannane), azobisisobutyronitrile (0·1–0·5 mg) and benzene in a thick-walled ampoule under nitrogen. The mixture was then frozen in liquid nitrogen, and the ampoule was sealed and placed in a thermostat at the required temperature. After the appropriate time (12–36 h) the ampoule was cooled, opened and analysed by g.l.c. on columns (A) [products from bromides (1a) and (2a)], (B) [bromide (3a)], (C) [bromides (1c) and (18)], (D) [bromide (2c)], and (G) [bromide (3c)].

Peak areas were integrated by cutting and weighing; total and relative yields were determined by reference to calibration curves. Each reaction was run in duplicate (in triplicate) over a range of temperatures and concentrations, and appropriate control experiments were carried out to check the stability of products under the reaction conditions. For computational purposes the total yields of products were normalized to 100% and the final concentration of stannane was assumed to be zero. Values of the rate constant ratio k_c/k_H were determined by solving the integrated rate equation,[10] a computer-based iterative technique being used. Arrhenius parameters were determined by a least-squares treatment of the rate constants according to a modified ACTENG program.[30]

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[29] Bourgain-Commercon, M., and Normant, J. F., *Bull. Soc. Chim. Fr.*, II, 1980, 289.
[30] DeTar, D. F., 'Computer Programs for Chemistry' Vol. 3, p. 6 (Benjamin: New York 1969).

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Stereoelectronic Effects in Hydrogen-atom Transfer Reactions of Substituted Cyclohexyl Radicals

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Thermolysis of the peroxyoxalates (1)—(7) and the diacyl peroxides (8)—(11) in cyclohexane at 100 °C gives cycloalkenes and cycloalkanes by hydrogen-atom transfer reactions of the initially formed conformationally biased 4-t-butyl-, 4-t-butyl-*cis,cis*-2,6-dimethyl-, 4-t-butyl-*cis,trans*-2,6-dimethyl-, 4-t-butyl-*cis*-2-methyl-, 4-t-butyl-*trans*-2-methyl-, and 5-t-butyl-*cis*-2-methylcyclohexyl radicals (12)—(17). The composition of the product mixtures indicates that transfer of axial β-hydrogen atoms occurs more rapidly than does transfer of equatorial β-hydrogen atoms. These results support the hypothesis that homolytic fission of a C–H bond is favoured when it lies close to the plane of an adjacent semi-occupied orbital.

In a previous communication [1] we presented evidence that disproportionation of substituted cyclohexyl radicals, like homolytic fission of C_β–C, C_β–O, and other βγ-bonds in suitable carbon-centred radicals, is influenced by stereoelectronic factors, in that it proceeds most readily when the C–H bond undergoing fission can assume coplanarity with the adjacent semi-occupied orbital.[2,3] Similar conclusions were reached by Agosta and Wolff [4] and by Greenlee *et al.*[5] who found that the course of hydrogen-atom transfer in various photochemically generated biradicals can be rationalised on stereoelectronic grounds. A contrary result was reported by Livant and Lawler [6] who studied the disproportionation of cyclohexyl radicals by the CIDNP technique and obtained evidence for the selective fission of those C_β–H bonds (equatorial) which lie furthest from the plane of the semi-occupied orbital. We now give further examples of stereoelectronic effects on disproportionation of substituted cyclohexyl radicals together with full details of our earlier work.

Extensive e.s.r. studies [7–9] have indicated that the cyclohexyl radical at ordinary temperatures undergoes rapid interconversion between two chair-like conformers in each of which the β-protons are stereochemically non-equivalent.[7,8] The two axial C_β–H bonds lie close to the axis of the semi-occupied orbital (θ 22°) whereas the two equatorial C_β–H bonds are almost orthogonol to it (θ 82°)].[8] For most substituted cyclohexyl radicals the two possible chair-like conformations will differ in free energy and will not be equally populated. For example, the e.s.r. spectrum of the 4-t-butyl-1-hydroxycyclohexyl radical shows the presence of only one conformer.[10] As is the case for cyclohexanes the t-butyl substituent strongly favours that conformer in which it occupies an equatorial position. It is reasonable to assume, therefore, that the t-butyl groups in the radicals (12)—(17) studied in the present work will provide a strong conformational bias and will effectively ensure that each radical is conformationally homogeneous under our experimental conditions. Consequently, any difference in reactivity towards abstraction between the equatorial and axial β-hydrogen atoms in, for example, the radical (15) will be reflected in the distribution of products.

The radicals (12)—(17) were generated by thermolysis in dilute cyclohexane solution of the appropriate monoperoxyoxalates [11] (1)—(7) and, in some cases, of the diacyl peroxides (8)—(11). Product mixtures were analysed by g.l.c. and individual components were identified, after separation by chromatography on silver nitrate-impregnated silica gel, by comparison with authentic compounds.

Results and Discussion

The yields of cyclohexanes and cyclohexenes formed when the peroxides (1)—(11) were heated in dilute cyclohexane solution (0.25M) at 100 °C for 2 h are given in the Table. Since the peroxides were too unstable to be handled safely in pure form they were prepared *in situ* and the yields of products are based on the amounts of acid chlorides used for their synthesis. The total yield given in the Table for each peroxide is the highest obtained from at least two experiments. Although there was considerable variation in total yield the agreement between the relative yields of products determined in duplicate or triplicate experiments with each substrate was satisfactory (s.d. ±20%). Cyclohexene was detected in most reaction mixtures but the yields (generally >0.6 mol per mol of peroxyoxalate or >0.2 mol per mol of diacyl peroxide) were not accurately determined. Products of higher g.l.c. retention time, presumably esters and dimers, were also formed but were not unambiguously identified or determined. However, the fact that monomeric products were formed in good yield (generally 70—90%) indicates that radical coupling is not an important reaction pathway.

The results given in the Table show that each peroxide affords the appropriate substituted cyclohexane in >50% yield. Such products must arise, at least in part, by hydrogen-atom abstraction from solvent or some other donor. Although the present results do not allow the extent of this type of reaction to be estimated, the formation of cyclohexene, particularly in reactions involving diacyl peroxides, verifies that cyclohexyl radicals are generated from solvent and are involved in hydrogen-atom transfer processes.

The substituted cyclohexenes (24)—(31) formed by thermolysis of the peroxides (1)—(11) must arise by hydrogen-atom transfer from the appropriate substituted cyclohexyl radicals, either by disproportionation or by reaction with cyclohexyl radicals. Hydrogen-atom transfer to t-butoxyl radicals, although possible in experiments involving peroxyoxalates, is improbable since their rapid reaction with solvent will ensure that their stationary concentration remains very low. The fact that the diacyl peroxides (8)—(11) give product distributions very similar to those from their corresponding peroxyoxalates lends support to this view and provides evidence that reactions of both types of precursor proceed through the same radical intermediates. Also it indicates that decarboxylation of the substituted cyclohexyloxycarbonyl radicals formed initially from peroxyoxalates, although

Products from heating of peroxyoxalates and diacyl peroxides in cyclohexane at 100 °C

Peroxide	Radical	Cycloalkane	Relative yield (%)	Cycloalkene	Relative yield (%)	Total yield (%)
(1)	(12)	(18)	83	(24)	17	84
(8)	(12)	(18)	78	(24)	22	71
(2)	(13)	(20)	76	(27)	24	91
(4)	(15)	(22) *	82	(27)	1.9	88
				(29)	16.0	
(3)	(14)	(21)	81	(25)	8.3	85
				(28)	11.2	
(5)	(14)	(21)	81	(25)	9.0	83
				(28)	10.2	
(9)	(14)	(21)	80	(25)	9.5	74
				(28)	10.8	
(10)	(14)	(21)	79	(25)	9.2	71
				(28)	11.3	
(6)	(16)	(23)	81	(25)	2.9	88
				(30)	16.0	
(11)	(16)	(23)	77	(25)	4.4	68
				(30)	19.0	
(7)	(17)	(19)	83	(26)	4.5	78
				(31)	12.9	

* The product mixture also contained *ca.* 1% of the cycloalkane (20) formed from the isomeric impurity in the peroxide (4)

expected to be relatively slow,[12] is still sufficiently rapid to compete effectively with other possible processes. In summary, it appears that the major course of these thermolyses is consistent with the Scheme.

$$(RCO_2)_2 \longrightarrow 2\,R^{\cdot} + 2\,CO_2$$

$$ROCOCO_2OBu^t \longrightarrow ROCO^{\cdot} + CO_2 + {}^{\cdot}OBu^t$$

$$ROCO^{\cdot} \longrightarrow R^{\cdot} + CO_2$$

$$C_6H_{12} + {}^{\cdot}OBu^t \longrightarrow C_6H_{11}^{\cdot} + Bu^tOH$$

$$C_6H_{12} + R^{\cdot} \longrightarrow C_6H_{11}^{\cdot} + RH$$

$$2\,R^{\cdot} \longrightarrow R(-H) + RH$$

$$R^{\cdot} + C_6H_{11}^{\cdot} \longrightarrow R(-H) + C_6H_{12}\ (\text{or } RH + C_6H_{10})$$

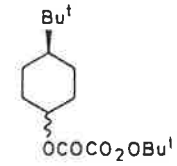
$$2\,C_6H_{11}^{\cdot} \longrightarrow C_6H_{10} + C_6H_{12}$$

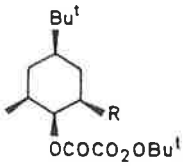
Scheme. R = Substituted cyclohexyl radical

The relative yields of cyclohexenes formed by thermolysis of the peroxides (1)—(11) indicates that there is preferential loss of axial β-hydrogen atoms from the appropriate intermediate cyclohexyl radicals. The most clear-cut demonstration of this phenomenon is provided by thermolysis of the peroxyoxalate (4). The substituted cyclohexyl radical (15) so generated has two non-equivalent β-hydrogen atoms. Abstraction of the equatorial hydrogen should be less sterically hindered and is thermodynamically favoured since it relieves non-bonded interactions of the axial methyl substituent. Nevertheless, the results show that the preferred process is the loss of the axial β-hydrogen to give the *trans*-substituted cyclohexene (29). The approximate relative values of the rate constants for β-hydrogen atom abstraction are given by the ratio of yields of the cyclohexenes (29) and (27), *i.e.* k(axial-H)/k(equatorial-H) ≈ 8.

The results of reactions involving the disubstituted radicals (14) and (16) can be similarly rationalised on the assumption that axial C_β–H bonds are more reactive than their equatorial counterparts towards hydrogen-atom transfer. Disproportionation of the radical (14) which has one axial hydrogen on each β-carbon affords approximately equal amounts of the olefins (25) and (28), whereas disproportionation of the radical (16) in which the sole axial β-hydrogen is bonded to C-6 gives a large preponderance of that olefin (30) formed by reaction at C-6. While neither of these results taken individually provides unambiguous support for the concept of enhanced reactivity for axial C_β–H bonds a comparative consideration of the data certainly points in this direction. Since each contains one tertiary and two secondary β-hydrogen atoms the statistical factor is the same for disproportionation of both of the radicals (14) and (16). Nor can the difference in the ratios of cyclohexenes, (28) : (25) and (30) : (25), formed respectively from the radicals (14) and (16) be attributed to steric or thermodynamic factors. Steric interactions should disfavour abstraction of axial β-hydrogen atoms from either radical (14) or (16) but particularly from the latter because of the proximity of the reaction centre to the axial methyl substituent. Also, fission of the tertiary C_β–H bond in (16) should be favoured thermodynamically since it relieves nonbonded interactions of the axial methyl group. Thus, both steric and thermodynamic considerations suggest that the ratio of the yield of olefin formed by homolysis of the tertiary C_β–H bond to that formed by cleavage of a secondary C_β–H bond should be greater for (16) than for (14). However, the opposite was observed. We conclude that disproportionation of the radicals involves preferentially the loss of axial β-hydrogen atoms. It is noteworthy that the radical (17) which has its sole axial β-hydrogen atom at the 6-position affords mainly the olefin (31) formed by hydrogen abstraction from C-6.

A number of experiments were conducted with the aim of confirming that the observed products do indeed arise by hydrogen atom transfer reactions of substituted cyclohexyl radicals. Thus, solvolysis of the tosylate (32h) afforded the


(1)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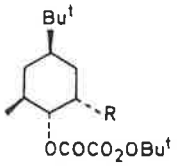
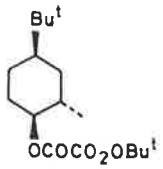
(3) R = H


(4) R = Me
(5) R = H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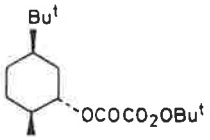
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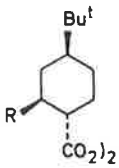
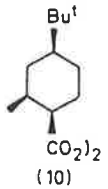

(8) R = H
(9) R = Me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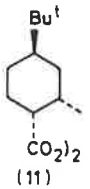
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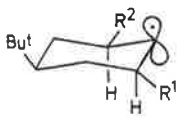
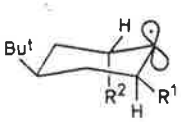
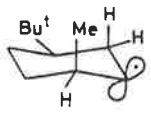

(12) $R^1 = R^2 = H$
(13) $R^1 = R^2 = Me$
(14) $R^1 = Me, R^2 = H$


(15) $R^1 = R^2 = Me$
(16) $R^1 = H, R^2 = Me$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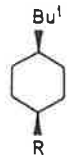
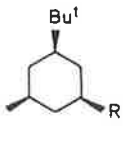
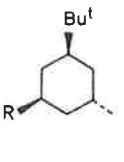
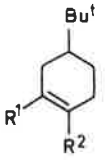
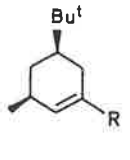
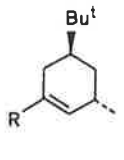

(18) R = H
(19) R = Me


(20) R = Me
(21) R = H


(22) R = Me
(23) R = H


(24) $R^1 = R^2 = H$
(25) $R^1 = Me, R^2 = H$
(26) $R^1 = H, R^2 = Me$


(27) R = Me
(28) R = H

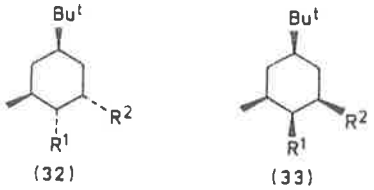

(29) R = Me
(30) R = H


(31)

cis-disubsubstituted cyclohexene (27) and rearranged olefins but none of the *trans*-compound (29), whereas decomposition of the peroxide (4) gives mainly the *trans*-disubstituted cyclohexene (29) and no rearranged products. Nor were rearranged products detected from reactions of the peroxides (1)—(11) although ionic transformations of cyclohexyl derivatives often involve rearrangement.[13] We conclude, therefore, that cationic intermediates do not play a part in the thermal decomposition of either the peroxyoxalates (1)—(7) or the diacyl peroxides (8)—(11). Likewise, both the acetate (32g) and the chloroglyoxalate of the alcohol (32d) were found to be stable to the conditions employed for thermolysis of the peroxyoxalates (1)—(7). It appears unlikely, therefore, that peroxyoxalates will undergo formation of olefins by concerted elimination of carbon dioxide and t-butyl alcohol. Finally it is noteworthy that there is very close agreement between the results of the four experiments in which the radical (14) was generated from precursors of different types and stereochemistry. This provides compelling evidence that the radical (14) is a discrete intermediate common to each of these four reactions and that the peroxide functionality is not directly involved in product formation.

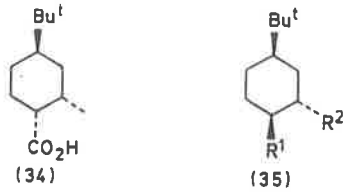
In summary, the results presented above clearly show that β-hydrogen atoms in appropriately substituted cyclohexyl radicals are chemically non-equivalent with respect to transfer processes. The most obvious interpretation is that which we have adumbrated above and has been advanced previously,[4,5] namely, that axial C_β–H bonds in chair conformations of cyclohexyl radicals undergo fission more readily than their equatorial counterparts (in our experiments some four to eight times). However, it is also possible that the apparent loss of equatorial hydrogen atoms actually involves fission of pseudo-axial C_β–H bonds in twist-boat conformations. If this were true the real preference for axial β-hydrogen atom abstraction may be greater than indicated. In any event our results suggest that it is those bonds which more effectively overlap with the adjacent semi-occupied orbital that undergo preferential fission. They are fully consistent with the view that radical disproportionation is influenced by stereoelectronic factors and conforms to a general pattern, namely, homolytic fission is most favoured when the bond concerned can assume coplanarity with an adjacent semi-occupied orbital.[2,3]

Synthesis of Radical Precursors and Reference Compounds.—The peroxyoxalates (1)—(7) were prepared by a standard procedure [11] from the appropriate alcohols *via* their chloroglyoxalates, ROCOCOCl, while the diacyl peroxides (8)—(11) were obtained by interaction of the appropriate acid chlorides with sodium peroxide.[14] *trans*-4-t-Butylcyclohexanecarboxylic acid [15] was prepared from 4-t-butylcyclohexanol *via* the bromide,[16] while the acids (32a), (33a), and (34) required for the synthesis of diacyl peroxides were obtained from 4-t-butyl-2-methylcyclohexanone [17] *via* the cyanohydrin.[18] However, attempts to obtain the acids (32b) and (33b) or their epimers at C-1 by this method failed as did a number of other



(32) (33)

a ; R^1 = CO_2H , R^2 = H
b ; R^1 = CO_2H , R^2 = Me
c ; R^1 = OH , R^2 = H
d ; R^1 = OH , R^2 = Me
e ; R^1 = OAc , R^2 = H
f ; R^1 = OTos , R^2 = H
g ; R^1 = OAc , R^2 = Me
h ; R^1 = OTos , R^2 = Me



(34) (35)

a ; R^1 = OH , R^2 = Me
b ; R^1 = Me , R^2 = OH
c ; R^1 = OTos , R^2 = Me
d ; R^1 = Me , R^2 = OAc

attempts based on nucleophilic attack on appropriate substrates. The rigidity and steric hindrance associated with such pseudo-neopentyl systems are known [19] to leave them prone to hydride shifts, alkyl rearrangements, and elimination processes in preference to nucleophilic substitution.

The alcohols (33c) and (32c) required for synthesis of the peroxyoxalates (3) and (5) were prepared by hydrogenation of 4-t-butyl-2-methylphenol,[20] while the alcohols (35a and b) were obtained by treatment of a mixture of the stereoisomers of 4-t-butylcyclohexene oxide with dimethylmagnesium.[21,22]

Although other 2,4,6-trisubstituted phenols have been hydrogenated in the presence of platinum oxide [23] this method failed when applied to 4-t-butyl-2,6-dimethylphenol. Hydrogenation over Raney nickel [24] was also unsuccessful as was attempted Birch reduction.[25] Eventually, the alcohol (33d) was obtained by treatment of the phenol in ethanol at 100 °C with hydrogen at 2 500 lb in^{-2} over 5% rhodium on alumina. At lower temperatures and pressures no hydrogenation occurred, whilst at higher temperatures the catalyst was deactivated. The alcohol (33d) was the only isomer formed. Its stereochemistry was assigned on the basis of its ^{13}C and ^1H n.m.r. spectra. Oxidation of the alcohol (33d) gave a single ketone the structure of which was assigned as *c*-4-t-butyl-*r*-2,*c*-6-dimethylcyclohexanone by n.m.r. spectroscopy. Its failure to undergo acid equilibration to a stereoisomer under the reaction conditions is surprising.[26] This ketone was epimerised at C-6 *via* its semicarbazone [27] to give *c*-4-t-butyl-*r*-2,*t*-6-dimethylcyclohexanone, which underwent highly stereoselective reduction with lithium aluminium hydride to afford the alcohol (33d).

Authentic samples of cycloalkenes required for comparison with free radical reaction products were made by appropriate elimination reactions. The trisubstituted cyclohexene (27), for example, was obtained by treatment of the alcohol (33d) with thionyl chloride in pyridine,[28] by flash pyrolysis [29] of the acetate (33g) and by interaction of the tosylate (33h) with potassium t-butoxide.[29] The same olefin (27) was the major component of the mixture formed by treatment of the alcohol (32d) with thionyl chloride or by base promoted elimination of the tosylate (32h). However, pyrolysis of the acetate (32g) proceeded, as expected,[30] mainly by *cis*-elimination to give the olefin (29).

Mixtures of the olefins (25) and (28) were obtained by similar methods from mixtures of the alcohols (32c) and (33c) or their appropriate derivatives. Interestingly, pyrolysis of the acetates (32e) and (33e) gave more of the olefin (25) than can reasonably be accounted for by a *cis*-elimination mechanism. Presumably, under vigorous conditions *trans*-elimination can occur from distorted chair conformations of the acetates (32e) and (33e). More direct evidence for this is the formation of the cyclohexene (27) from the pyrolysis of the acetate (32g). Similarly, base treatment of a mixture of the tosylates (32f) and (33f) gave more of the olefin (25) than expected on the basis of a *trans*-elimination process. It has been suggested previously [29,31] that *cis*-eliminations may occur in these systems *via* boat conformations. This is observed more directly in the formation of the cyclohexene (27) from the tosylate (32h). Pure samples of the olefins (25) and (28) were obtained from product mixtures by chromatography on silver nitrate-impregnated silica gel. A pure sample of the olefin (30) was similarly separated from the mixture with the olefin (25) obtained by base treatment of the tosylate (35c). As expected, flash vacuum pyrolysis of the acetate (35d) gave a mixture of the olefins (26) and (31) which could be separated chromatographically.

Each of the cycloalkanes (19), (21), and (23) required for comparison with free-radical reaction products was prepared by catalytic hydrogenation of the respective cycloalkenes (31), (28), and (30). Similar treatment of the olefin (27) gave a mixture of the cycloalkanes (20) and (22) in which the former predominated. Catalytic hydrogenation of a mixture of the olefins (27) and (29) gave only the cycloalkanes (20) and (22).

The assignment of stereochemistry to many of the compounds described above rests heavily on the correlation of observed ^{13}C n.m.r. shifts with those predicted by the use of addivity factors and the data already available for a variety of substituted cyclohexenes and cyclohexanes in their chair conformations.[32] Both observed and predicted values are given in the Experimental section.

Experimental

I.r. spectra for liquid films, unless otherwise stated, were recorded on either a Jasco IRA-1 or Unicam SP200 spectrometer. Mass spectra were measured on an AEI MS30 or a Hitachi–Perkin-Elmer RMU-6D instrument operating at 70 eV. ^1H N.m.r. spectra were recorded, in carbon tetrachloride unless otherwise stated, on either a Varian T60 or a JEOL JNM-PMX 60 spectrometer. ^{13}C N.m.r. spectra were recorded for solutions in deuteriochloroform on a Bruker WP-80 Fourier transform spectrometer. ^{13}C Chemical shifts were measured relative to tetramethylsilane; assignments and predicted values of chemical shifts are given in parentheses Microanalyses were performed by the Australian Microanalytical Service, Melbourne.

G.l.c. was conducted on a Perkin-Elmer 881 or 990 instrument using the following columns; A, 0.75% FFAP on Chromosorb W (100—120), 6.0 m × 3.0 mm stainless steel; B, 5% Carbowax 20M on Gaschrom P (80—100), 3.0 m × 3.0 mm stainless steel; C, 5% FFAP on Chromosorb W (80—100) (base washed), 3.0 m × 3.0 mm glass; D, 20% FFAP on Chromosorb W (80—100), 2.6 m × 4.0 mm glass; E, SCOT Carbowax 20M, 58 m × 0.5 mm glass; F, 15% SE30 on

Chromosorb W (60—80), 2.0 m × 6.0 mm glass; and G SCOT Carbowax 20M, 68.6 m × 0.5 mm glass. Products were identified by comparison of their retention times with those of authentic samples and confirmed by peak enhancement. The areas of peaks were determined with either a Perkin-Elmer 194B printing integrator or a disc integrator and were checked by triangulation. Relative response ratios were determined by standard techniques. High performance liquid chromatographic (h.p.l.c.) separations were carried out on a Spectra-Physics 3500B chromatograph equipped with a Spectra-Physics 230 detector and a Pye-Unicam LCM2 detector. Two Lichrosorb S1.60 (10μ, 50 cm × 1 cm) columns were used in series. Silver nitrate impregnated silica was prepared by the procedure of Gream *et al.*[33]

4-t-Butylcyclohexanol.—A sample of the commercial material after recrystallisation had m.p. 61—65 °C and was shown to be a mixture of the *cis*- and *trans*-isomers (30 : 70) by g.l.c. (column A).

c-4-t-Butyl-c-2-methylcyclohexan-r-1-ol (33c) *and t-4-t-Butyl-t-2-methylcyclohexan-r-1-ol* (32c).—When 4-t-butyl-2-methylphenol (prepared in 71% yield from 2-methylphenol)[34] was hydrogenated[20] a mixture of the alcohol (33c) (64%) and its isomer (32c) (36%) was obtained in 82% yield. Chromatography of the mixture on alumina afforded *c-4-t-butyl-c-2-methylcyclohexan-r-1-ol* (33c), homogeneous by g.l.c. (column C; 150 °C; 6.5 min), m.p. 79—81 °C (lit.,[20] 78—79 °C), and *t-4-t-butyl-t-2-methylcyclohexan-r-1-ol* (32c), homogeneous by g.l.c. (column C; 150 °C; 7.8 min), m.p. 69—70 °C (lit.,[20] 72—73 °C).

c-4-t-Butyl-t-2-methylcyclohexan-r-1-ol (35a) *and t-5-t-Butyl-t-2-methylcyclohexan-r-1-ol* (35b).—Treatment of a mixture of *cis*-4-t-butylepoxycyclohexane and *trans*-4-t-butylepoxycyclohexane (prepared in 74% yield from 4-t-butylcyclohexene)[35] with dimethylmagnesium[21,22] gave a mixture (57%) of the alcohols (35a and b) [g.l.c. column C; 150 °C; 6.5 min (68%) and 6.9 min (32%)] which were separated by h.p.l.c. [ethyl acetate–light petroleum (1 : 10); 16 ml min^{-1}; 12.5 min (68%) and 13.2 min (32%)]. The first was distilled to give an oil, b.p. 108—109 °C at 15 mmHg, which crystallised from light petroleum in needles (2.3 g, 32%), m.p. 70—72 °C (lit.,[21] 70—71 °C), of *c-4-t-butyl-t-2-methylcyclohexan-r-1-ol* (35a). The second crystallised from light petroleum in needles (0.9 g, 13%), m.p. 74—76 °C (lit.,[22] 75—76 °C), of *t-5-t-butyl-t-2-methylcyclohexan-r-1-ol* (35b).

c-4-t-Butyl-c-2,c-6-dimethylcyclohexan-r-1-ol (33d).—A solution of 4-t-butyl-2,6-dimethylphenol (10.0 g, prepared in 54% yield from 2,6-dimethylphenol)[36] in 95% aqueous ethanol over 5% rhodium on alumina (1.0 g) was hydrogenated at 2 500 lb in^{-2} and 100 °C for 72 h. The mixture was then filtered through Celite, concentrated, and distilled to give the required *alcohol* as an oil, b.p. 88—90 °C at 4 mmHg, which crystallised from light petroleum as needles (7.5 g, 68%), m.p. 62—63 °C, homogeneous by g.l.c. (column A; 150 °C, 5.45 min) (Found: C, 78.5; H, 12.9. $C_{12}H_{24}O$ requires C, 78.2; H, 13.1%); *m/e* 184 (*M*, 27%), 167 (14), and 109 (100); $\nu_{max.}$ (Nujol) 961, 1 363, and 3 446 cm^{-1}; δ_H 0.86 (9 H, s, Bu^t), 0.92 (6 H, d, *J* 6 Hz, 2 × CH_3), 1.0—2.2 (7 H), 2.35br (1 H, s, OH), and 3.43 (1 H, m, C*H*OH); δ_C 18.8 (2 × CH_3), 27.6 and 32.4 (Bu^t), 28.5 (C-3, C-5; 29.4), 37.4 (C-2, C-6; 38.9), 47.7 (C-4; 48.2), and 74.6 p.p.m. (C-1).

c-4-t-Butyl-r-2,c-6-dimethylcyclohexanone.—Jones' reagent (8_N)[37] was added dropwise to a solution of *c-4-t-butyl-c-2,c-6-dimethylcyclohexan-r-1-ol* (5.5 g) in acetone (50 ml) until the colour persisted. The mixture was then poured into water (150 ml) and extracted with ether (3 × 100 ml). The ether extracts were then washed with sodium hydrogencarbonate solution and with water, dried, and distilled to give the required *ketone* as a liquid (5.1 g, 93%), b.p. 95—98 °C at 10 mmHg, homogeneous by g.l.c. (column F; 150 °C; 4.20 min) (Found: C, 78.8; H, 12.1. Calc. for $C_{12}H_{22}O$: C, 79.1; H, 12.1%); *m/e* 182 (*M*, 11%), 126 (56), 57 (100), and 41 (27); $\nu_{max.}$ 1 365 and 1 718 cm^{-1}; δ_H 0.95 (9 H, s, Bu^t), 0.98 (6 H, d, *J* 7.5 Hz, 2 × CH_3), and 1.0—2.6 (7 H); δ_C 14.8 (2 × CH_3; 14.6), 27.7 and 32.4 (Bu^t; 27.6 and 32.4), 38.2 (C-3, C-5; 37.6), 44.3 (C-2, C-6; 44.5), 47.0 (C-4; 47.1), and 215.2 p.p.m. (C-1).

c-4-t-Butyl-r-2,t-6-dimethylcyclohexanone.—A solution of the preceding ketone (4.8 g), semicarbazide hydrochloride (5.6 g), and potassium acetate (5.0 g) in methanol (90 ml) was boiled under reflux for 18 h, then concentrated and diluted with water (100 ml). The resultant precipitate was dried and crystallised twice from methanol to give the semicarbazone as needles (5.2 g, 86%), m.p. 173—174 °C (Found: C, 64.9; H, 10.5. Calc. for $C_{13}H_{25}N_3O$: C, 65.2; H, 10.5%); δ_H 0.90 (9 H, s, Bu^t), 1.10 (6 H, d, *J* 7.5 Hz, 2 × CH_3), 1.4—3.6 (7 H), 6.0br (2 H, s, NH_2), and 8.86br (1 H, s, NH).

A solution of the semicarbazone (5.0 g) in acetic acid (30 ml) was maintained below 5 °C while sodium nitrite (4.2 g) in water (30 ml) was added during 30 min. After extraction of the mixture with ether, the extract was washed with sodium hydrogen carbonate solution and with water, dried, and distilled to afford *c-4-t-butyl-r-2,t-6-dimethylcyclohexanone* (3.4 g, 89%), b.p. 104—105 °C at 10 mmHg (Found: C, 79.4; H, 12.0. $C_{12}H_{22}O$ requires C, 79.1; H, 12.1%); *m/e* 182 (*M*, 16%), 126 (77), 57 (91), and 41 (100); $\nu_{max.}$ 1 373 and 1 716 cm^{-1}; δ_H 0.86 (9 H, s, Bu^t), 0.95 (3 H, d, *J* 7.5 Hz, equatorial CH_3), 1.12 (3 H, d, *J* 7.5 Hz, axial CH_3), and 1.3—2.8 (7 H); δ_C 15.1 (equatorial CH_3; 14.6), 17.5 (axial CH_3; 17.4), 27.5 and 32.2 (Bu^t; 27.6 and 32.4), 34.1 (C-5; 34.5), 36.3 (C-3; 36.2), 40.6 (C-2; 38.4), 41.3 (C-4; 41.0), 43.4 (C-6; 44.7), and 218.5 p.p.m. (C-1). The ketone was shown by g.l.c. to be contaminated with 1—2% of *c-4-t-butyl-r-2,c-6-dimethylcyclohexanone* [column F; 150 °C; 4.20 min (*ca.* 2%), 4.55 min (*ca.* 98%)].

t-4-t-Butyl-c-2,t-6-dimethylcyclohexan-r-1-ol (32d).—A solution of the preceding ketone (3.1 g) in dry ether (60 ml) was heated with lithium aluminium hydride (1.0 g) under reflux for 3 h. The cooled mixture was then worked-up in the usual way with sodium hydroxide solution, and the crude product was distilled to give the required *alcohol*, b.p. 106—108 °C at 10 mmHg, which crystallised from light petroleum as needles (2.1 g, 69%), m.p. 56—58 °C (Found: C, 78.5; H, 13.1. $C_{12}H_{24}O$ requires C, 78.2; H, 13.1%); *m/e* 184 (*M*, 14%), 167 (8), and 109 (100); $\nu_{max.}$ (Nujol) 1 043, 1 363, and 3 432 cm^{-1}; δ_H 0.86 (9 H, s, Bu^t), 0.92 (6 H, d, *J* 6 Hz, 2 × CH_3), 1.1—2.5 (7 H), 2.2br (1 H, s, OH), and 3.2 (1 H, dd, *J* 5 and 10 Hz, C*H*OH); δ_C 12.2 (equatorial CH_3), 19.1 (axial CH_3), 27.5 and 31.9 (Bu^t; 27.5 and 32.1), 32.1 (C-3; 30.6), 33.1 (C-5; 34.5), 34.6 (C-6; 35.9), 35.3 (C-2; 36.8), 40.1 (C-4; 41.9), and 78.8 p.p.m. (C-1). The alcohol (32d) was shown by g.l.c. to contain *ca.* 1—2% of its isomer (33d) [column A; 150 °C; 5.45 min (*ca.* 2%), 6.50 min (*ca.* 98%)].

cis-1-Bromo-4-t-butylcyclohexane.—A mixture of 4-t-butylcyclohexanol (3.0 g), phosphorus tribromide (5.4 g), pyridine (0.3 ml), and benzene (30 ml) was stirred at 55 °C for 12 h, then cooled, and poured onto ice. The layers were separated, the aqueous solution was extracted with benzene, and the combined organic extracts were washed with sodium carbonate solution and with water, dried, and distilled to give the required

bromide (3.5 g, 84%), b.p. 112—114 °C at 19 mmHg (lit.,[16] 104—110 °C at 14 mmHg).

trans-4-*t*-*Butylcyclohexanecarboxylic Acid*.—The Grignard reagent prepared from the preceding bromide (2.2 g), magnesium (0.48 g), and ether (50 ml) was poured onto solid carbon dioxide (12 g). After acidification of the mixture the organic phase was separated and the aqueous solution was extracted twice with ether. After being washed with water the combined ether solutions were extracted thrice with saturated sodium hydrogencarbonate solution. Acidification of the aqueous extracts gave a precipitate of the required carboxylic acid which crystallised from ethanol in plates (0.95 g, 52%), m.p. 176—178 °C (lit.,[15] 174—176 °C).

t-4-*t*-*Butyl*-t-2-*methylcyclohexane*-r-1-*carboxylic Acid* (32a), c-4-*t*-*Butyl*-c-2-*methylcyclohexane*-r-1-*carboxylic Acid* (33a), *and* t-4-*t*-*Butyl*-c-2-*methylcyclohexane*-r-1-*carboxylic Acid* (34).—4-t-Butyl-2-methylcyclohexanone [17] was converted as previously described [18] into a mixture of the three acids (32a), (33a), and (34), which was converted into a mixture of the appropriate methyl esters, and separated by h.p.l.c. Hydrolysis of the fractions [18] afforded pure samples of the acids (32a), (33a), and (34) having physical and spectral properties identical with those previously reported.[18]

c-5-*t*-*Butyl*-1,r-3-*dimethylcyclohexene* (27).—(a) A solution of the alcohol (33d) (1.0 g) in pyridine (20 ml) was stirred at < -5 °C while thionyl chloride (4 ml) was added dropwise. After being kept at −5 °C for 4 h the mixture was poured onto ice, extracted with ether, and worked up in the usual way to give an oil which was chromatographed on alumina. The fraction eluted with light petroleum was distilled to afford the *olefin* (27) (0.54 g, 60%), b.p. 45—47 °C (block) at 15 mmHg, homogeneous by g.l.c. (column E; 100 °C; 31.3 min) (Found: C, 86.5; H, 13.4. $C_{12}H_{22}$ requires C, 86.7; H, 13.3%); *m/e* 165 ($M - 1$, 100%) and 57 (42); δ_H 0.90 (9 H, s, Bu^t), 0.94 (3 H, d, J 8.5 Hz, CH_3), 1.70 (3 H, m, allylic CH_3), 1.2—2.6 (6 H), and 5.20 (1 H, m, vinylic CH); δ_C 22.2 (CH_3; 22.4), 23.7 (allylic CH_3; 23.8), 27.3 and 32.4 (Bu^t; 27.7 and 32.7), 31.8 (C-6; 31.6), 32.4 (C-3; 32.3), 33.6 (C-4; 33.7), 44.8 (C-5; 45.5), 127.9 (C-2), and 133.7 p.p.m. (C-1).

(b) Treatment of the alcohol (33d) (3.9 g) with toluene-*p*-sulphonyl chloride (7.2 g) in pyridine (50 ml) at 0—3 °C for 30 h in the usual way afforded *c*-4-t-butyl-*c*-2,*c*-6-dimethylcyclohexyl *r*-toluene-*p*-sulphonate (33h) which crystallised from light petroleum at −78 °C in needles (1.7 g, 63%), m.p. 117—118 °C; δ_H 0.87 (9 H, s, Bu^t), 0.98 (6 H, d, J 7 Hz, 2 × CH_3), 1.1—2.3 (7 H), 2.50 (3 H, s, $ArCH_3$), 4.9 (1 H, m, CHOTos), and 7.3—8.1 (4 H, m, ArH). A solution of this tosylate (0.65 g) in dimethyl sulphoxide (2 ml) and benzene (10 ml) was added dropwise to a stirred suspension of potassium t-butoxide (0.5 g) in dimethyl sulphoxide (8 ml), and the mixture was stirred for 16 h at ambient temperature, and then poured into ice–water. Extraction with pentane and distillation of the extract afforded the olefin (27) (0.13 g, 41%).

(c) The alcohol (33d) (6.2 g) was heated with acetic anhydride (15 ml) and pyridine (15 ml) at 50 °C for 6 h, and the mixture was worked up in the usual way to give *c*-4-t-butyl-*c*-2,*c*-6-dimethylcyclohexyl *r*-acetate (33g) (4.7 g, 61%), b.p. 141—144 °C at 18 mmHg, homogeneous by g.l.c. (column D; 100 °C; 3.30 min); δ_H 0.87 (9 H, s, Bu^t), 0.93 (6 H, d, J 6.5 Hz, 2 × CH_3), 1.1—2.6 (7 H), 2.10 (3 H, s, $OCOCH_3$), and 4.90 (1 H, m, CHOAc). This acetate (2.0 g) was slowly distilled at 15 mmHg through a Vycor tube (50 cm × 2.5 cm) packed with silica beads and maintained at 475 °C. The pyrolysate, which was collected in a trap at −78 °C, was taken up in light petroleum, washed with sodium carbonate solution and with water, dried, and distilled to afford the olefin (27) (0.68 g, 47%).

t-5-*t*-*Butyl*-1,r-3-*dimethylcyclohexene* (29).—The alcohol (32d) was converted as described above into its acetate (32g) (68%), b.p. 130—135 °C at 15 mmHg; δ_H 0.88 (9 H, s, Bu^t), 0.90 (3 H, d, J 8 Hz, equatorial CH_3), 0.93 (3 H, d, J 8 Hz, axial CH_3), 1.0—2.5 (7 H), 2.00 (3 H, s, $OCOCH_3$), and 4.42 (1 H, dd, J 4.5 and 11 Hz, CHOAc). The acetate (32g) was shown by g.l.c. to be contaminated with 1—2% of the acetate of the alcohol (33d) [column D; 100 °C; 3.30 min (*ca.* 2%) and 3.75 (*ca.* 98%)]. Pyrolysis of this acetate (see preceding experiment) gave the *olefin* (29) (51%), b.p. 47—48 °C (block) at 20 mmHg (Found: C, 86.3; H, 13.2. $C_{12}H_{22}$ requires C, 86.7; H, 13.3%); *m/e* 165 ($M - 1$, 52%) and 57 (100); δ_H 0.89 (9 H, s, Bu^t), 0.95 (3 H, d, J 9 Hz, CH_3), 1.1—2.6 (6 H), 1.68 (3 H, m, allylic CH_3), and 5.35 (1 H, m, vinylic H); δ_C 21.0 (CH_3), 24.0 (allylic CH_3; 23.8), 27.4, and 32.2 (Bu^t; 27.7 and 32.7), 30.0 (C-4; 30.0), 30.4 (C-3; 27.8), 32.2 (C-6; 31.8), 39.1 (C-5; 40.1), 126.9 (C-2), and 133.5 p.p.m. (C-1). The sample was shown by g.l.c. to contain 7% of the isomer (27) [column E; 100 °C; 31.3 min (7%) and 32.2 min (93%)].

(b) Treatment of the alcohol (32d) with thionyl chloride in pyridine as described above gave a mixture (0.24 g, 47%), b.p. 109—110 °C (block) at 13 mmHg, of the olefins (27) and (29) [column E; 100 °C; 31.3 min (93%) and 32.2 min (7%)].

(c) The toluene-*p*-sulphonate (32h), prepared in the usual way from the alcohol (32d), was obtained as a crystalline solid (1.65 g, 24%), m.p. 103—104 °C (decomp.); δ_H ($CDCl_3$) 0.90 (9 H, s, Bu^t), 1.04 (6 H, d, J 7.5 Hz, 2 × CH_3), 1.1—2.3 (7 H), 2.54 (3 H, s, $ArCH_3$), 4.35 (1 H, dd, J 5.5 and 11 Hz, CHOTos), and 7.3—8.1 (4 H, m, ArH). On treatment with potassium t-butoxide as described above it afforded a mixture (0.17 g, 34%), b.p. 50—55 °C (block) at 18 mmHg, of the olefins (27) and (29) [column E; 100 °C; 31.3 min (91%) and 32.2 min (9%)].

c-5-*t*-*Butyl*-r-3-*methylcyclohexene* (28) *and* 5-*t*-*Butyl*-1-*methylcyclohexene* (25).—(a) Treatment of a mixture of *c*-4-t-butyl-*c*-2-methylcyclohexan-*r*-1-ol (33c) (64%) and *t*-4-t-butyl-*t*-2-methylcyclohexan-*r*-1-ol (32c) (36%) with thionyl chloride and pyridine as described above afforded a mixture of two components [column G; 100 °C; 28.0 min (8%) and 32.4 min (92%)] which was chromatographed on silver nitrate impregnated silica. Elution with light petroleum gave 5-t-butyl-1-methylcyclohexene (25) (1.7 g, 54%), b.p. 59—63 °C at 20 mmHg (lit.,[38] 82.0—82.5 °C at 18 mmHg), homogeneous by g.l.c. (column G; 100 °C; 32.4 min). Continued elution with light petroleum afforded *c*-5-t-butyl-*r*-3-methylcyclohexene (28) (0.13 g, 4%), b.p. 60—62 °C (block) at 20 mmHg (lit.,[31] 85—87 °C at 35 mmHg), also homogeneous by g.l.c. (column G; 100 °C; 28.0 min).

(b) Treatment of a mixture of the alcohols (33c) (64%) and (32c) (36%) with toluene-*p*-sulphonyl chloride as described above gave a mixture of the toluene-*p*-sulphonates (33f) (64%) and (32f) (36%) as a crystalline solid (1.46 g, 35%), m.p. 52—59 °C (decomp.); δ_H ($CDCl_3$) 0.88 (9 H, s, Bu^t), 1.02 (3 H, d, J 7 Hz, CH_3), 1.0—2.1 (8 H), 2.53 (3 H, s, $ArCH_3$),4. 40 (0.36 H, m, $CH_{axial}OH$), 4.85 (0.64 H, m, $CH_{equatorial}OH$), and 7.3—8.1 (4 H, m, ArH). Treatment of this mixture with potassium t-butoxide afforded a mixture of the cyclohexenes (25) and (28) [column G; 100 °C; 28.0 min (12%) and 32.4 min (88%)] as an oil (0.24 g, 52%), b.p. 43—49 °C (block) at 12 mmHg, which were separated by chromatography on silver nitrate-impregnated silica.

(c) Acetylation of a mixture of the alcohols (33c) (64%) and (32c) (36%) in the usual way gave a mixture of the acetates (33e) and (32e) as an oil (3.7 g, 73%), b.p. 126—129 °C at 19

mmHg [column D; 100 °C; 3.1 min (64%) and 3.6 min (36%)], pyrolysis of which afforded a mixture of the olefins (28) (32%) and (25) (88%) as an oil (1.3 g, 72%), b.p. 51—52 °C at 16 mmHg. Chromatography of the mixture on silver nitrate-impregnated silica afforded the separate components.

t-5-*t-Butyl*-r-3-*methylcyclohexene* (30).—Treatment of the alcohol (35a) with toluene-*p*-sulphonyl chloride in the usual way gave the toluene-*p*-sulphonate (35c) which was stirred with potassium t-butoxide as described above to give a mixture of two components [column G; 100 °C; 28.8 min (33%) and 32.4 min (67%)] which was chromatographed on silver nitrate impregnated silica. Elution with light petroleum gave the major fraction, 5-t-butyl-1-methylcyclohexene (25) as an oil (17%), b.p. 63—67 °C (block) at 18 mmHg. Further elution gave the minor fraction, *t*-5-t-butyl-*r*-3-methylcyclohexene (30) as an oil (6%), b.p. 51—57 °C (block) at 16 mmHg (lit.,[31] 63 °C at 10 mmHg).

r-3-*t-Butyl*-c-6-*methylcyclohexene* (31) *and* 4-*t-Butyl*-1-*methylcyclohexene* (26).—Acetylation of the alcohol (35b) in the usual way afforded the corresponding acetate which was subjected to flash pyrolysis as described above to afford a mixture of two components [column G; 100 °C; 29.7 min (42%) and 33.5 min (58%)]. The major component (24%) separated by chromatography on silver nitrate-impregnated silica was 4-t-butyl-1-methylcyclohexene (26), b.p. 50—57 °C (block) at 17 mmHg (lit.,[39] 180—182 °C at 752 mmHg). The minor component (19%), similarly isolated, was the olefin (31), b.p. 62—64 °C (block) at 25 mmHg, which had spectral properties identical with those previously reported.[40,41]

t-Butylcyclohexane.—4-t-Butylcyclohexene [42] (1.0 g) in acetic acid (15 ml) was shaken with platinum oxide (0.3 g) under hydrogen at 50 lb in^{-2} for 12 h. The mixture was then filtered through Celite, and the filtrate was diluted with water (40 ml) and extracted thrice with light petroleum. The combined extracts were washed with sodium hydrogencarbonate solution and with water, dried, and distilled to afford t-butylcyclohexane (0.68 g, 67%), b.p. 69—72 °C at 15 mmHg (lit.,[43] 172 °C at 760 mmHg), homogeneous by g.l.c. (column E; 100 °C; 18.6 min).

r-1-*t-Butyl*-c-4-*methylcyclohexane* (19).—Hydrogenation of *r*-3-t-butyl-*c*-6-methylcyclohexene (31) as described above gave the cycloalkane (19), b.p. 76—82 °C (block) at 14 mmHg (lit.,[44] 189 °C at 760 mmHg), homogeneous by g.l.c. (column G; 100 °C; 26.0 min).

r-1-*t-Butyl*-c-3-*methylcyclohexane* (21).—Hydrogenation of the cycloalkene (28) as described above gave the cycloalkane (21), b.p. 77—80 °C (block) at 17 mmHg (lit.,[38] 75 °C at 20 mmHg), homogeneous by g.l.c. (column G; 100 °C; 23.0 min).

r-1-*t-Butyl*-t-3-*methylcyclohexane* (23).—Similar treatment of *t*-5-t-butyl-*r*-3-methylcyclohexene (30) gave the cycloalkane (23), b.p. 70—75 °C (block) at 15 mmHg (lit.,[26] 163—175 °C at 760 mmHg), homogeneous by g.l.c. (column G; 100 °C; 25.3 min).

r-1-*t-Butyl*-c-3,*c*-5-*dimethylcyclohexane* (20) *and* r-1-*t-Butyl*-c-3,t-5-*dimethylcyclohexane* (22).—(a) Hydrogenation of *c*-5-t-butyl-1,*r*-3-dimethylcyclohexene (27) as described above gave a mixture (76%) of the cycloalkanes (20) (89%) and (22) (11%) as an oil, b.p. 78—80 °C (block) at 20 mmHg (Found: C, 85.7; H, 14.5. $C_{12}H_{24}$ requires C, 85.6; H, 14.4%); δ_H 0.87 (9 H, s, Bu^t), 0.90 (6 H, d, *J* 9 Hz, 2 × CH_3), and 0.95—2.4 (9 H).

(b) Similar treatment of *t*-5-t-butyl-1,*r*-3-dimethylcyclohexene (30) gave a mixture (79%) (Found: C, 85.3; H, 14.5%) containing the olefins (20) (12%) and (22) (88%).

General Procedure [11] *for the Preparation and Thermolysis of Alkyl t-Butylperoxyoxalates*.—The appropriate cyclohexanol (1 mmol) was added in small portions over *ca.* 10 min to oxalyl chloride (2 mmol) under nitrogen at 0 °C. When the addition was complete the reaction mixture was allowed to warm to room temperature, excess oxalyl chloride was removed under reduced pressure, and the residue was distilled to give the chloroglyoxalate as an oil which was stored in the dark.

A solution of t-butyl hydroperoxide (0.5 mmol) and pyridine (0.5 mmol) in cyclohexane (1 ml) was kept below 0 °C during the dropwise addition of a solution of the chloroglyoxalate (0.5 mmol) in cyclohexane (1 ml). Pyridine hydrochloride began to precipitate immediately. When the addition was complete the solution was allowed to warm to room temperature and was then filtered. The residue was washed with cyclohexane (1 ml) and the combined cyclohexane solutions were placed in an ampoule, flushed with nitrogen, then sealed under nitrogen and heated at 100 °C for 2 h. The ampoule was then cooled in ice, opened, and an accurately weighed sample of an internal standard [one of the cyclohexanes (18)—(23)] was added. Each experiment was performed at least in duplicate.

Products were identified by comparison of their g.l.c. retention times with those of authentic samples on columns B, E, and G. For accurate calculation of yields molar response ratios were determined. Each analysis was performed at least in triplicate. Yields calculated by repeated analyses varied by <2%.

Mixtures were also analysed by comparison of physical and spectral properties of components separated by chromatography on silver nitrate-impregnated silica with those of authentic samples. Elution with light petroleum gave the appropriate cyclohexane. Continued elution with light petroleum gave the cyclohexene(s). When more than one cyclohexene was produced the one with the most substituted double bond eluted first. The cyclohexenes (27) and (29) formed in the reaction of the peroxide (4) could not be separated.

General Procedure [14] *for the Preparation and Thermolysis of Diacyl Peroxides*.—The appropriate carboxylic acid (5 mmol) and thionyl chloride (15 mmol) were heated under reflux for 0.5 h. Excess thionyl chloride was removed under reduced pressure and the residue was distilled to give the acid chloride as an oil.

To a suspension of sodium peroxide (1.3 mmol) in anhydrous ether (3 ml) a solution of the acid chloride (2 mmol) in ether (1 ml) was added. Reaction was initiated by adding a drop of water and was assumed to be complete when the yellow colour of the peroxide had disappeared and the addition of water no longer caused the temperature to rise. Cold water (5 ml) was then added, the ether layer was separated and washed with 10% aqueous sodium carbonate, dried ($MgSO_4$) and concentrated under reduced pressure at 0 °C. Cyclohexane (2 ml) was added to the residue and the resultant solution was placed in an ampoule, flushed with nitrogen, sealed under nitrogen, and heated at 100 °C for 2 h. The mixture was then cooled and analysed as described above.

Solvolysis of t-4-*t-Butyl*-c-2,t-6-*dimethylcyclohexyl* r-*Toluene*-p-*sulphonate* (32h).—A solution of the toluene-*p*-sulphonate (0.5 g) and anhydrous sodium acetate (0.25 g) in anhydrous acetic acid (15 ml) was heated in a sealed ampoule under nitrogen at 75 °C for 8 h, then cooled, diluted with

water (40 ml), and extracted thrice with light petroleum. The combined extracts were washed with sodium hydrogencarbonate solution and with water, dried, and concentrated. Analysis of the residue by g.l.c. (column E; 100 °C) showed it to contain *c*-5-t-butyl-1,*r*-3-dimethylcyclohexene (27) (47%) and two unidentified components [27.7 (36%) and 29.6 min (17%)].

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References

1 A. L. J. Beckwith and C. Easton, *J. Am. Chem. Soc.*, 1978, **100**, 2913.
2 A. L. J. Beckwith, C. J. Easton, and A. K. Serelis, *J. Chem. Soc., Chem. Commun.*, 1980, 482.
3 A. L. J. Beckwith, *Tetrahedron*, 1981, **37**, 3073.
4 W. C. Agosta and S. Wolff, *J. Am. Chem. Soc.*, 1976, **98**, 4316; 1977, **99**, 3355.
5 M. L. Greenlee, E. L. Fritzen, and J. S. Swenton, *J. Org. Chem.*, 1978, **43**, 4512.
6 P. Livant and R. G. Lawler, *J. Am. Chem. Soc.*, 1976, **98**, 6044.
7 R. W. Fessenden and R. H. Schuler, *J. Chem. Phys.*, 1963, **39**, 2147.
8 S. Ogawa and R. W. Fessenden, *J. Chem. Phys.*, 1964, **41**, 994.
9 R. W. Fessenden, *J. Phys. Chem.*, 1967, **71**, 74; P. R. Ayscough and C. Thomson, *Trans. Faraday Soc.*, 1962, **58**, 1477; N. Y. Cherniak, N. N. Bubnov, L. S. Poliac, Y. D. Tsvetkov, and V. V. Voevodski, *Opt. Spektrosk.*, 1959, **6**, 360; K. Leibler and H. Szwarc, *J. Chim. Phys.*, 1960, **57**, 1109; P. M. K. Leung and J. W. Hunt, *J. Phys. Chem.*, 1967, **71**, 3177; N. Y. Buben, Y. N. Molin, A. I. Pristupa, and V. N. Shamshev, *Dokl. Akad. Nauk. SSSR*, 1963, **152**, 352; T. Ohmae, S. Ohnishi, K. Kuwata, H. Sakurai, and I. Nitta, *Jpn. J. Chem.*, 1967, **40**, 226; L. Bonazzola, N. Leray, and R. Marx, *Chem. Phys. Lett.*, 1974, **24**, 88.
10 R. V. Lloyd and J. G. Causey, *J. Chem. Soc., Perkin Trans. 2*, 1981, 1143; J. C. Micheau, B. Despax, N. Paillous, A. Lattes, A. Castellano, J. P. Catteau, and A. Lablanche-Cambier, *Nouv. J. Chim.*, 1981, **5**, 257.
11 F. R. Jensen and T. I. Moder, *J. Am. Chem. Soc.*, 1975, **97**, 2281; and references cited.
12 D. Griller and B. P. Roberts, *J. Chem. Soc., Perkin Trans. 2*, 1972, 747; K. U. Ingold, B. Maillard, and J. C. Walton, *ibid.*, 1981, 970.
13 R. M. Delaney, S. Middleton, and W. F. Norfolk, *Aust. J. Chem.*, 1970, **23**, 1015.
14 H. Hart and R. A. Cipiani, *J. Am. Chem. Soc.*, 1962, **84**, 3697.
15 F. R. Jensen, L. H. Gale, and J. E. Rodgers, *J. Am. Chem. Soc.*, 1968, **90**, 5793.
16 E. L. Eliel and R. G. Haber, *J. Org. Chem.*, 1959, **24**, 143.
17 K. E. Harding and C.-Y. Tseng, *J. Org. Chem.*, 1975, **40**, 929.
18 J. Sicher, M. Tichy, and F. Sipos, *Collect. Czech. Chem. Commun.*, 1966, **31**, 2238.
19 J. Cason and J. S. Correia, *J. Org. Chem.*, 1961, **26**, 3654; H. R. Hudson, *J. Chem. Soc. B*, 1968, 664.
20 D. J. Pasto and J. A. Gontarz, *J. Am. Chem. Soc.*, 1971, **93**, 6909.
21 F. Sipos, J. Krupicka, M. Tichy, and J. Sicher, *Collect. Czech. Chem. Commun.*, 1962, **27**, 2079.
22 J. Sicher and M. Tichy, *Collect. Czech. Chem. Commun.*, 1967, **32**, 3687.
23 J. C. Dalton, K. Dawes, N. J. Turro, D. S. Weiss, J. A. Barltrop, and J. D. Coyle, *J. Am. Chem. Soc.*, 1971, **93**, 7213.
24 H. R. Billica and H. Adkins, *Org. Synth.*, 1949, **29**, 24.
25 J. Fried, N. A. Abraham, and T. S. Santhanakrishnan, *J. Am. Chem. Soc.*, 1967, **89**, 1044.
26 B. J. L. Huff, F. N. Tuller, and D. Caine, *J. Org. Chem.*, 1969, **34**, 3070; F. Johnson, L. G. Duquette, A. Whitehead, and L. C. Dorman, *Tetrahedron*, 1974, **30**, 3241.
27 L. F. Johnson and L. G. Duquette, *Chem. Commun.*, 1969, 1448.
28 W. S. Allen and S. Bernstein, *J. Am. Chem. Soc.*, 1955, **77**, 1028.
29 A. L. J. Beckwith and G. Phillipou, *Aust. J. Chem.*, 1976, **29**, 877.
30 E. L. Eliel, 'Stereochemistry of Carbon Compounds,' McGraw-Hill, New York, 1962, p. 231.
31 M. Pankova, J. Sicher, M. Tichy, and M. C. Whiting, *J. Chem. Soc. B*, 1968, 365.
32 J. B. Stothers, 'Carbon-13 NMR Spectroscopy,' Academic Press, New York, 1972; J. D. Roberts, F. J. Weigert, J. I. Kroschwitz, and H. J. Reich, *J. Am. Chem. Soc.*, 1970, **92**, 1338; J. B. Stothers and C. T. Tan, *Can. J. Chem.*, 1974, **52**, 308; S. H. Grover and J. B. Stothers, *ibid.*, 1975, **53**, 589.
33 G. E. Gream, M. H. Laffer, and A. K. Serelis, *Aust. J. Chem.*, 1978, **38**, 803.
34 H. Hart and E. A. Haglund, *J. Org. Chem.*, 1950, **15**, 396.
35 B. Rickborn and J. Quartucci, *J. Org. Chem.*, 1964, **29**, 2476.
36 K. Hultzsch, *Ber. Dtsch. Chem. Ges.*, 1941, **74**, 1539.
37 A. Bowers, T. G. Halsall, E. R. H. Jones, and A. J. Lemin, *J. Chem. Soc.*, 1953, 2548.
38 N. A. LeBel, R. F. Czaja, and A. DeBoer, *J. Org. Chem.*, 1969, **34**, 3112.
39 C. M. Rynard, C. Thankachan, and T. T. Tidwell, *J. Am. Chem. Soc.*, 1979, **101**, 1196.
40 T. Masamune, H. Matsue, and M. Fujii, *Jpn. J. Chem.*, 1972, **45**, 1812.
41 Y. Tanigawa, H. Kanamaru, A. Sonoda, and S. Murahashi, *J. Am. Chem. Soc.*, 1977, **99**, 2361.
42 J. Sicher, F. Sipos, and M. Tichy, *Collect. Czech. Chem. Commun.*, 1961, **26**, 847.
43 H. A. Smith and E. F. H. Pennekamp, *J. Am. Chem. Soc.*, 1945, **67**, 276.
44 A. L. Liberman, B. M. Lerman, G. N. Zhizhin, and Kh. E. Sterin, *Dokl. Akad. Nauk SSSR*, 1964, **156**, 375.

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Regioselective Chlorination of *N*-Benzoylvaline Methyl Ester

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Regioselective chlorination of valine derivatives establishes the chemical validity of a regiospecific hydrogen-atom abstraction proposed in penicillin biosynthesis and provides a viable synthetic method for direct and selective functionalisation of these compounds.

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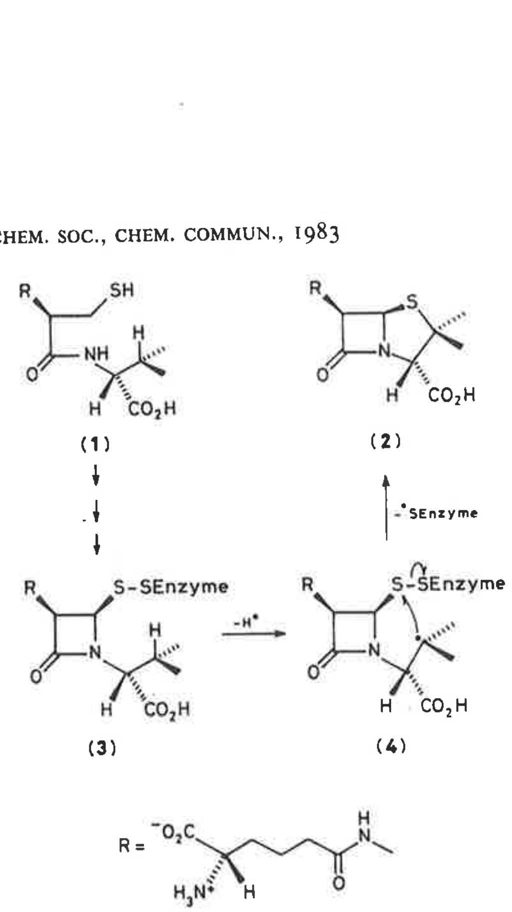
Regioselective chlorination of valine derivatives establishes the chemical validity of a regiospecific hydrogen-atom abstraction proposed in penicillin biosynthesis and provides a viable synthetic method for direct and selective functionalisation of these compounds.

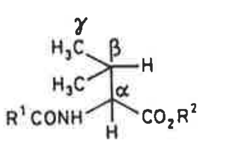
Details of the biosynthesis of penicillins and cephalosporins have not been elucidated. Oxidative cyclisation of Arnstein's tripeptide (**1**) affords isopenicillin N (**2**),[1] but the mechanism of this conversion remains unknown. On the basis of *in vitro* experiments with model compounds[2] and *in vivo* studies with labelled tripeptides,[1] a mechanism for formation of the carbon–sulphur bond has been proposed [(**4**) → (**2**)];[2,3] however, no consideration has been given to production of the radical (**4**) from (**3**), fundamental to this hypothesis. In this report we describe synthetically viable chlorinations of valine derivatives that establish the chemical validity of the hydrogen-atom transfer (**3**) → (**4**).

H.p.l.c.† of the mixture obtained when *N*-benzoylvaline methyl ester (**5a**)[4] (1 mmol), sulphuryl chloride (1.1 mmol), and benzoyl peroxide (5 mg) in dry CCl_4 (10 ml) under N_2 were

† H.p.l.c. analyses were performed on a Brownlee Laboratories OH-10A Diol column (26 cm × 4.6 mm i.d.) and a DuPont Zorbax cyanopropyl column (25 cm × 9.4 mm i.d.), using hexane–propan-2-ol (9:1) as eluant, monitoring at 220 nm. Product separations were achieved on the Zorbax column. Similar, but less efficient, separations were accomplished by chromatography on silica, eluting with ethyl acetate–dichloromethane (1:9).



Scheme 1



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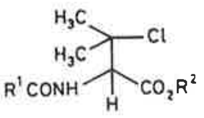

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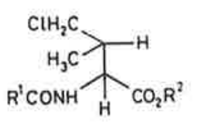

(7)

a $R^1 = Ph, R^2 = CH_3$
b $R^1 = Ph, R^2 = H$
c $R^1 = R^2 = CH_3$

heated under reflux for 0.5 h, afforded the β-chlorovaline (6a) [35—40%; oil; ^{1}H n.m.r. δ (CCl_4) 1.60 (s, 3H), 1.74 (s, 3H), 3.77 (s, 3H), 4.90 (d, *J* 9 Hz, 1H), 6.50 (br. d, *J* 9 Hz, 1H), and 7.10—8.00 (m, 5H)], identical with an authentic sample,[6] and diastereoisomers of the γ-chloro derivative (7a) [(i) 15—20%; m.p. 72—74 °C; ^{1}H n.m.r. δ (CCl_4) 1.09 (d, *J* 7 Hz, 3H), 2.50 (m, 1H), 3.50 (m, 2H), 3.80 (s, 3H), 4.95 (dd, *J* 4 and 8 Hz, 1H), 6.65 (br. d, *J* 8 Hz, 1H), and 7.30—7.90 (m, 5H), and (ii) 15—20%; m.p. 108—110 °C; ^{1}H n.m.r. δ (CCl_4) 1.14 (d, *J* 7 Hz, 3H), 2.50 (m, 1H), 3.60 (m, 2H), 3.84 (s, 3H), 5.00 (dd, *J* 5 and 9 Hz, 1H), 6.80 (br. d, *J* 9 Hz, 1H), and 7.30—8.00 (m, 5H)].‡ ^{1}H N.m.r. spectroscopy and h.p.l.c. analysis of crude mixtures at 10—50% reaction of (5a) showed ratios of (6a):(7a) (i): (7a) (ii) of *ca.* 2:1:1, and no other products were detected. More extensive reactions afforded small amounts of unidentified secondary products.

Presumably this peroxide-initiated chlorination proceeds by initial hydrogen-atom transfer, with subsequent chlorine incorporation at the site of hydrogen abstraction.[6] The lack of *N*-chlorinated product is consistent with reports that formation of acylamino radicals by hydrogen-atom loss is not a facile process,[7] and the phenyl and ester-methyl groups were unreactive as expected.[8] The absence of α-chlorinated product is surprising since the amide function in (5a) would be expected to facilitate Cα–H bond homolysis[9] and prevail over the deactivating effect of the ester group.[8] In fact, since sulphuryl chloride is a relatively random halogenating agent,[8] the lack of α-chlorinated product indicates a strong preference for abstraction of β- and γ-hydrogens.

Production of equal amounts of (6a) and (7a) indicates a 6:1 selectivity for homolysis of the Cβ–H bond, which can be attributed to the relative reactivities of the tertiary and primary hydrogens.[8] Reactions of (5a) with sulphuryl chloride in benzene, a more selective free-radical halogenating system,[8] afforded near quantitative yields of (6a) and (7a) in ratios of *ca.* 3:2. This represents a 9:1 selectivity for Cβ–H bond homolysis. Reactions of the acid (5b)[10] with sulphuryl chloride afforded complex mixtures; however, (5c)[4] afforded (6c) and (7c) in yields comparable to those of the products obtained from (5a). Again a clear preference for Cβ–H bond homolysis was observed.

To the extent that (5a) and (5c) may be considered as models of (3), these chlorinations proceeding *via* regioselective Cβ–H bond homolysis establish the chemical validity of the hydrogen-atom abstraction (3) → (4) and support the proposed mechanism for carbon–sulphur bond formation in penicillin biosynthesis shown in Scheme 1. Reactions of (5a) and (5c) with sulphuryl chloride provide a viable synthetic procedure for direct and selective β-chlorination, with relevance to the synthesis of penicillins. It should be noted that the synthesis of cephalosporins from valine derivatives requires γ-functionalisation, the other process observed in these reactions.

The authors thank Professor A. L. J. Beckwith for valuable discussions.

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‡ All new compounds gave satisfactory n.m.r., i.r., and mass spectral, and microanalytical data.

References

1 D. J. Aberhart, *Tetrahedron*, 1977, **33**, 1545; J. O'Sullivan, R. C. Bleaney, J. A. Huddleston, and E. P. Abraham, *Biochem. J.*, 1979, **184**, 421; Y. Sawada, J. E. Baldwin, P. D. Singh, N. A. Solomon, and A. L. Demain, *ibid.*, p. 465.
2 J. E. Baldwin and T. S. Wan, *J. Chem. Soc., Chem. Commun.*, 1979, 249.
3 A. L. J. Beckwith and C. J. Easton, *Tetrahedron*, in the press.
4 T. H. Applewhite, H. Waite, and C. Niemann, *J. Am. Chem. Soc.*, 1958, **80**, 1465.
5 J. Oh-Hashi and K. Harada, *Bull. Chem. Soc. Jpn.*, 1966, **39**, 2287.
6 H. C. Brown and A. B. Ash, *J. Am. Chem. Soc.*, 1955, **77**, 4019; G. A. Russell and H. C. Brown, *ibid.*, p. 4031.
7 E. Hayon, T. Ibata, N. N. Lichtin, and M. Simic, *J. Am. Chem. Soc.*, 1970, **92**, 3898; R. Livingston and H. Zeldes, *J. Chem. Phys.*, 1967, **47**, 4173.
8 M. L. Poutsma, in 'Free Radicals,' ed. J. K. Kochi, Wiley, New York, 1973, vol. 2, p. 159.
9 R. S. Neale, N. L. Marcus, and R. G. Schepers, *J. Am. Chem. Soc.*, 1966, **88**, 3051.
10 S. W. Fox and M. Winitz, *Arch. Biochem. Biophys.*, 1952, **35**, 419.

Rearrangement of an Isothiazolidinone to a β-Lactam. A Model for Penicillin Biosynthesis

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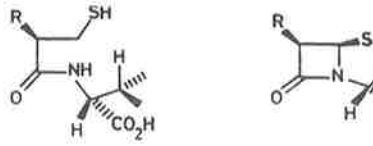
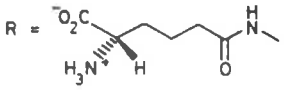
Based on novel syntheses of a β-lactam from an isothiazolidinone, a mechanism of formation of the β-lactam ring in penicillin biosynthesis is proposed.

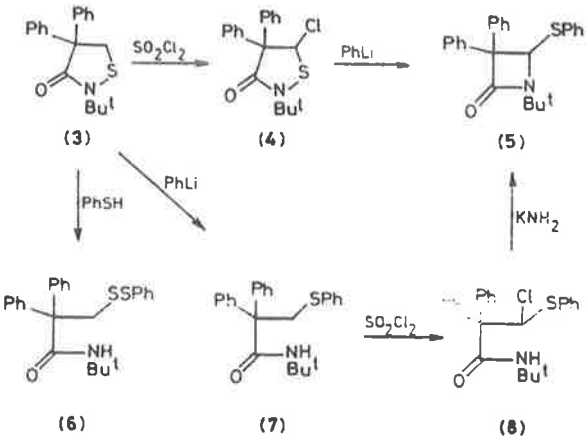
Investigation of the biosynthesis of penicillins and related β-lactam antibiotics has shown that isopenicillin N (2) is derived from the tripeptide,[δ-(L-α-aminoadipoyl)]-L-cysteinyl-D-valine (1),[1]† but the mechanism of this transformation has not been elucidated. Although isothiazolidinones have been proposed as intermediates in this conversion, attempts to prepare β-lactams from isothiazolidinones, *in vitro*, have failed to date.[2] In this report the preparation of β-lactam (5) from isothiazolidinone (3) is described and a mechanism for the formation of the β-lactam ring in penicillin biosynthesis is proposed.



(1)

(2)

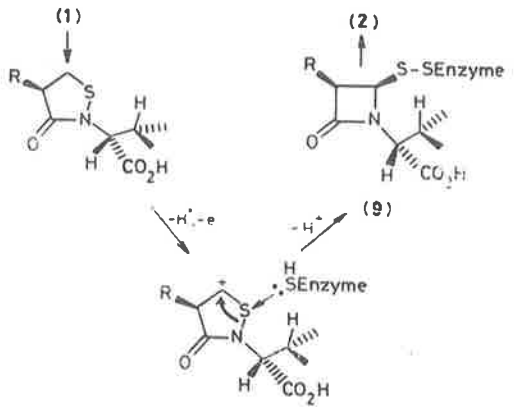




(3) (4) (5) (6) (7) (8)

† δ-(α-Aminoadipoyl) = 5-amino-5-carboxypentanoyl.

Treatment of (3)[2] with sulphuryl chloride (CCl_4, 20 °C) afforded (4)‡ [71%; m.p. 92—94 °C (decomp.); ^{1}H n.m.r. δ(CCl_4) 1.49 (s, 9H), 6.18 (s, 1H), and 7.0—7.5 (m, 10H)], which reacted with phenyl-lithium (Et_2O, −78 °C) to give the β-lactam (5) [86%; oil; ^{1}H n.m.r. δ(CCl_4) 1.42 (s, 9H), 5.53 (s, 1H), and 6.9—7.3 (m, 15H)]. Reaction of (3) with phenyl-lithium (Et_2O, −78 °C) afforded the amide (7) [82%; m.p. 80—81 °C; ^{1}H n.m.r. δ(CCl_4) 1.25 (s, 9H), 3.83 (s, 2H), 5.3 (br. s, 1H), and 6.9—7.4 (m, 15H)], identical to a sample prepared from 2,2-diphenyl-3-phenylthiopropanoic acid.[3] The chloride (8), produced by treatment of (7) with sulphuryl chloride (CCl_4, 20 °C) [60%; oil; ^{1}H n.m.r. δ(CCl_4) 1.22 (s,



Scheme 1

‡ All new compounds gave satisfactory n.m.r., i.r., and high resolution mass spectral data and, with the exceptions of (4) and (8), satisfactory microanalytical data.

9H), 5.3 (br. s, 1H), 6.22 (s, 1H), and 6.9—7.6 (m,15 H)], reacted with potassium amide (NH_3, −78 °C) to give (**5**) in 82% yield. These syntheses establish that a β-lactam can be prepared from an isothiazolidinone.

Rearrangement of the oxidized isothiazolidinone (**4**) to the β-lactam (**5**) most likely proceeds by nucleophilic attack of the phenyl anion at sulphur. An analogous mechanism could be involved in the *in vivo* transformation (**1**) → (**2**) (Scheme 1). A most attractive hypothesis is that the nucleophile promoting the biological rearrangement could be a thiol residue of the penicillin synthetase enzyme, as formation of the disulphide (**9**) is fundamental to a proposed mechanism for the formation of the thiazolidine ring.[4,5] Support for this hypothesis comes from the spontaneous reaction of (**3**) with thiophenol (CCl_4, 20 °C) to give disulphide (**6**) [94%; hard oil; ^{1}H n.m.r. δ(CCl_4) 1.23 (s, 9H), 3.75 (s, 2H), 5.1 (br. s, 1H), and 7.0—7.4 (m, 15H)].

The only alternative mechanism proposed for the formation of the β-lactam ring in (**2**),[5,6] that is consistent with all biosynthetic studies, does not rationalize formation of a disulphide. The mechanism proposed in Scheme 1 accounts for the decisive role of the thiol group in (**1**) in binding the substrate to the enzyme during penicillin biosynthesis.[7]

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References

1 D. J. Aberhart, *Tetrahedron*, 1977, **33**, 1545; J. O'Sullivan, R. C. Bleaney, J. A. Huddleston, and E. P. Abraham, *Biochem. J.*, 1979, **184**, 421; Y. Sawada, J. E. Baldwin, P. D. Singh, N. A. Solomon, and A. L. Demain, *ibid.*, p. 465.
2 J. E. Baldwin, A. L. J. Beckwith, A. P. Davis, G. Procter, and K. A. Singleton, *Tetrahedron*, 1981, **37**, 2181.
3 H. E. Zaugg, H. J. Glenn, R. J. Michaels, R. U. Schock, and L. R. Swett, *J. Am. Chem. Soc.*, 1957, **79**, 3912.
4 J. E. Baldwin and T. S. Wan, *J. Chem. Soc., Chem. Commun.*, 1979, 249.
5 A. L. J. Beckwith and C. J. Easton, *Tetrahedron*, in the press.
6 J. E. Baldwin and A. P. Davis, *J. Chem. Soc., Chem. Commun.*, 1981, 1219.
7 R. M. Adlington, R. T. Aplin, J. E. Baldwin, B. Chakravarti, L. D. Field, E. M. John, E. P. Abraham, and R. L. White, *Tetrahedron*, 1983, **39**, 1061.

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Stereoelectronic effects in free-radical reactions

Christopher J. Easton

Many free-radical reactions preferentially follow the most exothermic pathway. While this thermochemical criterium is often adequate to rationalize and even predict results of radical reactions, other factors affect the course of reaction. Conformational constraints on the geometry of reaction transition states resulting from the energy requirement of maximum electron delocalization in the transition states can dominate thermochemical effects. These stereoelectronic effects can be exploited in regio- and stereo-selective synthesis.

The branch of organic chemistry involving free radicals [atoms or groups of atoms possessing an odd (unpaired) electron] dates only to 1900 when Gomberg reported his observation of a species he believed to be the free triphenylmethyl radical. He wrote 'This work will be continued and I wish to reserve the field for myself'. The development of free-radical chemistry is testament to the numerous persons who ignored his reservation. This review deals with a particular area of free-radical chemistry – the recognition and exploitation of stereoelectronic effects.[1]

Development of free-radical chemistry resulted in a need to predict and rationalize results of reactions involving free-radical intermediates. Thermochemical criteria have been used generally in this regard, with assessment of free-radical reactions being based on the relative stabilities of reactants, products and intermediates. Yet even for relatively simple systems these criteria are often inadequate. Consider, for example, ring closure of hex-5-en-1-yl radical (1).

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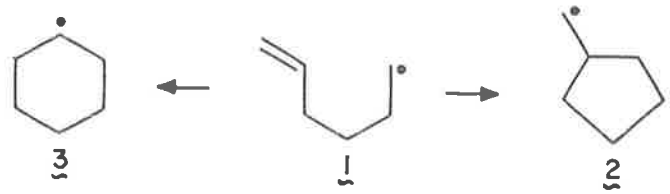


3 1 2

Intramolecular addition of the radical to the double bond could produce the primary cyclopentylmethyl radical (2) or the secondary cyclohexyl radical (3). Although the secondary radical 3 is more stable thermodynamically (the general order of stability of alkyl radicals is tertiary > secondary > primary), product ratios indicate that formation of the primary radical 2 is strongly preferred.

Stereoelectronic effects

The preferred transition state of a reaction has the lowest energy of all possible transition states and, in the absence of steric constraints, is attained by maximum delocalization of the electrons involved in the reaction. For each reaction the requirement of maximum electron delocalization is met by a particular orientation of molecular orbitals, and therefore by a particular geometry of the transition state. Results of reactions which are attributed to this restricted geometry of the transition state are termed stereoelectronic effects. A reaction that exhibits these effects is said to be stereoelectronically controlled. Stereoelectronic effects in ionic reactions are well known, but their importance in free-radical reactions has only recently been realized.

(a) *Addition to olefins*

Ring closure of hexenyl radical 1 is an example of a stereoelectronic effect in a free-radical reaction, specifically in the intramolecular addition of a radical to an olefin. The proposed transition state for addition of alkyl radicals to olefins is one formed by initial coplanar interaction of the semioccupied p orbital of the radical with the π^* antibonding orbital of the olefin (Fig. 1) because this allows maximum delocalization of the three electrons (one p, two π) involved in the redistribution process. In geometrical terms this corresponds to perpendicular attack of the radical from above or below the plane of the double bond. Studies have shown that this transition state is readily accommodated in the reaction of hexenyl radical 1 to give the *exo*-cyclic radical 2 but not on the pathway to the *endo*-cyclic radical 3. The strain energy associated with the transition state leading to the *endo*-cyclic radical 3 outweighs the normal thermodynamic preference for formation of a secondary radical. Intramolecular ring closures of many substituted alkenyl radicals exhibit similar stereoelectronic effects, with *exo*-ring closure to the thermodynamically less stable product radical being the preferred reaction.

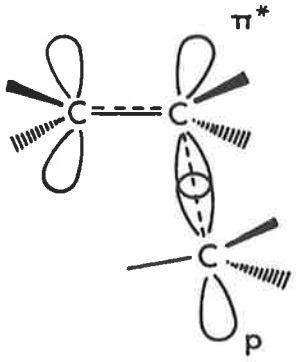
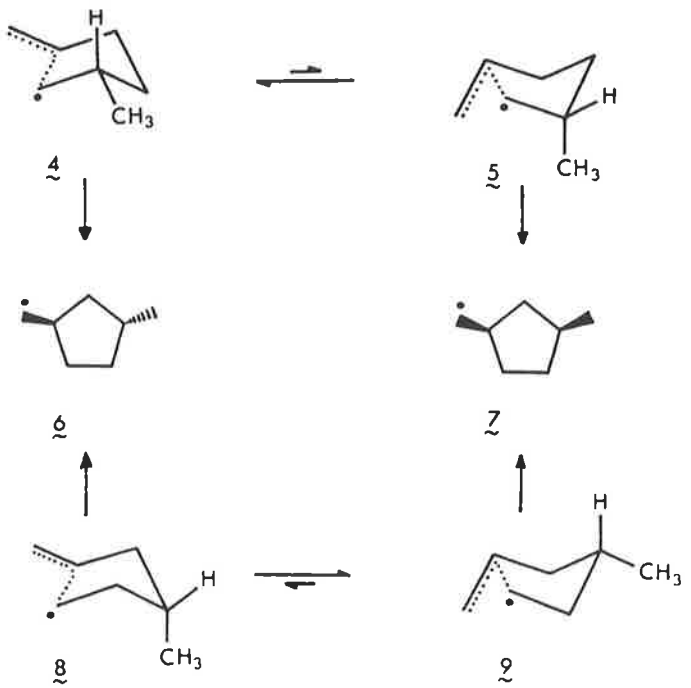


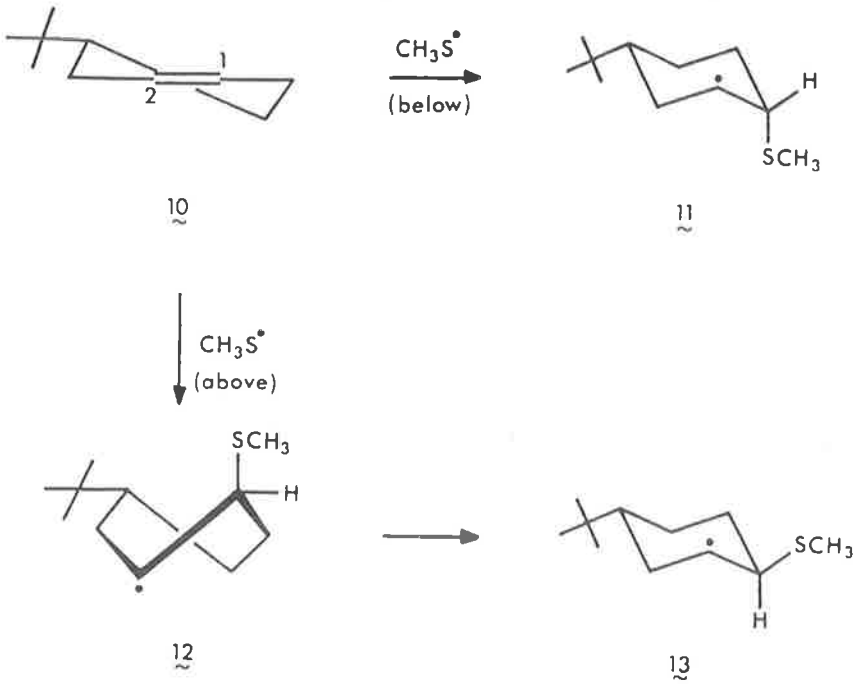
Fig. 1. Proposed transition state for addition of alkyl radicals to olefins.

The stereochemistry of products obtained from ring closure of alkenyl radicals has been rationalized by considering possible conformations of the transition state. For cyclization of hexenyl radicals the transition state resembles the chair-form of cyclohexane (e.g. 4), and for any monosubstituted hexenyl radical there will be two possible conformations of the transition state (e.g. 4 and 5), the most stable



(predominant) being that with the substituent in a pseudo-equatorial position (e.g. 4). Thus reaction of 2-methylhex-5-en-1-yl radical (4 and 5) affords predominantly the *trans*-product 6, whereas the 3-substituted radical 8 and 9 yields mostly the *cis*-product 7 despite its being of higher energy than 6.

Stereoelectronic effects are not restricted to addition of alkyl radicals to olefins, nor are they restricted to intramolecular reactions. They are exemplified in the preferred axial radical incorporation observed in intermolecular additions of thiyl radicals to conformationally rigid cyclohexenes. Consider the example of methanethiyl radical addition to 4-*tert*-butylcyclohexene (10). The transition state for this reaction should be similar to that proposed for addition of alkyl radicals to olefins. Attack of the radical perpendicular to the plane of the double bond would be energetically preferred and this could occur at either end of the double bond and from either above or below the double bond. Attack at C-1 from below the double bond will lead directly to the axially substituted cyclohexyl radical 11 having the chair conformation, while attack at C-1 from above the double bond will give initially the twist-boat radical intermediate 12 which will undergo conformational change to form 13. As 11 is of lower energy than 12 its rate of formation should be faster than that of 12 and a preference for axial addition of methanethiyl radical should result. This is indeed the case despite the fact that in the absence of any stereoelectronic effect a preference for equatorial radical incorporation would be observed. In this case both 11 and 13 would form directly with a predominance of



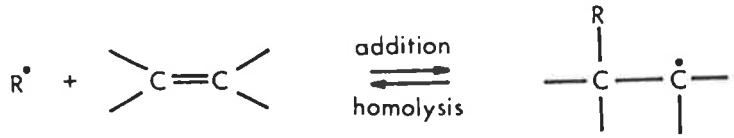
10 11 12 13

13 since it is more stable thermodynamically than 11 because of less favourable steric interactions in 11. Similar stereoelectronic arguments rationalise the preferred axial incorporation of methanethiyl radical at C-2.

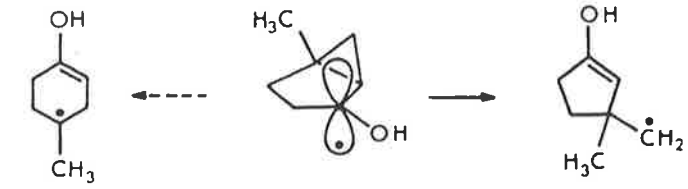
Stereoelectronic effects have also been observed in addition of aryl, vinyl and secondary and tertiary alkyl radicals, of bromine radical and of oxygen, nitrogen, phosphorus and silicon centred radicals to olefins.

(b) *Homolysis adjacent to a radical centre*

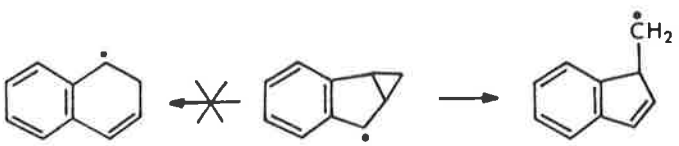
The reverse of addition of radicals to olefins is homolysis adjacent to a radical centre.



Many homolytic reactions of this type are stereoelectronically controlled. Numerous examples of C-C bond homolysis adjacent to a radical centre occurring by the thermodynamically less favourable reaction pathway have been reported. As examples, bond homolysis in the radical 14 results, almost exclusively, in a final product derived from the primary radical 15 and specific rearrangement of the radical 17 occurs to give 18. On thermodynamic grounds formation of the tertiary radical 16 should be preferred to formation of the primary radical 15 and rearrangement of 17 to the conjugatively-stabilized benzylic radical 19 should be preferred to the observed reaction. The proposed transition state for bond homolysis adjacent



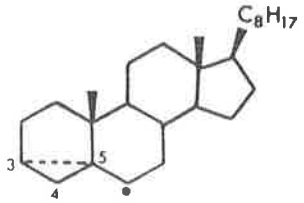
16 14 15



19 17 18

to a carbon centred radical is one formed by initial coplanar interaction of the semi-occupied p orbital of the radical with the σ^* antibonding orbital of the bond undergoing fission. Since the spatial orientation of a bond is equivalent to that of its σ^* antibonding orbital the transition state may be considered as involving initial coplanar interaction of the semioccupied p orbital with the bond undergoing fission. For both 14 and 17 the most stable conformation is that in which the semi-occupied p orbital and the bond which preferentially undergoes fission are almost coplanar. Thus rearrangements of 14 and 17 to 15 and 18 respectively can be attributed to stereoelectronic effects.

The clearest demonstration that C-C bond homoylsis adjacent to a radical centre is stereoelectronically controlled is provided by reactions of the 3α,5- and 3β,5-cyclopropyl ring. There is maximum coplanarity of the C_3-C_5 bond with the fixed spatial arrangement of the semioccupied p orbital and the bonds of the cyclopropyl ring. There is maximum coplanarity of the C_3-C_5 bond with the semioccupied p orbital in the radical 20, while there is maximum coplanarity of the C_4-C_5 bond with the semioccupied p orbital in the radical 22. The 3α,5-cyclocholestanyl radical 20 undergoes specific fission of the C_3-C_5 bond to give the cholesteryl radical 21 and the 3β,5-cyclocholestanyl radical 22 undergoes specific fission of the C_4-C_5 bond to give the thermodynamically less stable radical 23. These rearrangements provide compelling evidence that in homolytic cleavage of a C-C bond adjacent to a radical centre the bond preferentially broken is the one which can attain the maximum degree of coplanarity with the semioccupied p



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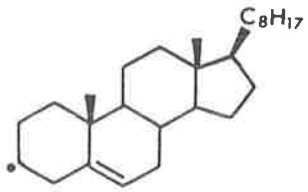

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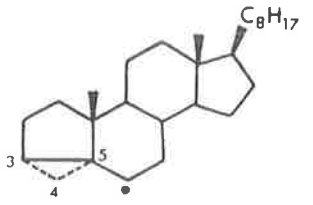

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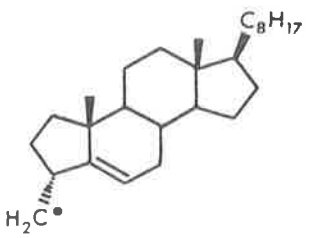


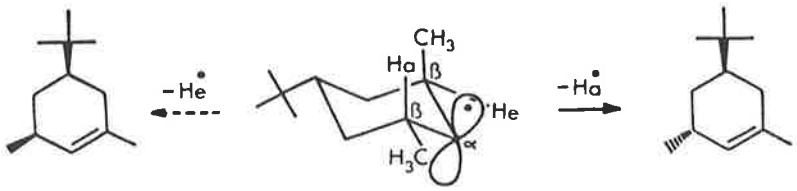
23

orbital. It is with this bond that the primary interaction involved in forming the transition state is stereoelectronically favoured.

It has also been demonstrated that C-H bond homolysis adjacent to a radical centre is stereoelectronically controlled. Deuterium labelled substrates were used to show that conversion of the biradical 24 into the olefin 25 involves preferential transfer of the pseudo-axial hydrogen atom (Ha). This was attributed to stereoelectronic control of the reaction since the axial C_β-H bond is more nearly coplanar with the semioccupied p orbital than is the equatorial C_β-H bond. More recent experiments with conformationally biased cyclohexyl radicals also showed

24 25

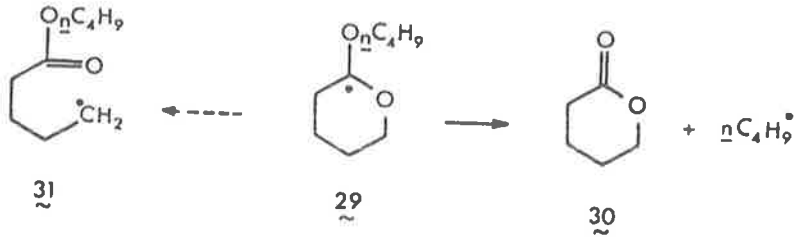
preferential homolysis of axial C_β-H bonds. Thus 26 underwent disproportionation to give the *trans*-olefin 27 as the predominant product olefin, despite the fact that disproportion to give the *cis*-olefin 28 should be favoured on thermodynamic



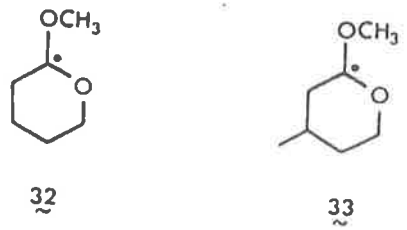
28 26 27

grounds since it would relieve non-bonded interactions of the axial methyl group in 26. Formation of the *trans*-olefin 27 involves fission of the axial C_β-H bond which is favoured on stereoelectronic grounds.

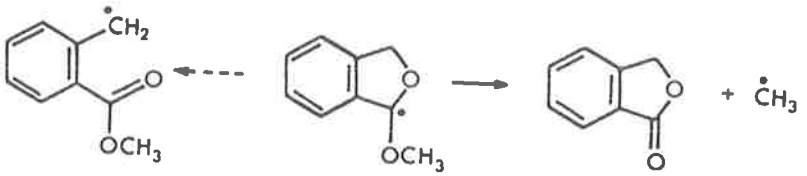
Stereoelectronic control of C-O bond homolysis adjacent to a semioccupied p orbital has been used to explain the preferred exocyclic fission of the radical 29 to form the lactone 30. The preferential exocyclic C-O bond fission of the radicals 32, 33 and 34 has been similarly rationalized. The energetically preferred coplanar interaction of the semioccupied p orbital and the σ^* antibonding orbital of the C-O bond can only occur with the exocyclic C-O bond.



31 29 30



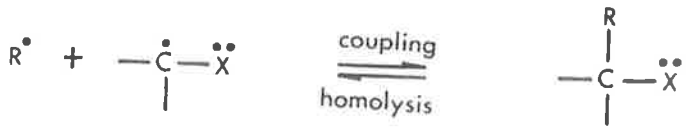
32 33



36 34 35

(c) *Coupling and homolysis adjacent to heteroatoms*

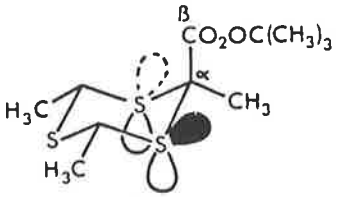
Stereoelectronic effects have also been observed in coupling and homolysis adjacent to heteroratoms.



X = S,O,N

The energetically preferred coplanar interaction of the filled non-bonding orbitals of heteroatoms with the σ^* antibonding orbital of an adjacent bond undergoing homolytic fission, or with the semioccupied p orbital of an incoming radical in a coupling reaction at the adjacent position, places geometrical constraints on the transition states of those reactions similar to the constraints described above.

The greater rate of thermolytic decomposition of the axial perester 37 as compared with its equatorial epimer 38, to the common intermediate 43, has been attributed to stereoelectronic control in C-C bond homolysis adjacent to a filled non-bonding orbital on sulphur. There is a greater degree of coplanarity of filled non-bonding orbitals on sulphur with the C_α-C_β bond undergoing fission in 37 than in 38. As was the case with homolysis adjacent to a radical centre in cyclohexyl systems, stereoelectronic control here results in preferential reactivity of axial bonds. Decomposition of each of the epimers 37 and 38 in toluene afforded similar mixtures of the products 39–42, the formation of which was attributed to reactions of the common intermediate radical 43 with solvent ($H^\cdot$ abstraction) and solvent derived benzyl radical (coupling). The high yields of 39 and 41 as compared to their respective epimers 40 and 42 were attributed to stereoelectronic control of C-C and C-H bond formation adjacent to a lone pair of electrons on sulphur. The rationalization for this is similar to that described above for axial methanethiyl radical additon to 4-*tert*-butly-cyclohexene (10): axial radical incorporation occurs directly through a chair conformation to produce 39 and 41 whereas equatorial radical incorporation to yield 40 and 42 proceeds indirectly through a high energy boat conformation.



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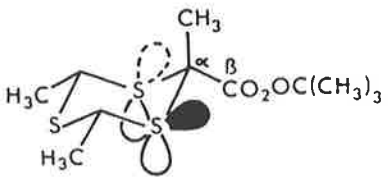

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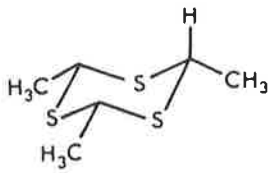

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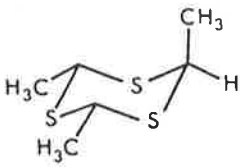

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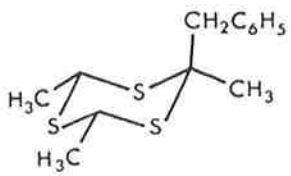

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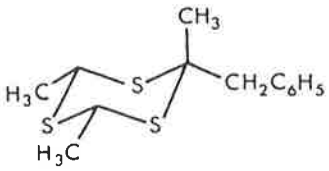

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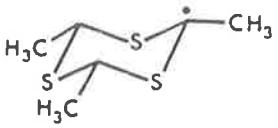
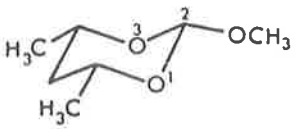


43

Similar stereoelectronic effects have been observed in C-H bond homolysis adjacent to a lone pair of electrons of oxygen. For example, the enhanced reactivity of the substituted 1,3-dioxanes 44 and 46 as compared to their respective epimers 45 and 47 towards hydrogen atom abstraction form C-2 by tert-butoxy radical is consistent with these reactions being stereoelectronically controlled. Since the preferred conformations of the dioxanes 44 and 46 are those in which the substituent at C-2 is equatorially oriented, while those of the dioxanes 45 and 47 have the substituent at C-2 axially oriented, the reactivity of these species clearly demonstrates that the axial C-H bonds at C-2 in the dioxanes 44 and 46 are more reactive than the equatorial C-H bonds at C-2 in the dioxanes 45 and 47.



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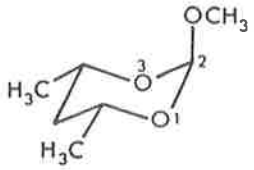

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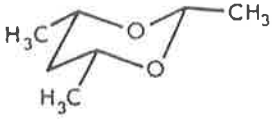

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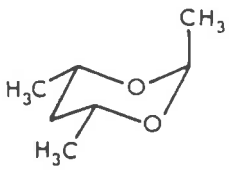

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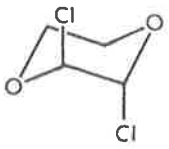

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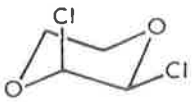
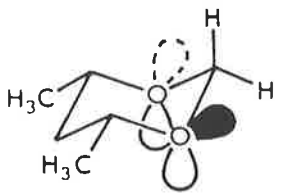
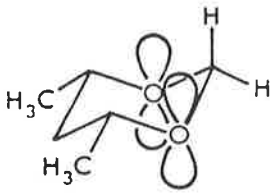


49

The greater reactivity of the dioxanes 44 and 46 as compared to their respective epimers 45 and 47 cannot be associated with the differences between the ground state energies of these compounds. Although the less stable 2-methoxy substituted dioxane 44 reacts preferentially so does the more stable 2-methyl substituted dioxane 46. Nor can the reactivity be attributed to product stability as each pair of epimers reacts to give a common product radical. It therefore seems that the enhanced reactivity of the axial C-H bonds at C-2 in the dioxanes 44 and 46 is a direct consequence of stereoelectronic control arising from favourable interactions between the bond being broken and the filled non-bonding orbitals on adjacent oxygen atoms. If, as is usually assumed, ethereal oxygen is sp^3 hydridized, then in the 1,3-dioxane system each oxygen would have one non-bonding orbital in a coplanar orientation with respect to the axial C-H bond at C-2, but there would be no such relationship between the equatorial bond at C-2 and the oxygens' non-bonding orbitals. Using this model the enhanced reactivity of the axial C-H bond at C-2 in the dioxanes 44 and 46 can be attributed to stabilization of the transition state by favourable overlap of the σ^* antibonding orbital of the bond undergoing fission and one of the non-bonding orbitals on each adjacent ring oxygen. If, on the other hand, it is assumed that the two lone pairs of electrons on oxygen are non-equivalent, with one in an essentially pure p-type orbital and the other in an s-type orbital, then in the 1,3-dioxane system the axial C-H bond at C-2 has a much greater degree of coplanarity with the adjacent oxygens' p-type non-bonding orbitals than does the equatorial C-H bond.



sp^3

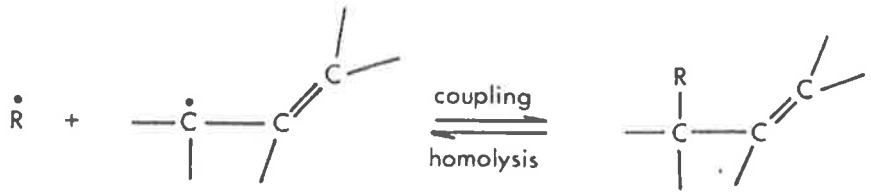


1s,1p

From studies of the reversible hydrogen atom transfer from the dioxanes 44 and 45 it has been concluded that C-H bond formation adjacent to a filled non-bonding orbital on oxygen is stereoelectronically controlled. Stereoelectronic control has also been observed in C-H bond cleavage adjacent to a lone pair of electrons on nitrogen, and in C-Cl bond homolysis adjacent to a lone pair of electrons on oxygen. Thus 48 undergoes C-Cl bond fission almost twice as rapidly as does 49, despite the fact that 49 is less stable thermodynamically than 48 and reaction of each compound gives the same product radical.

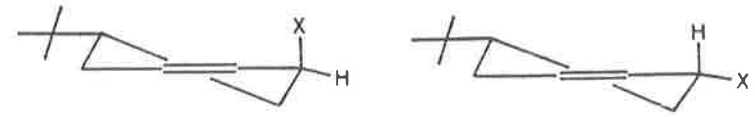
(d) *Coupling and homolysis adjacent to olefins*

Stereoelectronic effects in coupling and homolysis adjacent to olefins result from similar interactions to those described above in section (c) except that whereas



the interactions in section (c) are with filled non-bonding orbital of a heteroatom, here it is with the π orbitals of the olefin. Stereoelectronically controlled C-H bond homolysis is observed in reactions of the conformationally fixed methylenecyclohexanes 50–54 with *tert*-butoxy radical. The relative rates of reaction of these olefins are closely related to the number of axial C_β-H bonds in each species (Fig. 2). This is attributed to preferential homolysis of axial C_β-H bonds because of favourable stereoelectronic interactions as described above. This reactivity is the reverse of that expected on thermodynamic grounds; homolysis of equatorial C_β-H bonds in 52 and 54 would relieve unfavourable steric interactions.

The observation that allylic free-radical halogenation of 4-*tert*-butylcyclohexene (10) affords mainly the pseudoaxial halide 55, and that this compound undergoes C-X bond homolysis much more readily than does the equatorial epimer 56, indicates that C-X bond formation and homolysis adjacent to an olefin is stereoelectronically controlled.

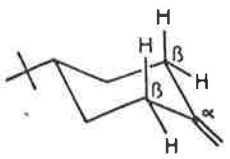


X = Cl, Br

55 56

(e) *Regio- and stereo-selective synthesis*

The predictive utility of stereoelectronic effects in free-radical reactions is demonstrated by their exploitation in synthesis. Stereoelectronic control of vinyl radical addition to olefins was utilized by Stork in the synthesis of the methyleneindanol 58 by ring closure of the radical 57.[2] Hart has used ring closure of the radical 59 in the synthesis of (±)-heliotridane (61) *via* 60.[3] Buchi used the chloride 62 as a radical precursor in the preparation of 63, a precursor of the fragrant dihydroagarofuran 64.[4] The furan 64 was also obtained directly, but as a mixture with 65, by ring closure of 66.



	k_{rel}	k_{rel}/axial β-hydrogen
50	1·00	0·50
51	1·22	0·61
52	0·62	0·62
53	1·28	0·64
54	0·50	0·50

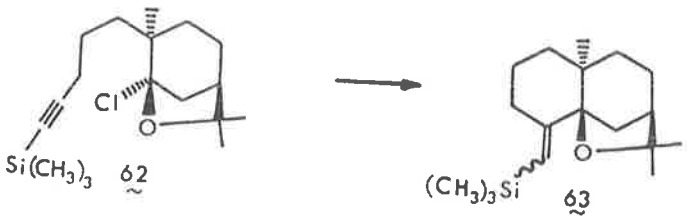
Fig. 2. Relative reactivities of methylenecyclohexanes 50–54 to *tert*-butoxy radical at 80°.

These examples of regio- and stereo-selective synthesis illustrate the use of free-radical reactions which occur under stereoelectronic control to give the thermodynamically less stable of the possible cyclic products. Although each example involves radical addition to a multiple bond as discussed in section (a) of this review, undoubtedly stereoelectronic control of other types of free-radical reactions will also be applied in synthesis.

57 58

59

60 X = O
61 X = H_2



62 63

66

64 $R_1 = CH_3$, $R_2 = H$
65 $R_1 = H$, $R_2 = CH_3$

Conclusion

A considerable but by no means comprehensive list of stereoelectronic effects in free-radical reactions has been discussed. Clearly, stereoelectronic effects are important and must be considered in conjunction with thermochemical criteria when predicting and/or rationalizing the outcome of free-radical reactions. In

synthesis design stereoelectronic control may be utilized particularly in the synthesis of thermodynamically disfavoured products.

Acknowledgment

The author gratefully acknowledges the considerable assistance, advice and encouragement of Professor A. L. J. Beckwith, and the help of colleagues at the University of Canterbury in the preparation of this manuscript.

References

1. For advanced texts the interested reader is referred to: Beckwith A.L.J., Easton C.J. & Serelis A.K. (1980) *J. Chem. Soc., Chem. Commun.* 482; Beckwith A.L.J. (1981) *Tetrahedron* 37, 3073; Easton C.J. (1980) Ph.D. thesis, University of Adelaide.
2. Stork G. & Baine N.H. (1982) *J. Am. Chem. Soc.* 104, 2321.
3. Hart D.J. & Tsai Y.M. (1982) *J. Am. Chem. Soc.* 104, 1430.
4. Buchi G. & Wuest H. (1979) *J. Org. Chem.* 44, 546.

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FORMATION OF β-LACTAMS FROM 3-PHENYLTHIOPROPIONAMIDE DERIVATIVES

A POSSIBLE MODEL FOR PENICILLIN BIOSYNTHESIS

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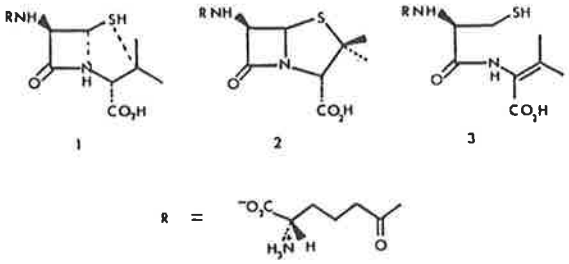
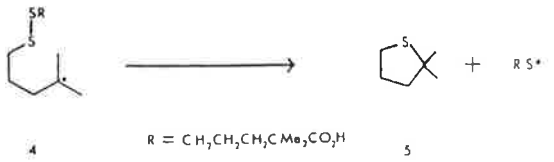
Abstract—The Cu-catalysed reaction of the substituted 3-phenylthiopropianamide (**10a**) with di-t-butyl peroxide gives the β-lactam (**14a**) *via* oxidative cyclisation of the α-thioalkyl radical (**11a**). Similar reactions of the propionamides (**10a**, **10b**) with t-butyl perbenzoate give benzoates (**15**, **17**) which can be readily converted into the β-lactams (**14a**, **14b**), but neither β-lactams nor benzoates can be obtained from the thiazepines (**23a**, **23b**). Dimethyl disulfide is benzoyloxylated on treatment with t-butyl perbenzoate. The relevance of these results to penicillin biosynthesis is discussed.

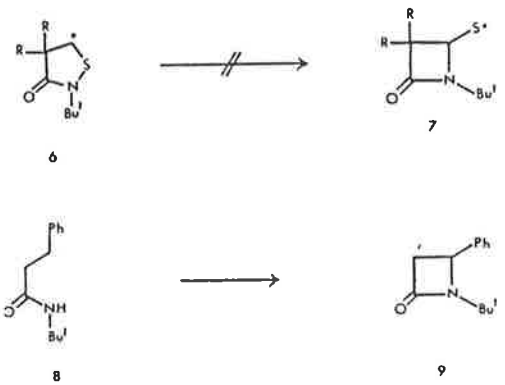
Despite intensive investigation[1] the intimate details of the biosynthesis of penicillin and related β-lactam antibiotics have not yet been elucidated. Although it is generally accepted that the Arnstein tripeptide, δ (L-α-aminoadipyl)-L-cysteinyl-D-valine (**1**), is a precursor of isopenicillin N (**2**),[1–3] the mechanism of the conversion *in vivo* of the former into the latter remains obscure. Labelling studies[4] and related experiments[5] have revealed that the ring closures affording the β-lactam and thiazolidine moieties occur with retention of configuration at the appropriate C atoms, and do not involve the intermediacy of unsaturated compounds (e.g. **3**). Most attempts to detect stable intermediates *in vivo* on the pathway from **1** to **2** have been unsuccessful and evidence suggesting that C–N bond formation precedes C–S bond formation[3] has not been confirmed.[6]



Each of the ring closures involved in the conversion of **1** into **2** requires the loss of two H atoms. The suggestion[7,8] that these oxidative cyclisations are homolytic processes seems plausible in the light of evidence that enzymatic hydroxylation[9] and other biological oxidations[10] proceed *via* free-radical intermediates. The fact that the penicillin synthetase enzyme is dependent on Fe^{2+} and oxygen[1] gives further credence to this hypothesis.

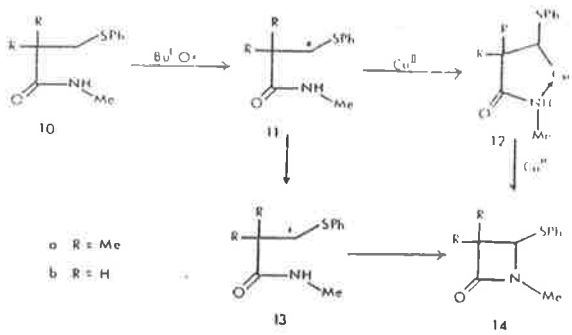


An adequate model (**4**→**5**) for formation of the thiazolidine ring of penicillin by an intramolecular S_H2 reaction of a carbon centred radical with an S–S bond has already been described.[9] Attempts to provide *in vitro* examples of free-radical processes leading to β-lactams by C–N bond formation have been somewhat less successful. Appropriate model experiments[8] showed that the putative radical rearrangement (**6**→**7**) does not occur. However, treatment of the amide (**8**) with di-t-butyl peroxide and a Cu catalyst, reagents expected to generate radical intermediates,[11] gave the lactam (**9**), albeit in very small yield.[12] In the present work, which was independently conceived and initiated, we have conducted similar experiments with suitable S containing substrates, and have shown that arylthio-substituted β-lactams can be generated either directly or indirectly from appropriate acyclic amides (e.g. **10a**) but not from thiazepines (e.g. **23a**).



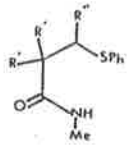
Our experiments were based on the knowledge that α-thioalkyl radicals (e.g. **11a**) can be readily generated by H-atom transfer from the corresponding sulfide (e.g. **10a**) to t-butoxy radicals,[13–15] and on the hypothesis that interaction of such radicals (**11a**) with cupric species should afford the β-lactam (**14a**) either by intramolecular ligand transfer involving intermediates such as **12a**, or *via* the cation (**13a**) generated by electron transfer. It is noteworthy that the Cu-catalysed reactions of simple dialkyl sulfides with t-butyl perbenzoate afford moderate

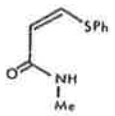
yields of α-benzoyloxy sulfides.[11] However, the mechanistic details of such reactions have not yet been elucidated.



RESULTS AND DISCUSSION

The propionamide derivative (**10a**), chosen as substrate for our initial experiments because the presence of the two Me substituents precludes side reactions involving deprotonation at C-2, was readily prepared from chloropivalic acid by reaction with thiophenol and potassium fluoride in dimethylformamide followed by amide formation. When **10a** was heated for 6 hr in boiling benzene with t-butyl perbenzoate and a catalytic amount of cuprous bromide, the major product (71%) was the benzoate (**15**) but the required β-lactam (**14a**) was also obtained in small yield. In the absence of cuprous bromide the reaction proceeded more slowly and after 18 hr afforded only the benzoate (**15**; 37%) and starting material (54%). No β-lactam (**14a**) was detected.





15 R' = Me, R" = OCOPh
16 R' = Me, R" = Br
17 R' = H, R" = OCOPh
18 R' = H, R" = Br

19

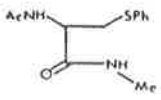
The β-lactam (**14a**) was obtained in good yield when the benzoate (**15**) was treated consecutively with hydrogen bromide and with potassium amide in liquid ammonia. This route to **14a** involves formation of the bromo-compound (**16**) followed, presumably, by intramolecular nucleophilic displacement of bromide.

The predominant formation of the benzoate (**15**) in the Cu-catalysed reaction of **11a** with t-butyl perbenzoate suggests that the benzoate ion competes effectively with the amide function either as a nucleophile towards the positive centre of **13a** or as a ligand to copper in ligand transfer reactions of **11a**. Accordingly, the reaction was repeated with di-t-butyl peroxide as the source of t-butoxy radicals. As expected (because di-t-butyl peroxide is less susceptible than t-butyl perbenzoate towards reduction by cuprous species) this reaction was much slower and a higher temperature was required. At low conversion (38%) only the β-lactam (**14a**) was formed in 18% yield (47%, based on unrecovered starting material). However, attempts to improve the yield by increasing the conversion afforded complex mixtures, presumably because **14a** is more susceptible than the starting material (**10a**) towards attack by t-butoxy radicals.[16] Similar yields of **14a** were obtained when the reaction was conducted at ambient temperature under UV irradiation.

Heating of the amide (**10b**) bearing no substituents at C-2 with t-butyl perbenzoate and cuprous bromide afforded the benzoate (**17**) in good yield (>70%). Some starting material was recovered but neither the β-lactam (**14b**) nor the unsaturated amide (**19**) could be detected. Treatment of the benzoate (**17**), first with hydrogen bromide, then with potassium amide, gave **14b**, an authentic specimen of which was prepared by an unambiguous route. Interestingly, the reaction involving potassium amide gave no detectable amount of the unsaturated amide (**19**). Presumably, this indicates that deprotonation of the bromoamide (**18**) with strong base is confined to the amide function, and that the resultant ion undergoes very rapid ring closure by intramolecular nucleophilic displacement.

When the amide (**10b**) was treated with di-t-butyl peroxide and cupric bromide in naphthalene at 130° no β-lactam (**14b**) could be detected. The only product isolated was the unsaturated amide (**19**) which was assigned the *cis* configuration because of the magnitude of the vicinal coupling of the olefinic protons.[8]† Similarly, UV irradiation of **10b** with cupric bromide in di-t-butyl peroxide gave only the unsaturated amide (**19**).

Attempts to generate a β-lactam from the acetylamino compound (**20**), a good analogue of the Arnstein tripeptide (**1**), were unsuccessful. Treatment of **20** in the usual way with di-t-butyl peroxide or t-butyl perbenzoate and a Cu salt gave complex mixtures in which neither β-lactam nor benzoyloxylated products could be detected. However, when the reaction of **20** with t-butyl perbenzoate and cuprous bromide was run to low conversion it gave a single product which was isolated in slightly impure form. Its spectral properties support its formulation as the acrylamide derivative (**22**) formed, presumably, by loss of phenylthio radical from the radical (**21**) generated by attack of t-butoxy radical at C-2 of **20**.



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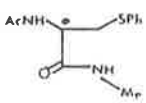

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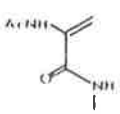

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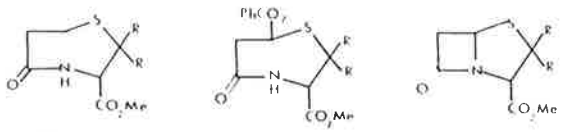
In an attempt to mimic the generation of the β-lactam ring in a hypothetical biosynthetic process involving C–S bond formation prior to C–N bond formation, the thiazepines (**23a** and **23b**) were treated in the usual way with di-t-butyl peroxide or t-butyl perbenzoate. Both compounds were considerably less reactive than the acyclic sulfides (**10a** and **10b**) towards H-abstraction by t-butoxy radicals. Thus, when either was heated with 2.5 molar equiva of t-butyl perbenzoate and cuprous bromide in benzene until <10% of the perester remained, the thiazepine could be recovered in >90% yield. The major product was methyl benzoate. This indicates that β-fission of t-butoxy radicals, $Bu^tO\cdot \rightarrow MeCOMe + Me\cdot$, competes effectively with H-abstraction from **23a** or **23b**. We are unable to provide an

†In the absence of the other double bond isomer this assignment is based on tenuous evidence.

explanation for the low reactivity of the thiazepines towards attack by t-butoxy radicals, since inspection of models reveals no steric or stereo-electronic inhibition of the reaction. A quantitative examination of reactivity in these and related systems appears to be warranted.



23 24 25

a R = H
b R = Me

When either of the thiazepines **23a** or **23b** was heated, or irradiated with UV light, in the presence of a very large excess of di-t-butyl peroxide or t-butyl perbenzoate and a Cu salt catalyst, a complex mixture of products was formed which contained neither the benzoate (**24**) nor the β-lactam (**25**), an authentic sample of which was prepared from 6-aminopenicillanic acid. However, from reactions of the thiazepine (**23a**) it was possible to isolate small amounts of the two olefins (**26** and **27**).

26 27

Finally, we examined the reaction of dimethyl disulfide, MeSSMe, with t-butyl perbenzoate. The reaction in the absence of a Cu salt proceeded slowly in boiling benzene and afforded the benzoate, $PhCO_2CH_2SSMe$, in small yield (1.5%). Addition of cuprous bromide had little effect upon either the rate or the outcome of the reaction. Treatment of the benzoate with hydrogen bromide in the usual way gave the bromosulfide, $BrCH_2SMe$, after chromatography of the crude product. However, NMR examination of the mixture indicated that the primary product is the bromo-disulfide, $BrCH_2SMe$, which undergoes facile rearrangement during the course of the reaction and subsequent work-up.

Mechanism

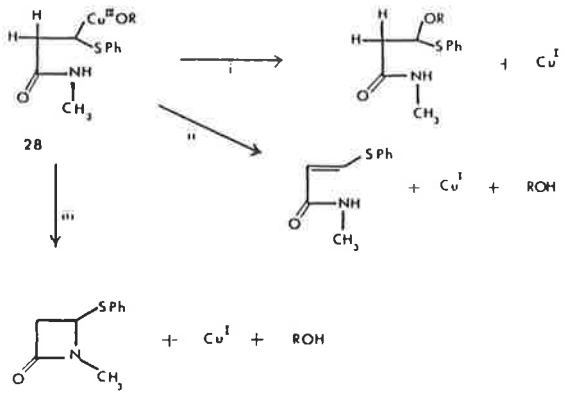
Most of the results described above accord well with previous observations[13,14] that H-atom abstraction by t-butoxy radicals from thioethers occurs preferentially at positions adjacent to the S atom, presumably because of the stabilisation of the resultant radicals through interaction of the unpaired electron with sulphur lone pairs. The only substrate which did not conform to this pattern was that **20** in which H-atom abstraction from the position adjacent to both N and C=O affords a capto-dative radical[17] (**21**) stabilised by extended conjugation over both groups.

There is no firm information available concerning the mechanisms of the reactions whereby the α-thioalkyl radicals generated in the first step are converted into the final products. It has been suggested[16] that outer-sphere one-electron oxidation by cupric species affords carbocations (e.g. **13**) which then undergo coupling with suitable nucleophiles. However, if such a mechanism applies to all our substrates it is difficult to see why deprotonation to afford the unsaturated compound, a process which should be favoured for carbocations containing a β-amido function (e.g. **13b**), occurs in some cases, but not in others. For example the Cu-catalysed reaction of **10b** with di-t-butyl peroxide gave only the unsaturated amide (**19**), but the latter (**19**) could not be detected when t-butyl perbenzoate was used. Conversely, the thiazepine (**23a**) when treated with the perester and a Cu salt gave only the olefinic compounds (**26** and **27**).

It is well known that some radicals undergo direct ligand transfer with suitable cupric species.[18] Thus, the formation of benzoates in our experiments could be envizaged as involving S_H2 attack of the radical on cupric benzoate:

$$-\dot{S}CHR + Cu^{II}OCOPh \rightarrow -SCHROCOPh + Cu^{I}.$$

However, the formation of the β-lactam (**14a**) by a similar intramolecular process would require *either* that t-butoxy radicals react only with those substrate molecules already complexed to Cu, *or* that the radical (**11a**) forms a Cu-amide complex more rapidly than it undergoes any other reaction. We consider both hypotheses to be unlikely.



28

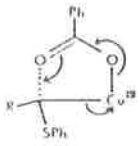
Simple alkyl radicals react with cupric species by coupling to give organo-Cu (III) complexes.[18,19] Our results can be accomodated by mechanisms involving the intermediacy of such species. The putative Cu(III) intermediate (**28**) generated from **11a** or **11b** should be potentially capable of undergoing three distinct reactions: (i) displacement of Cu by the RO (R=Bu^t or PhCO) group either by intermolecular nucleophilic substitution,

$$RO^- + R'Cu^{III}OR \rightarrow ROR' + Cu^{I} + OR^-,$$

or by intramolecular ligand transfer, $R'Cu^{III}OR \rightarrow R'OR + Cu^{I}$; (ii) deprotonation and elimination to afford an olefin; (iii) intramolecular displacement of Cu to give the lactam (**14a**), either by direct nucleophilic attack or *via* formation of the cyclic complex **12a**. The relative rates of theses three competing processes should depend in a predictable manner on the nature of the substrate and the reaction conditions.

When benzoate is present, i.e when t-butyl perbenzoate is employed, reactions of type (i) are favoured both because benzoate ion is a relatively good nucleophile and because cyclic mechanisms (e.g. **29**) are available.

In reactions involving di-t-butyl peroxide the intermediate (**28**, R=Bu^t) preferentially undergoes reaction (ii)



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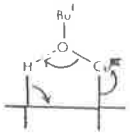

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presumably because t-butoxide is a strong protic base or because of the accessibility of a cyclic process (e.g. **30**). We prefer the latter since the formation of β-lactams (e.g. **14b**) by direct treatment of bromo-amides (e.g. **18**) with strong base suggests that the intermediate (**28**) should undergo preferential deprotonation at the amide function.

The ring-closure (iii) appears to be the slowest of all the processes available to the Cu(III) complexes in our reactions, presumably because of the strain-energy engendered in the formation of the β-lactam ring. It is important, therefore, only when benzoate is absent and when deprotonation is prevented by substitution of the position adjacent to the CO group.

Previous workers[20] have suggested that reactions of thioethers with diacyl peroxides or peresters might involve radical and/or ionic intermediates. The fact that we were unable to isolate products usually associated with the ionic route from uncatalysed perester reactions suggests that formation of benzoyloxylated products (e.g. **17** from **10b**) involves radical intermediates. The chain propagation step could proceed either by an S_Hi mechanism,

$$ArS\dot{C}HR + Bu^tOOCOPh \rightarrow ArSCHROCOPh + Bu^tO^{\cdot},$$

or by electron transfer,

$$ArS\dot{C}HR + Bu^tOOCOPh \rightarrow ArS\overset{+}{C}HR + Bu^tO^{\cdot} + PhCO_2^{-}.$$

We prefer the former since the latter would be expected to afford some unsaturated products.

CONCLUSION

Our experiments show that suitable β-arylthio-alkanecarboxamides can be converted directly into β-lactams by treatment with di-t-butyl peroxide and a Cu salt catalyst. The same transformation can be effected in better overall yield and greater generality by the indirect route involving consecutive treatment with hydrogen bromide and with sodium amide of the benzoates obtained when t-butyl perbenozoate is employed as the radical precursor. Both routes offer scope for synthetic exploitation.

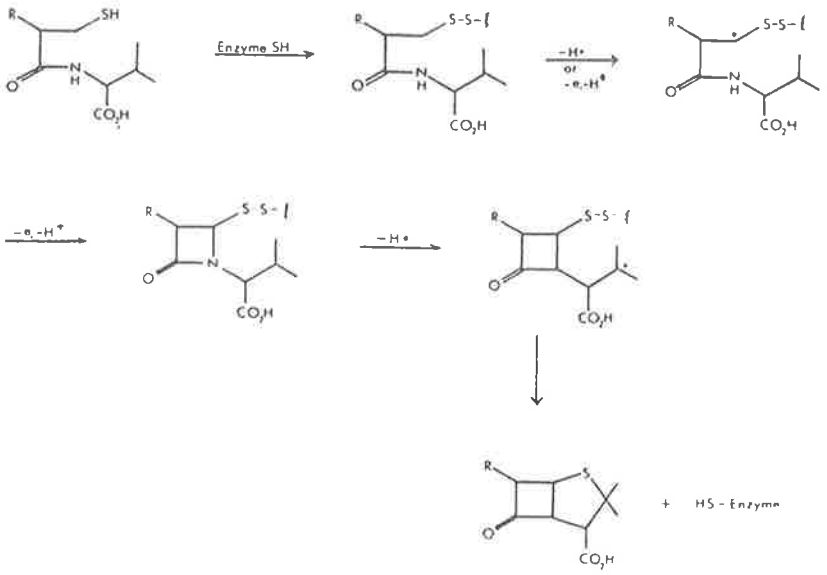
The observation that β-lactams can be generated by a direct oxidative cyclisation of suitable amides gives further credence to the suggestion[8,12] that the biosynthesis of β-lactam antibiotics might involve radical intermediates. Indeed, it now appears that the biosynthetic pathway set out in Scheme 1 is consistent with all the evidence available from labelling and other biosynthetic studies, and involves plausible oxidative ring closures for which appropriate *in vitro* models are available.

EXPERIMENTAL

M.ps were measured on a Reichert hot-stage melting apparatus and are uncorrected. Microanalyses were performed by the Australian National University Microanalytical Unit. IR spectra of neat liquids or of nujol mulls of solids were measured on a Perkin–Elmer 683 spectrophotometer. NMR spectra were measured relative to TMS as internal standard on a Jeol Minimar spectrometer operating at 100 MHz or on a Joel JNM–PMX 60 spectrometer operating at 60 MHz. Mass spectra were measured at 70 eV on an AEI MS902 spectrometer. Accurate mass measurements were carried out with heptacosane as a reference compound. Distillations were carried out on a Buchi GKR-50 glass oven and b.ps are the temperatures required for distillation. UV irradiation was achieved with a G.E.C. 250-W mercury lamp. Solvents were purified by standard procedures. Light petroleum refers to the fraction b.p. 40–60°. Flash chromatography was performed on Merck Kieselgel 60 (0.04–0.063 mm).

2,2-*Dimethyl*-3-(*phenylthio*)*propionic acid*

A mixture of 3-chloro-2,2-dimethylpropionic acid[21] (2.73 g, 20.0 mmol), KF (1.36 g, 23.4 mmol) and thiophenol (4.44 g, 40 mmol) was heated in dimethylformamide (20 ml) under N_2 at 120–130° for 12 hr, then poured into cold water (100 ml). The resultant ppt was dissolved in 1N NaOH, washed with CH_2Cl_2, and reprecipitated with dil HCl. Crystallisation from aqueous EtOH afforded colourless needles of 2,2-dimethyl-3-(phenylthio)propionic acid (3.02 g, 72%), m.p. 116–118° (lit.[22] m.p. 116–117°).



N-2,2-Trimethyl-3-(phenylthio)propionamide (**10a**)

A mixture of 2,2-dimethyl-3-(phenylthio)propionic acid (3.00 g, 14.4 mmol) and $SOCl_2$ (1.25 ml) was heated at 80° for 2 hr then dissolved in CH_2Cl_2 (30 ml) and added dropwise over 1 hr to a soln of 40% NH_2MeAq (30 ml) at −5–−10°. After the mixture had been stirred for 2 hr at 0°, water (30 ml) was added, and the organic layer was separated, washed with water, and with 5N H_2SO_4 and water, then dried and concentrated. Crystallisation of the residual oil from EtOAc-light petroleum gave N-2,2-*trimethyl*-3-(*phenylthio*)*propionamide* **10a** (2.46 g, 77%) as needles, m.p. 61.5–62.0°. (Found: C, 64.24; H, 7.55; N, 6.49; S, 14.12. $C_{12}H_{17}NOS$ requires: C, 64.54; H, 7.67; N, 6.27; S, 14.36%). δ ($CDCl_3$) 1.30 [6H, s, $C(CH_3)_2$], 2.70 (3H, d, *J* 5 Hz, NCH_3), 3.15 (2H, s, CH_2), 6.0 (1H, b, NH), 7.1–7.5 (5H, m, Ar), ν_{max} 3342, 1643, 1555 cm^{-1}, *m/e* 223 (M^+, 70%), 165 (13%), 123 (88%), 114 (100%).

The reactions of N-2,2-*trimethyl*-3-(*phenylthio*)*propionamide* (**10a**) *with t-butyl perbenzoate*

(a) A mixture of **10a** (2.23 g, 0.01 mol), t-butyl perbenzoate (2.13 g, 0.012 mol), cuprous bromide (*ca* 10 mg) and dry benzene (50 ml) under N_2 was heated under reflux for 6 hr, then cooled, washed with $Na_2S_2O_5$ aq and water, dried ($MgSO_4$), and concentrated. Flash chromatography of the residue in EtOAc-CH_2Cl_2 (20:80) afforded an oil which was distilled to give 1,3,3-*trimethyl*-4-*phenylthioazetidin*-2-*one* **14a** (86 mg, 4%), b.p. 65–70° at 0.2 mm Hg (GKR). δ ($CDCl_3$) 1.33 (3H, s, C–CH_3), 1.37 (3H, s, C–CH_3), 2.76 (3H, s, NCH_3), 4.64 (1H, s, CH), 7.1–7.5 (5H, m, Ar), ν_{max} 1752 cm^{-1}, *m/e* 221 (M^+, 5%), 164 (M^+–CH_3NCO, 9%), 112 (M^+–SC_6H_5, 100%), *m/e* 22.0879 (M^+) (calc for $C_{12}H_{15}NOS$ (M^+) *m/e* 221.0874).

Continued elution of the column afforded 3-*benzoyloxy*-N-2,2-*trimethyl*-3-(*phenylthio-propionamide* (**15**) which crystallised from EtOH–light petroleum as colourless plates (2.42 g, 71%), m.p. 134–136° dec. δ ($CDCl_3$) 1.44 [6H, s, $C(CH_3)_2$], 2.72 (3H, d, *J* 5 Hz, NCH_3), 6.0 (1H, b, NH), 6.52 (1H, s, CH), 7.0–8.0 (10H, m, Ar), ν_{max} 3357, 1717, 1633, 1538 cm^{-1}, *m/e* 343 (M^+, 1%), 234 (M^+–SC_6H_5, 20%), 222 (M^+–$OCOC_6H_5$, 2%), 105 (C_6H_5CO, 100%), *m/e* 343.1228 (M^+) (Calc for $C_{19}H_{21}NO_3S$ (M^+) *m/e* 343.1242). Further elution of the column afforded the starting amide **10a** (0.17 g, 8%).

(b) When the amide (**10a**) and the perester were heated without a Cu salt in benzene under reflux for 18 hr **15** (37%) was isolated and **10a** (54%) was recovered. The lactam (**14a**) could not be detected by TLC.

Preparation of 1,3,3-*trimethyl*-4-*phenylthioazetidin*-2-*one* (**14a**) *from* **15**

A soln of **15** (0.45 g, 1.3 mmol) in dry CH_2Cl_2 (10 ml) was added dropwise with stirring to an ice-cold soln of dry HBr in CH_2Cl_2 (50 ml). After the addition the soln was allowed to stand for 2 hr at 0–5°. Subsequent removal of the solvent afforded a pale yellow solid which was dissolved in CH_2Cl_2 (10 ml) and cooled to −78°, then added dropwise to a soln of potassium amide at −78° prepared from ammonia (50 ml), K (65 mg, 1.7 mmol) and ferric nitrate (*ca* 2 mg). After the mixture had been kept for 1 hr at −78° ammonia was removed under reduced pressure, CH_2Cl_2 (25 ml) and water (25 ml) were added, and the organic layer was separated, washed with water, dried ($MgSO_4$) and concentrated. Distillation of the residue gave **14a** as a colourless oil (0.21 g, 73%), b.p. 65° at 0.5 mm Hg (GKR).

The reaction of N-2,2-*trimethyl*-3-*phenylthiopropionamide* (**10a**) *with di-t-butyl peroxide and cupric bromide*

(a) A mixture of the amide (220 mg, 1 mmol), di-t-butyl peroxide (1.0 g, 6.8 mmol), cupric bromide (220 mg, 1 mmol) and naphthalene (10 g) was heated under N_2 at 120–130° for 4 hr. Flash chromatography of the mixture as described above afforded **14a** (39 mg, 18%) and starting **10a** (136 mg, 62%).

(b) A mixture of the amide (220 mg, 1 mmol), di-t-butyl peroxide (1.0 g, 6.8 mmol), and cupric bromide (220 mg, 1 mmol) in refluxing benzene (10 ml) was irradiated under N_2 with a mercury lamp for 3 hr. Work-up as described above afforded **14a** (33 mg, 15%) and **10a** (81 mg, 37%).

No β-lactam (**14a**) was formed when methods (a) or (b) were applied in the absence of the Cu salt.

3-(Phenylthio)propionic acid

Reaction of thiophenol with acrylic acid by the method of Hogeveen and Montanari[23] afforded 3-(phenylthio)propionic acid in 86% yield, m.p. 58–60° (lit.[23] m.p. 59.5–61°).

N-Methyl-3-(*phenylthio*)*propionamide* (**10b**)

Treatment of 3-(phenylthio)propionic acid with $SOCl_2$ then $MeNH_2$ as described above gave a solid which crystallised from EtOAc–light petroleum as flakes of *N-methyl*-3-(*phenylthio*)*propionamide* **10b** (3.67 g, 82%), m.p. 89–91°. (Found: C, 61.75; H, 6.60; N, 7.42; S, 16.62. $C_{10}H_{13}NOS$ requires: C, 61.51; H, 6.71; N, 7.17; S, 16.42%). δ ($CDCl_3$) 2.38 (2H, t, *J* 7 Hz, CH_2CO), 2.67 (3H, d, *J* 4 Hz, CH_3), 3.12 (2H, t, *J* 7 Hz, CH_2S), 5.9 (1H, b, NH), 7.0–7.4 (5H, m, Ar), ν_{max} 3286, 1641, 1568 cm^{-1}, *m/e* 195 (M^+, 100%), 137 (13%), 123 (38%), 109 (43%), 86 (74%).

The reaction of N-methyl-3-(*phenylthio*)*propionamide* (**10b**) *with t-butyl perbenzoate*

Treatment of **10b** with t-butyl perbenzoate in the presence of cuprous bromide as described above for **10a** afforded 3-*benzoyloxy*-N-*methyl*-3-(*phenylthio*)*propionamide* (**17**) which crystallised from EtOAc-light petroleum as colourless plates in 72% yield, m.p. 123–127° dec. δ ($CDCl_3$) 2.75 (3H, d, *J* 4 Hz, NCH_3), 2.85 (2H, d, *J* Hz, CH_2), 6.1 (1H, b, NH), 6.6 (1H, t, *J* 7 Hz, CH), 7.1–8.2 (10H, m, Ar), ν_{max} 3300, 1721, 1650, 1552 cm^{-1}, *m/e* 315 (M^+, 1%), 206 (M^+–SC_6H_5, 15%), 194 (M^+–$C_6H_5CO_2$, 2%), 105 (C_6H_5CO, 100%), *m/e* 315–0929 (M^+) (Calc for $C_{17}H_{17}NO_3S$ (M^+) *m/e* 315.0929). The amide (**10b**) (16%) was recovered but no β-lactam or **19** was detected by HPLC.

The reaction of (**10b**) *with di-t-butyl peroxide*

(a) The thermolytic reaction of methyl 3-phenylthiopropionamide with di-t-butyl peroxide as described above, followed by flash chromatography of the product mixture in EtOAc CH_2Cl_2 (20:80) afforded cis-N-*methyl*-3-(*phenylthio*)*propenamide* (**19**) which crystallised from EtOAc-light petroleum in 28% yield, m.p. 120–121°. (Found: C, 61.95; H, 5.76; N, 7.12; S, 16.47. $C_{10}H_{11}NOS$ requires: C, 62.14; H, 5.73; N, 7.24; S, 16.58%). δ ($CDCl_3$) 2.84 (3H, d, *J* 4 Hz, NCH_3), 5.85 (1H, d, *J* 10 Hz, CHCO), 6.2 (1H, b, NH), 6.90 (1H, d, *J* 10 Hz, CHS), 7.1–7.6 (5H, m, Ar), ν_{max} 3308, 1636, 1572, 1440 cm^{-1}, *m/e* 193 (M^+, 96%), 163 (M^+–$NHCH_3$, 100%). Continued elution of the column afforded starting material (46%). No β-lactam (**14b**) was detected.

(b) The photolytic reaction of **10b** with di-t-butyl peroxide as described above for **10a** gave similar results to the thermolytic reaction. The propenamide (**19**) was obtained in 21% yield and **10b** (43%) was recovered. No β-lactam (**14b**) was detected.

4-Phenylthioazetidin-2-one

This compound, prepared from vinyl acetate and chlorosulfonylisocyanate *via* 2-oxoazetidin-4-yl acetate in 32% yield by the method of Claub *et al.*[24] had m.p. 72–73° (lit.[24] m.p. 72°).

1-Methyl-4-*phenylthioazetidin*-2-*one* (**14b**)

(a) Treatment of 3-benzoyloxy-N-methyl-3-(phenylthio)-propionamide with HBr and subsequently with potassium amide as described above afforded 1-*methyl*-4-*phenylthioazetidin*-2-*one* (59%), b.p. 60–70° at 0.6 mm Hg (GKR). δ ($CDCl_3$) 2.83 (3H, s, CH_3), 2.5–3.5 (2H, m, CH_2), 4.80 (1H, dd, *J* 2.5 Hz, CH), 7.0–7.5 (5H, m, Ar), ν_{max} 1760 cm^{-1}, *m/e* 193 (M^+, 1%), 136 (M^+–CH_3NCO, 1%), 84 (M^+–SC_6H_5, 100%), *m/e* 193.0556 (M^+) (Calc for $C_{10}H_{11}NOS$ (M^+) *m/e* 193.0561). No **19** was detected.

(b) 4-Phenylthioazetidin-2-one (0.38 g, 2.1 mmol) was dissolved in CH_2Cl_2 (10 ml), cooled to −78°, then added dropwise to a soln of potassium amide at −78° which had been prepared from ammonia (50 ml), K (90 mg, 2.3 mmol) and ferric nitrate (*ca* 2 mg). After the mixture had been kept for 1 hr at −78° a soln of MeI (0.5 g, 3.5 mmol) in CH_2Cl_2 (5 ml) was added dropwise at −78°. After being kept at −78° for a further 1 hr the mixture was worked-up as described above to afford an oil flash chromato-

graphy of which in EtOAc-CH_2Cl_2 (20:80) afforded 1-methyl-4-phenylthioazetidin-2-one (0.26 g, 63%) identical in all respects to the sample obtained as described above. Continued elution of the column afforded starting material (8%).

2-Acetylamino-3-(phenylthio)propionic acid

The reaction of 2-acetylamino-2-propenoic acid with thiophenol by the method of Goodman *et al.*[25] afforded 2-acetylamino-3-(phenylthio)propionic acid (67%), m.p. 151–152° (lit.[25] m.p. 150–151°).

2-Acetylamino-N-methyl-3-(phenylthio)propionamide (**20**)

Treatment of 2-acetylamino-3-(phenylthio)propionic acid with diazomethane in the usual way afforded the methyl ester in 94% yield. δ ($CDCl_3$) 1.85 (3H, s, $COCH_3$), 3.36 (2H, d, *J* 5 Hz, CH_2), 3.52 (3H, s, OCH_3), 4.80 (1H, dt, *J* = 7.5 Hz, CH), 6.45 (1H, b, NH), 7.2–7.4 (5H, m, Ar).

The crude ester was treated with $MeNH_2$ as described above for the acid chlorides. The ppt which formed was dried under vacuum, washed with light petroleum, and crystallised from EtOAc to give needles (87%) of *2-acetylamino-N-methyl-3-(phenylthio)propionamide*, m.p. 160–161°. (Found: C, 56.97; H, 6.49; N, 11.29; S, 12.46. $C_{12}H_{16}N_2O_2S$ requires: C, 57.12; H, 6.39; N, 11.10; S, 12.71%). δ ($CDCl_3$) 1.93 (3H, s, $COCH_3$), 2.70 (3H, d, *J* 5Hz, NCH_3), 3.2 (2H, d, *J* 6 Hz, CH_2), 4.40 (1H, m, CH), 6.3 (2H, b, 2 × NH), 7.1–7.3 (5H, m, Ar), (35% DCl in D_2O) 2.23 (3H, s, $COCH_3$), 2.42 (3H, s, NCH_3), 3.3 (2H, d, *J* 6 Hz, CH_2), 4.6 (1H, t, *J* 6 Hz, CH), 7.1–7.3 (5H, m, Ar), *m/e* 252 (M^+, 17%), 193 (100%), 163 (26%), 135 (30%), 110 (35%), 109 (19%).

Reactions of 2-acetylamino-N-methyl-3-(phenylthio)propionamide (**20**)

(a) Treatment of **20** (100 mg, 0.4 mmol) with t-butyl perbenzoate (100 mg, 0.52 mmol) and cuprous bromide (10 mg) in refluxing benzene for 6 hr gave a complex product mixture from which no pure compound could be isolated. However, when the reaction was repeated and worked up after 20 min by flash chromatography in EtOAc-CH_2Cl_2 (20:80), it afforded a single product as an oil (4%), tentatively assigned the structure 2-acetylamino-N-methyl-propenamide, δ ($CDCl_3$) 2.10 (3H, s, CH_3CO), 2.90 (3H, d, *J* 4 Hz, NCH_3), 5.18 and 6.36 (2H, m, m, CH_2), 7.6 (2H, m, 2 × NH). Attempts to obtain an analytically pure sample or to prepare the authentic compound were unsuccessful. The starting amide (83%) was also isolated.

(b) Treatment of **20** (100 mg, 0.4 mmol) with di-t-butyl peroxide (400 mg, 2.75 mmol) and cupric bromide, *either* in naphthalene (50 g) at 120–130°, *or* in benzene (100 ml) under UV irradiation gave an intractable complex mixture of products in each case.

3-Carbomethoxy-5-oxoperhydro-1,4-thiazepine (**23a**)

The reaction of methyl acrylate and L-cysteine hydrochloride hydrate[26] gave **23a** (42%) which crystallized from ether as needles, m.p. 109–110° (lit.[26] m.p. 91–92°). Spectra were consistent with those reported.[26]

3-Carbomethoxy-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine (**23b**)

3-Carboxy-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine (27%), prepared from methyl acrylate and D-penicillamine,[27] was treated with diazomethane in the usual way to give **23b** (92%) which crystallised from hexane as needles, m.p. 107–110° (lit.[28] 103.5–105.5°). Spectra were consistent with those reported.[28]

Methyl penicillinate

Penicillanic acid (51%), obtained from 6-aminopenicillanic acid *via* the bromide,[29] was treated with diazomethane in the usual way to give methyl penicillinate (82%), which crystallised from light petroleum as colourless plates, m.p. 48–50° (lit.[30] m.p. 52–53°).

Reactions of 3-carbomethoxy-5-oxoperhydro-1,4-thiazepine (**23a**)

(a) The thiazepine **23a** (500 mg, 2.7 mmol), t-butyl perbenzoate (1.0 g, 51 mmol) and cuprous bromide (100 mg) were heated in benzene (1 l.) under reflux for 6 hr. Flash chromatography of the product mixture in ether afforded 3-carbomethoxy-5-oxo-4,5,6,7-tetrahydro-1,4-thiazepine (54 mg, 11%), m.p. 88–89° (lit.[26] m.p. 88–89°), 3-carbomethoxy-5-oxo-2,3,4,5-tetrahydro-1,4-thiazepine (70 mg, 13%), m.p. 125–126° (lit.[26] m.p. 131–132°), and starting material (40%). No β-lactam or benzoyloxylated product could be detected.

(b) Treatment of **23a** (50 mg, 0.27 mmol) with di-t-butyl peroxide (1.5 g, 10.2 mmol) and cupric bromide (*ca* 10 mg) *either* in naphthalene (50 g) at 120–130°, *or* in benzene (100 ml) with UV irradiation gave a complex intractable mixture in each case.

Reactions of 3-carbomethoxy-2,2-dimethyl-5-oxoperhydro-1,4-thiazepine (**23b**)

Treatment of **23b** (50 mg, 0.23 mmol) with t-butyl perbenzoate or di-t-butyl peroxide as described for **23a** gave only intractable mixtures of products. The expected β-lactam (methyl penicillinate) could not be detected.

The reaction of dimethyl disulfide with t-butyl perbenzoate

A soln of Me_2S_2 (47.1 g, 0.5 mmol) and t-butyl perbenzoate (97.1 g, 0.5 mol) in benzene (500 ml) under N_2 was heated under reflux for 60 hr, then cooled and concentrated under reduced pressure. Flash chromatography of the residue in light petroleum–CH_2Cl_2 (1:1), afforded benzoyloxymethyl methyl disulfide as a pale yellow oil (1.6 g, 1.5%). δ ($CDCl_3$) 2.46 (3H, s, CH_3), 5.50 (2H, s, CH_2), 7.2–8.3 (5H, m, Ar), ν_{max} 1728 cm^{-1}, *m/e* 214 (M^+, 7%), 184 (M^+–OCH_2, 100%), 135 (M^+–$SSCH_3$, 45%), 122 ($C_6H_5CO_2H$, 82%), 105 (M^+–OCH_2SSCH_3, 51%); *m/e* 184.0014 (M^+–OCH_2) (Calc for $C_8H_8OS_2$ (M^+–OCH_2) *m/e* 184.0017). The product decomposed upon attempted distillation. When this reaction was repeated with added cuprous bromide the result was the same.

The reaction of benzoyloxymethyl methyl disulfide with hydrogen bromide

Flash chromatoggraphy (CH_2Cl_2) of the product obtained by treatment of benzoyloxymethyl methyl disulfide in CH_2Cl_2 with HBr for 10 hr as described above afforded an oil which was distilled to give bromomethyl methyl sulfide as a colourless oil (0.44 g, 72%), b.p. 30–35° at 18 mg Hg (GKR) (lit.[31] b.p. 29–32° at 13 mm Hg). δ ($CDCl_3$) 4.60 (2H, s, CH_2), 2.55 (3H, s, CH_3). The ^1H NMR spectrum of the crude mixture contained resonances assigned to this sulfide and benzoic acid, and singlets at δ 5.23 and 2.36 which integrated in the ratio 2:3 and were assigned to bromomethyl methyl disulfide. Integration of the ^1H NMR spectrum indicated a ratio of sulfide to disulfide of about 1:1 which, on taking into account the high yield of sulfide obtained from the chromatography column, indicates the rearrangement of the disulfide to the sulfide on the column. Sampling of the reaction mixture during the course of the reaction indicated that the disulfide was intermediate in the conversion of the benzoate to the sulfide. Distillation of the crude mixture afforded only the sulfide.

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REFERENCES

[1]For reviews see D. J. Aberhart, *Tetrahedron* 33, 1545 (1977); J. O'Sullivan, R. C. Bleaney, J. A. Huddleston and E. P. Abraham, *Biochem. J.* 184, 421 (1979); Y. Sawada, J. E. Baldwin, P. D. Singh, N. A. Solomon and A. L. Demain, *Ibid.* p. 465.
[2]E. P. Abraham, *Jap. J. Antibiotics* 30, 51 (1977).
[3]P. Adriaens, B. Meesschaert, L. Duman and H. Eyssen, *J. Pharm. Belg.* 34, 222 (1979); B. Meesschaert, P. Adrians and H. Eyssen, *J. Antibiotics* 23, 722 (1980).
[4]D. J. Morecombe and D. W. Young, *Chem. Commun.* 198 (1975); D. J. Aberhart, L. J. Lin and J. Y. R. Chu, *J. Chem. Soc.* Perkin I, 2157 (1975); N. Neuss, C. H. Nash, J. E. Baldwin, P. A. Lemke and J. B. Grutzner, *J. Am. Chem. Soc.* 95, 3797 (1973); H. Kluender, C. H. Bradley, C. J. Sih, P. Fawcett and E. P. Abraham, *Ibid.* 95 6149 (1973); D. J. Aberhart and L. J. Lin, *J. Chem. Soc.* Perkin I, 2320 (1974).
[5]J. E. Baldwin, M. Jung, J. J. Usher, E. P. Abraham, J. A. Huddleston and R. L. White, *Chem. Commun.* 246 (1981); J. E.

Baldwin, reported at *11th Ann. Chemical Society Symp. Modern Aspects of Stereochemistry*, 18 December 1974; *Chem. Brit.* **11**, 369 (1975).
[6]J. E. Baldwin, Private communication.
[7]J. E. Baldwin and T. S. Wan, *Chem. Commun.* 249 (1979).
[8]J. E. Baldwin, A. L. J. Beckwith, A. P. Davis, G. Proctor, and K. E. Singleton, *Tetrahedron* **37**, 2181 (1981).
[9]J. T. Groves, G. A. McClusky, R. E. White and M. J. Coon, *Biochem. Biophys. Res. Commun.* **81**, 154 (1978).
[10]See, for example, W. A. Pryor, *Free Radicals in Biology* **1**, 1 (1976); R. P. Mason, *Ibid.* **5**, 161 (1982).
[11]D. J. Rawlinson and G. Sosnovsky, *Synthesis* 1 (1972).
[12]J. E. Baldwin and A. P. Davis, *Chem. Commun.* 1219 (1981); J. E. Baldwin, A. P. Davis and L. D. Field, *Tetrahedron* **38**, 2777 (1982).
[13]W. B. Gara, J. R. M. Giles, and B. P. Roberts, *J. Chem. Soc.* Perkin II 1444 (1979); E. A. C. Lucken and B. Ponceni, *Ibid.*, Perkin II 777 (1976).
[14]P. J. Krusic and J. K. Kochi, *J. Am. Chem. Soc.* **93**, 846 (1971).
[15]Experiments conducted in these laboratories by D. G. Boate indicate that alkylphenyl thioethers are at least 5 times more reactive than their oxygen counterparts towards attack by t-butoxy radicals.
[16]H. Matsumura, T. Yano, M. Ueyama, K. Tori and W. Nagata, *Chem. Commun.* 485 (1979).
[17]H. G. Viehe, R. Merenyi, L. Stella and Z. Janousek, *Angew. Chem. Int. Ed. Engl.* **18**, 917 (1979).
[18]J. K. Kochi, *Free Radicals* (Edited by S. K. Kochi), Vol. I, p. 591, Wiley, New York (1973).
[19]W. A. Mulac and D. Meyerstein, *Chem. Commun.* 893 (1979), and refs. cited.
[20]W. A. Pryor and H. T. Bickley, *J. Org. Chem.* **37**, 2885 (1972); L. Homer and E. Jurgens, *Liebigs Ann.* **602**, 135 (1957); L. Homer and B. Anders, *Chem. Ber.* **95**, 2470 (1962); D. I. Davies, D. H. Hey and B. Summers, *J. Chem. Soc.* (C), 2653 (1970).
[21]M. S. Kharasch and H. C. Brown, *J. Am. Chem. Soc.* **82**, 925 (1960).
[22]W. E. Parham, W. D. McKown, V. Nelson, S. Kajigaeshi and N. Ishikawa, *J. Org. Chem.* **38**, 1361 (1973).
[23]H. Hogeveen and F. Montanari, *J. Chem. Soc.* 4864 (1963).
[24]K. Claub, D. Grimm and G. Prossel, *Liebigs Ann.* 539 (1974).
[25]L. Goodman, L. O. Ross and B. R. Baker, *J. Org. Chem.* **23**, 1251 (1958).
[26]P. Blondeau, R. Gauthier, C. Berse and D. Gravel, *Can. J. Chem.* **49**, 3866 (1971).
[27]T. Wieland, G. Ohnacker and W. Ziegler, *Chem. Ber.* **90**, 194 (1957).
[28]N. J. Leonard and G. E. Wilson, *J. Am. Chem. Soc.* **86**, 5307 (1964).
[29]D. G. Brenner and J. R. Knowles, *Biochemistry* **20**, 3680 (1981).
[30]J. P. Clayton, *J. Chem. Soc.* (C) 2123 (1969).
[31]F. Boberg, G. Winter and G. R. Schultze, *Chem. Ber.* **89**, 1160 (1956).

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Correlation of the Effect of β-Lactamase Inhibitors on the β-Lactamase in Growing Cultures of Gram-Negative Bacteria with Their Effect on the Isolated β-Lactamase

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The effectiveness of clavulanic acid, sulbactam, quinacillin sulfone, and the carbapenems MM13902 and MM4550 as inhibitors of TEM-2 β-lactamase in growing cultures of gram-negative bacteria has been studied. Each of these β-lactams inhibited the enzyme in intact cells, and the nature of the inhibition correlated with studies on the purified enzyme. The potency of these compounds as inhibitors of the β-lactamase in vivo can be correlated with the amounts hydrolyzed by the purified enzyme under saturating conditions during the inhibition of the enzyme in vitro.

From studies in these laboratories with purified TEM-2 β-lactamase from *Escherichia coli* (7), the characteristics of the inhibition of the enzyme by clavulanic acid (3, 5, 13), sulbactam (2, 15), quinacillin sulfone (12), and the carbapenems MM13902 and MM4550 (4, 9) have been delineated. However, the relevance of these investigations to the use of the inhibitors in synergy with β-lactam antibiotics in vivo is not clear. For example, English et al. (10) found that whereas clavulanic acid and sulbactam are about equally effective as inhibitors of cell-free β-lactamases, clavulanic acid is considerably more potent than sulbactam when used in synergy with the β-lactamase-susceptible antibiotic ampicillin against the bacteria from which the β-lactamases were derived.

The work described here examines the relationship between the effectiveness of β-lactamase inhibitors in vivo and the known characteristics of inhibition of the purified β-lactamase in vitro.

MATERIALS AND METHODS

Materials. Clavulanic acid and the olivanic acid derivatives MM4550 and MM13902 were the generous gifts of Beecham Pharmaceuticals, Brockham Park, Betchworth, Surrey, United Kingdom. Each was supplied as the sodium salt, and clavulanate was supplied as the tetrahydrate. Quinacillin sulfone was synthesized as previously described (12) and stored as the free acid. Ampicillin was purchased as the free acid from Sigma Chemical Co., St. Louis, Mo. The sodium salt of sulbactam (penicillanic acid sulfone) and a culture of *E. coli* UB1005 (*nal*^{r} *met*$^-$) (17) were kindly supplied by Pfizer, Inc., Groton, Conn. A culture of *Proteus mirabilis* F67 was generously provided by G. Jacoby, Massachusetts General Hospital, Boston, Mass.

The RP4 plasmid was transferred into *E. coli* UB1005 and *P. mirabilis* F67, by mating with *E. coli* W3110(RP4) as donor under standard conditions for an overnight mating experiment at 37°C (14). The mating mixture was plated on nutrient agar to select recipient colonies that had received the RP4 plasmid. Strain UB1005(RP4) was selected on 3% agar containing nalidixic acid (50 μg/ml) and tetracycline (10 μg/ml). Strain F67(RP4) was selected on 5% agar containing nicotinic acid (50 μg/ml), rifampin (25 μg/ml), and tetracycline (10 μg/ml).

The purification of the TEM-2 β-lactamase that was used has been previously described (4).

Methods. Solutions of the β-lactams in 0.1 M sodium phosphate buffer (pH 7.0) were prepared by weight with a Cahn 25 electrobalance. UV spectroscopic measurements were made with a Perkin-Elmer 554 or 575 spectrophotometer.

To determine the MIC of an antibiotic, nutrient broth (10 ml) equilibrated at 37°C was inoculated to ca. 20 cells per ml from a culture in exponential-phase growth. A small inoculum was used to avoid problems of antibiotic depletion by the β-lactamase during the incubation. After 1 h at 37°C, the β-lactam solution was added. The MICs given in Table 1 are the lowest concentrations of β-lactam at which no cell growth could be detected by absorbance at 550 nm after 24 h at 37°C. The limit of detection is ca. 10^6 cells per ml. In each case, cell growth was detected at a β-lactam concentration 20% lower than the MIC. Very similar MICs were obtained by plating bacteria as single CFU on the surface of agar containing the β-lactam in question.

To measure growth curves for *E. coli* W3110(RP4), nutrient broth equilibrated at 37°C was inoculated to ca. 10^5 cells per ml from a culture in exponential growth phase. After 1 h at 37°C, the β-lactam solution was added. Bacterial growth was determined by measuring the change in absorbance at 550 nm.

Viable cell counts were performed by determination of the number of CFU (on nutrient agar plates at 37°C, overnight) from a range of dilutions of the broth being assayed.

β-Lactamase catalytic activity was measured as described earlier (13).

RESULTS AND DISCUSSION

To examine the effectiveness of the β-lactamase inhibitors in growing cultures, we examined *E. coli* and *P. mirabilis*. To investigate the consequence of β-lactamase production, we introduced the RP4 plasmid into both bacterial strains. We thus ensured that each β-lactamase-producing organism would elaborate the same β-lactamase as that used in all our studies with the purified enzyme.

The target enzymes of β-lactam antibiotics are involved in the biosynthesis of the bacterial cell wall, and in gram-negative bacteria, these enzymes are thought to face into the

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TABLE 1. MICs for various antibiotics and β-lactamase inhibitors, alone and in combination

Drug	MIC for strain:			
	E. coli		*P. mirabilis*	
	βla⁻	βla⁺	βla⁻	βla⁺
Ampicillin	9	5,000	0.05	100
Clavulanate	25	25	10	10
Ampicillin + clavulanate		20 + 5		0.1 + 2 2.5 + 0.5
Enhancement ratio		200		800
Sulbactam	50	200	50	50
Ampicillin + sulbactam		2,000 + 40		2.5 + 10
Enhancement ratio		2		32
Quinacillin sulfone	>5,000	>5,000	1,000	1,000
Ampicillin + quinacillin sulfone				0.1 + 200 2.5 + 150
Enhancement ratio				800
MM13902	1.0	8	0.2	0.5
Ampicillin + MM13902		1.800 + 1.6		1.5 + 0.1 2.5 + 0.08
Enhancement ratio		2.2		53
MM4550	2.0	4	1.0	1.0
Ampicillin + MM4550		2.500 + 1		0.2 + 0.2 2.5 + 0.14
Enhancement ratio		1.5		400

(μg/mL)

periplasm on the outer face of the inner membrane. It can reasonably be assumed that the affinity of the target enzymes for β-lactam antibiotics is not affected by the production of a β-lactamase by the cell. The higher concentration of antibiotic required to kill a β-lactamase-producing (βla⁺) cell compared with that needed for the β-lactamase-deficient (βla⁻) variety is therefore directly related to the rate of hydrolysis of the antibiotic by the periplasmic β-lactamase. Table 1 lists, for both bacterial strains, the MICs of ampicillin (an antibiotic that is very susceptible to β-lactamase-catalyzed hydrolysis: $k_{cat} = 2{,}000\ s^{-1}$; $K_m = 20\ \mu M$), the β-lactamase inhibitors, and mixtures of ampicillin with these inhibitory agents. With ampicillin alone, introduction of the RP4 plasmid and the consequent production of β-lactamase caused an increase in the MIC of more than 500-fold. This increase would presumably be nullified if the β-lactamase were to be inhibited or inactivated, and the reagents that effect such inhibition of the purified enzyme are now considered.

Clavulanic acid. Although clavulanate is not a very potent antibiotic against the bacterial species examined, the fact that its MIC for the βla⁺ strain roughly equaled that for the βla⁻ strain shows that the β-lactamase does not significantly affect the periplasmic concentration of clavulanate at its MIC. This indicates that clavulanate may be an effective β-lactamase inhibitor in vivo and could therefore show synergy in combination with a susceptible antibiotic against βla⁺ strains. Indeed, it is generally true that only compounds for which the MICs for βla⁺ and βla⁻ strains are equal will have significant potential as synergists with β-lactamase-susceptible antibiotics.

When clavulanate and ampicillin were used together, and the concentration of clavulanic acid was 20% of its MIC for the βla⁺ strain (at which level there would be no discernible effect on bacterial growth rate), the amount of ampicillin required at the MIC was reduced sharply (Table 1). For the βla⁺ *E. coli* strain, the MIC of ampicillin alone was 5,000 μg/ml compared with 20 μg/ml in the presence of 5 μg of clavulanate per ml. Although such synergy can be quantitated by the use of the fractional inhibitory concentration index (11), we shall define a simpler quantity here, the enhancement ratio, to provide a measure of the effectiveness of anti-β-lactamase agents. If the effects of the two antibacterial agents were merely additive, then since for βla⁺ the MIC of ampicillin alone is 5,000 μg/ml and that of clavulanate alone is 25 μg/ml, the MIC of a mixture (in the absence of synergy) would be, for example, 2,500 μg of ampicillin per ml plus 12.5 μg of clavulanate per ml or 4,000 μg of ampicillin per ml plus 5 μg of clavulanate per ml. The synergistic effect can then be quantified as the enhancement ratio, which is the ratio of antibiotic predicted in the absence of synergy to that actually required. In the case of the βla⁺ *E. coli* strain, 5 μg of clavulanate per ml needed only 20 μg of ampicillin per ml rather than the 4,000 μg/ml that would be needed if the β-lactams acted additively. The enhancement ratio is therefore 200. It should be noted that the ampicillin level required in the mixture (20 μg/ml) is only twice that needed for the defenseless (βla⁻) strain, and it is clear that clavulanate almost completely protects ampicillin from the β-lactamase. (It should be stressed that a large enhancement ratio is a necessary but insufficient condition for a synergist to have clinical utility. The absolute concentration of synergist is an important factor in any clinical regimen, and the total β-lactam burden is not expressed in the enhancement ratio as defined.)

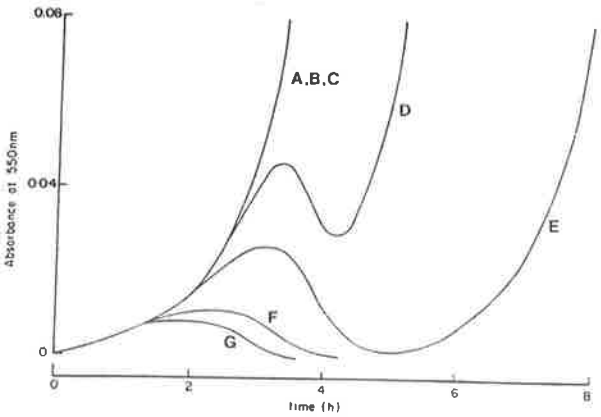

FIG. 1. Growth curves for *E. coli* W3110(RP4). Plots describe the growth of cultures in which the growth medium contained the following amounts of ampicillin and clavulanate, respectively (in micrograms per milliliter): curve A, 0 and 0; curve B, 2,000 and 0; curve C, 0 and 20; curve D, 10 and 5; curve E, 10 and 10; curve F, 10 and 15; curve G, 10 and 20.

In Fig. 1 are plotted growth curves for βla⁻ *E. coli* cells (strain W3110 carrying the RP4 plasmid) grown in the presence of ampicillin alone, clavulanic acid alone, and various mixtures of ampicillin and clavulanate. For this strain, the MIC of ampicillin alone was 5,000 μg/ml, and the MIC of clavulanate alone was 80 μg/ml. Neither 2,000 μg of ampicillin per ml (Fig. 1, curve B) nor 20 μg of clavulanate per ml (Fig. 1, curve C) detectably affected the growth rate. However, in the presence of 10 μg of ampicillin per ml plus 5 or 10 μg of clavulanate per ml, growth occurred in a manner described by a triphasic curve (Fig. 1, curves D and E). Such curves may be interpreted as follows. At the start, cell division is not inhibited significantly, since the β-lactamase can hydrolyze enough of the ampicillin that diffuses into the periplasm to prevent any significant inactivation of the cell wall-synthesizing enzymes. During this first phase, however, clavulanic acid both inhibits and inactivates the β-lactamase, and after 2 to 2.5 h, the β-lactamase activity has been so reduced that the ampicillin continuing to diffuse through the outer membrane starts to affect the growth rate. It may be noted that a very high proportion of the β-lactamase has to be inactivated before ampicillin starts to affect cell growth. This is due both to the relatively massive amount of β-lactamase synthesized by *E. coli* carrying RP4 and to the extreme lability of ampicillin towards β-lactamase-catalyzed hydrolysis. In the second phase of curves D and E (Fig. 1), the number of cells falls, because the rate of new β-lactamase production by cell growth and cell division is inadequate to counteract the enzyme inhibition by clavulanate and the killing of cells by ampicillin. If, however, before all the cells have been killed, the external ampicillin concentration has been reduced to levels at which its effect on cell growth is negligible, logarithmic growth will resume. This is the third phase of growth curves D and E (Fig. 1). For curves F and G, no third phase is seen: the higher levels of clavulanate are sufficient to prevent the β-lactamase from hydrolyzing all the ampicillin before all the cells have lysed.

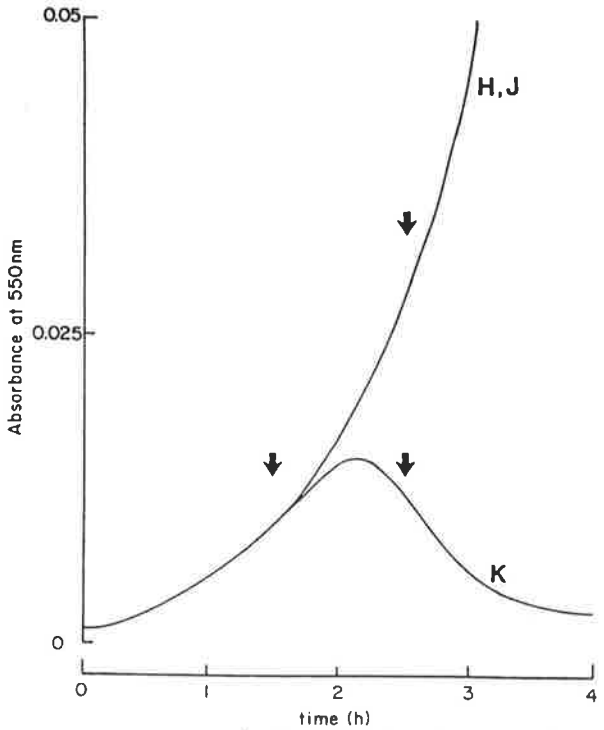

FIG. 2. Growth curves for *E. coli* W3310(RP4) under conditions used to assay for β-lactamase activity. The growth medium contained the following amounts of ampicillin and clavulanate, respectively (μg/ml): (curve H) 10, 0; (curve J) 0, 10; and (curve K) 10, 10. Arrows indicate values shown in Table 2.

TABLE 2. Relative amounts of active β-lactamase per viable cell[a] of *E. coli* W3110(RP4) under various conditions of bacterial growth

Cells grown in the presence of:		Growth curve[b]	Amt of active β-lactamase per cell after:	
Ampicillin (μg/ml)	Clavulanate (μg/ml)		90 min	150 min
10	0	H	100	97
0	10	J	19	22
10	10	K	23	4

[a] Active β-lactamase assayable after repeated washing of cells, followed by cell lysis in a French press. Viable cell counts were made as described in the text, and the β-lactamase activity per viable cell was calculated. All values are relative to the activity measured in a culture 90 min after addition of ampicillin (10 μg/ml), which is set at 100.

[b] Growth curves H, J, and K are shown in Fig. 2.

To discover whether the above interpretation is consistent with our studies on the interaction of clavulanate with the purified β-lactamase (3, 5, 13), we measured the amount of active β-lactamase under various conditions of cell growth. Illustrated in Fig. 2 are growth curves for incubations from which cell samples were taken at times shown by the arrows. The cells were repeatedly washed, the concentration of viable cells was measured, and the cells were then lysed in a French press. The amount of β-lactamase per viable cell in the sample was then determined by enzymatic assay. The results are shown in Table 2. First, in the presence only of 10 μg of ampicillin per ml (at which concentration there was no discernible effect on cell growth), the amount of β-lactamase per viable cell was constant, as expected for constitutive synthesis. Second, in the presence only of 10 μg of clavulanate per ml (again, there was no detectable effect on cell growth), the average amount of active β-lactamase was about 20% of that seen in the absence of clavulanate. It appears that clavulanic acid at 10 μg caused the irreversible inactivation of 80% of the β-lactamase. Is this consonant with what we know about the kinetics of inactivation of the purified β-lactamase by clavulanate?

Let us assume that a parent cell just before division contains n molecules of active β-lactamase and that each daughter cell inherits $n/2$ molecules of enzyme and synthesizes $n_0/2$ molecules during the time (t) between one cell division and the next. At a particular extracellular level of clavulanic acid, we assume that some fraction f of the total number of β-lactamase molecules, $(n + n_0)/2$, is inactivated during time t, so that the daughter cell just before division contains $(1 - f)(n + n_0)/2$ molecules of active β-lactamase. After several generations we shall reach a steady state in which each generation of cells inherits the same number of active β-lactamase molecules as did its parent, at which time:

$$n/2 = (1 - f)(n + n_0)/4$$

whence:

$$n/n_0 = (1 - f)/(1 + f) \quad (1)$$

In the absence of clavulanate (Fig. 2, curve H), $f = 0$ and

n/n_0 = 1. In the presence of clavulanate (Fig. 2, curve J), there is at the steady state only about 20% of the normal amount of β-lactamase per cell (Table 2), and so (since the steady-state amount of enzyme per cell is proportional to n, and n_0 is constant for constitutive synthesis) n/n_0 = 0.2. Hence, from equation 1, f = 0.67. That is, ca. 70% of the β-lactamase of a cell is irreversibly inactivated during one generation time, which for curves H and J of Fig. 2 is about 35 min. This is equivalent to an enzyme half-life of about 20 min. We have shown earlier (3) that at saturating concentrations of clavulanic acid, the half-life for irreversible inactivation of the purified enzyme is about 15 min. Considering the approximations involved, there is remarkably good agreement between the half-life for inactivation of purified enzyme of 15 min and the half-life for inactivation of β-lactamase in growing cells of 20 min.

When clavulanic acid interacts with the purified enzyme, both transient inhibition and irreversible inactivation are observed (13). Further, since the acyl enzyme derived from clavulanate partitions more towards the transiently inhibited form than towards the inactivated form, under steady-state conditions in which 80% of the enzyme is irreversibly inactivated (Table 2, experiment J), almost all of the remaining 20% of the enzyme would be transiently inhibited (see, for example, Fig. 1 of reference 13). There is therefore an essentially complete blockade of the β-lactamase in experiment J (Fig. 2, curve J).

When clavulanic acid and ampicillin are used in combination, we see the biphasic growth curve K (Fig. 2). During the first few generation times, the growth rate does not change, presumably while the steady-state level of β-lactamase activity is established. As the clavulanic acid takes hold, the cell can no longer destroy the periplasmic ampicillin, and cell division slows down (Fig. 2, curve K; 1.5 to 2 h). Clavulanic acid continues to inactivate the β-lactamase that remains, and the amount of active enzyme per viable cell in the dying culture (Fig. 2, curve K; 2.5 h) falls to 4% (Table 2). These results provide a clear picture of the molecular consequences of the application of mixtures of clavulanic acid and ampicillin to a growing culture.

Sulbactam. When sulbactam was used alone, the MIC for the βla$^+$ *E. coli* strain was larger than the MIC for the βla$^-$ strain (Table 1), showing that the sulfone is susceptible to β-lactamase-catalyzed hydrolysis at a rate that is competitive with (but not much greater than) its rate of diffusion into the periplasm at these extracellular concentrations. Since the sulfone cannot completely protect itself from hydrolysis, it is unlikely that it will act as an effective synergist with a susceptible antibiotic such as ampicillin, and the synergy factor is close to unity, as expected.

For *P. mirabilis*, in contrast, sulbactam had an MIC for the βla$^-$ strain equal to the MIC for the βla$^+$ strain, showing that with this organism, the periplasmic concentration of the sulfone is not significantly affected by the presence of the lactamase. One reason for this difference between the *P. mirabilis* and *E. coli* strains is the amount of lactamase produced. Whereas a viable cell of *E. coli* produces, on average, 6.5×10^4 molecules of the β-lactamase, each viable cell of *P. mirabilis* produces only about 1.5×10^3 molecules of the enzyme. (These numbers are derived from the measured amount of β-lactamase activity per viable cell and the known specific catalytic activity of the purified TEM-2 β-lactamase [4].) The smaller amount of enzyme produced by *P. mirabilis* will obviously be less effective at reducing the periplasmic level of the sulfone. There may additionally be differences in the permeability of the *E. coli* and *P. mirabilis* strains towards sulbactam that result in the observed behavior.

Since for *P. mirabilis* the MIC for the βla$^+$ strain was equal to the MIC for the βla$^-$ strain, sulbactam may act as a synergist with β-lactamase-susceptible antibiotics against this organism. Indeed, the enhancement ratio with ampicillin is 32 (Table 1), and it is clear that sulbactam can inhibit the β-lactamase in these cells.

Qualitatively, clavulanic acid is a more potent inhibitor of the TEM β-lactamase in growing cells than is sulbactam. To prevent growth of the βla$^+$ *P. mirabilis* strain with 2.5 μg of ampicillin per ml, only 0.5 μg of clavulanic acid per ml is required, compared with 10 μg of sulbactam per ml. The concentrations of clavulanate and sulbactam used are each well below the levels at which these materials are themselves bactericidal, and they presumably act primarily as lactamase inhibitors under these conditions. It should, however, be stressed that β-lactamases from different bacterial sources differ in both substrate specificity and susceptibility to inhibition, and the relative effectiveness of clavulanate and sulbactam will depend upon the organism being challenged.

Quinacillin sulfone. Although quinacillin sulfone rapidly inactivates the purified TEM-2 β-lactamase (the half-life for enzyme inactivation at saturating inhibitor concentrations is <1 min [12] compared with a half-life for sulbactam of 44 min [2]), it is hardly effective, either alone or in combination with ampicillin, against growing bacteria (Table 1). It is an ineffective antibiotic against *E. coli*, and the MICs are too high (>5,000 μg/ml) to be informative. This behavior is

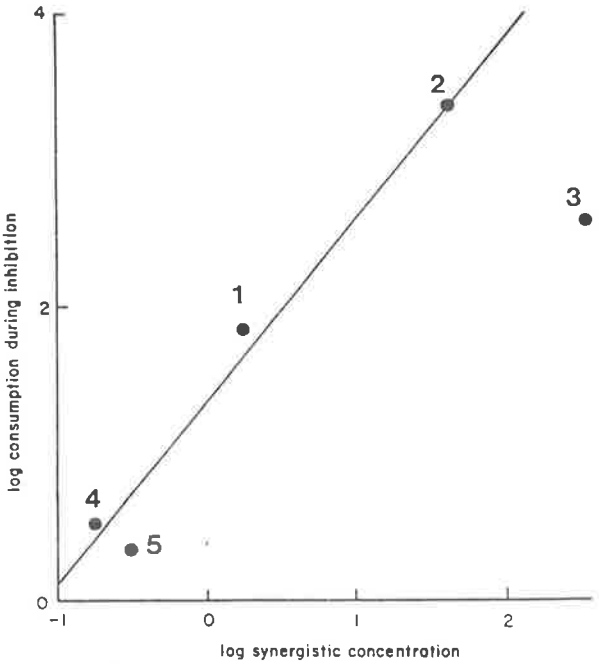

FIG. 3. Plot of the logarithm of the molar excess of the β-lactamase inhibitor required to inhibit the purified enzyme in vitro (Table 3) versus the logarithm of the concentration of the reagent required in synergy with 2.5 μg of ampicillin per ml (Table 1) against *P. mirabilis* F67(RP4). For details, see the text. Plots: 1, clavulanic acid; 2, sulbactam; 3, quinacillin sulfone; 4, MM13902; and 5, MM4550. The least-squares line is drawn omitting the data for quinacillin sulfone.

TABLE 3. Number of molar equivalents of β-lactamase inhibitor hydrolyzed by the purified TEM-2 β-lactamase under saturating conditions in 20 min

β-Lactamase inhibitor	Molar equivalents hydrolyzed per mol of enzyme	Method (reference)
Clavulanate	70	Fig. 1 and 3 (reference 13)
Sulbactam	2,400	Fig. 1 and 3 (reference 13)
Quinacillin sulfone	400	(12)
MM13902	3.3	Fig. 2 (reference 3)
MM4550	2.1	Fig. 1 (reference 9)

presumably due to the fact that quinacillin sulfone cannot penetrate the outer membrane and has access to neither the antibiotic targets nor the β-lactamase (see below). This sulfone is also a poor antibiotic against *P. mirabilis*, though the fact that the MIC of sulfone for the βla$^+$ strain is equal to its MIC for the βla$^-$ strain suggests the possibility of its acting as a synergist. The enhancement ratio with ampicillin is indeed 800 (Table 1), so it is clear that quinacillin sulfone does inhibit the β-lactamase in growing cells. However, even against the βla$^+$ *P. mirabilis* strain, quinacillin sulfone is 300 times less effective than clavulanic acid and 15 times less effective than sulbactam in terms of the amount required in combination with 2.5 μg of ampicillin per ml.

Olivanic acids MM13902 and MM4550. The carbapenems MM13902 and MM4550 are both potent antibiotics, and these compounds show only weak synergy with ampicillin against *E. coli* (Table 1). Against the *P. mirabilis* strain, however, the enhancement ratios are considerable. Low concentrations of either carbapenem can evidently inhibit the lactamase in growing cells of *P. mirabilis*. Both MM13902 and MM4550 are considerably more effective than clavulanic acid in combination with 2.5 μg of ampicillin per ml against the βla$^+$ *P. mirabilis* strain. The low in vivo synergy shown by the olivanates towards *E. coli* is presumably a reflection of the competition between the target enzymes and the β-lactamase for these carbapenems (which are considerably more effective as antibiotics than the other β-lactamase inhibitors considered in Table 1). With *P. mirabilis*, the much smaller amount of periplasmic β-lactamase present reduces this competition problem, and more significant synergy is observed. Since MM13902 and MM4550 act on the purified enzyme only by transient inhibition of the β-lactamase (4, 9) and do not inactivate it, if the enzyme behaves analogously in vivo, irreversible inactivation of the β-lactamase is not required to overcome the bacterial defense mechanism. Synergy is still possible if the β-lactamase is merely preoccupied while the antibiotic reaches its target.

All of the β-lactam derivatives discussed are inhibitors of both the purified TEM β-lactamase and the enzyme in growing cells. However, the relative in vivo potencies vary greatly. Why do these differences exist?

It is unlikely that there is much variation in the ease with which clavulanic acid, sulbactam, and the two carbapenems MM13902 and MM4550, pass through the outer membrane of gram-negative bacteria (1, 6, 8, 16, 20). Indeed, the difference between the antibiotic effectiveness of each antibiotic against the wild type and that against a permeable mutant of *E. coli* (DC2) (17, 18) (data not shown) is less than threefold. Presumably the differences in potency among these antibiotics as inhibitors of the β-lactamase in growing cells are a consequence of the fact that different amounts of each material are required to inhibit the enzyme. Consider, for example, the concentrations of clavulanate, sulbactam, MM13902, and MM4550 required in combination with 2.5 μg of ampicillin per ml against the βla$^+$ *P. mirabilis* strain. For this low ampicillin concentration to be effective, the defense provided by the β-lactamase must be almost completely overcome, and the enzyme must be almost totally preoccupied with the inhibitor. We can then compare the number of inhibitor molecules that are consumed in inhibiting one molecule of the purified enzyme with the extracellular molarity required in combination with 2.5 μg of ampicillin per ml against the βla$^+$ *P. mirabilis* strain (Fig. 3). The number of molar equivalents of the β-lactamase inhibitors consumed in inhibiting one molar equivalent of the purified β-lactamase was determined (Table 3) by monitoring the hydrolysis of the inhibitor by the enzyme for 20 min under saturating conditions. After this period, essentially all of the catalytic activity of the enzyme is lost when each inhibitor is used.

It is clear that the number of molecules of clavulanic acid, sulbactam, MM13902, and MM4550 hydrolyzed during the inhibition of the purified β-lactamase in vitro correlates with the effectiveness of these compounds in inhibiting the enzyme in growing *P. mirabilis* cells sufficiently to enable 2.5 μg of ampicillin per ml to bring about bacteriostasis (Fig. 3). The best inhibitors in vivo are those that inhibit the purified enzyme with the concurrent hydrolysis of the least number of molar equivalents of inhibitor.

In general, the correlation represented in Fig. 3 can only be valid if (i) there is no selective restriction of access of the inhibitors to the periplasmic β-lactamase and (ii) studies with the purified enzyme are relevant to the situation in growing cells. It appears that these conditions hold for clavulanate, sulbactam, MM13902, and MM4550. Indeed, Nikaido et al. have elegantly shown that a variety of cephems penetrate *E. coli* outer membranes surprisingly rapidly (16). Yet it is clear that quinacillin sulfone is exceptional. The synergistic dose of quinacillin sulfone required against the βla$^+$ *P. mirabilis* strain is more than 25 times higher than that expected on the basis of studies with the purified enzyme (and predicted from Fig. 3). This presumably reflects the fact that the outer membrane of *P. mirabilis* selectively excludes quinacillin sulfone, a conclusion that is consistent with the large size and hydrophobicity of this sulfone compared with that of the other inhibitors. It would seem that against these gram-negative bacteria, the benefits associated with the 6-substituent in quinacillin sulfone in terms of β-lactamase inactivation (12) are more than offset by a decrease in outer membrane permeability also associated with this 6-substituent (16).

In summary, it appears that studies with the purified enzyme (2–5, 9, 12, 13, 15) are relevant to the situation in growing cells and that the amount of β-lactam required to inhibit the purified enzyme is directly related to the amount required for enzyme inhibition in vivo. On this basis, studies with the purified enzyme can be used to screen for effective in vivo β-lactamase inhibitors. Finally, it should be emphasized that the present work concerns the inhibition of a constitutive plasmid-encoded β-lactamase and does not bear upon the problems of the inducible chromosomal enzymes often found in such genera as *Enterobacter* and *Pseudomonas*. The question of whether β-lactamase inactivators will be useful in overcoming the resistance developed even towards slowly hydrolyzed cephalosporins (19) has yet to be examined.

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LITERATURE CITED

1. **Boman, H. G., K. Nordstrom, and S. Normark.** 1974. Penicillin resistance in *Escherichia coli* K-12. Synergism between penicillinase and a barrier in the outer part of the envelope. Ann. N.Y. Acad. Sci. **235**:569–585.
2. **Brenner, D. G., and J. R. Knowles.** 1981. Penicillanic acid sulfone: an unexpected isotope effect in the interaction of 6α- and 6β-monodeuterio and of 6,6-dideuterio derivatives with RTEM β-lactamase from *Escherichia coli*. Biochemistry **20**:3680–3687.
3. **Charnas, R. L., J. Fisher, and J. R. Knowles.** 1978. Chemical studies on the inactivation of *Escherichia coli* RTEM β-lactamase by clavulanic acid. Biochemistry **17**:2185–2189.
4. **Charnas, R. L., and J. R. Knowles.** 1981. Inhibition of the RTEM β-lactamase from *Escherichia coli*. Interaction of enzyme with derivatives of olivanic acid. Biochemistry **20**:2732–2737.
5. **Charnas, R. L., and J. R. Knowles.** 1981. Inactivation of RTEM β-lactamase from *Escherichia coli* by clavulanic acid and 9-deoxy-clavulanic acid. Biochemistry **20**:3214–3218.
6. **Costerton, J. W., and K. J. Cheng.** 1975. The role of the bacterial cell envelope in antibiotic resistance. J. Antimicrob. Chemother. **1**:363–377.
7. **Datta, N., and P. Kontomichalou.** 1965. Penicillinase synthesis controlled by infectious R factors in *Enterobacteriaceae*. Nature (London) **208**:239–241.
8. **Decad, G. M., and H. Nikaido.** 1976. Outer membrane of gram-negative bacteria. XII. Molecular sieving function of cell wall. J. Bacteriol. **128**:325–336.
9. **Easton, C. J., and J. R. Knowles.** 1982. Inhibition of the RTEM β-lactamase from *Escherichia coli*. Interaction of the enzyme with derivatives of olivanic acid. Biochemistry **21**:2857–2862.
10. **English, A. R., J. A. Retsema, A. E. Girard, J. E. Lynch, and W. E. Barth.** 1978. CP45,899, a β-lactamase inhibitor that extends the antibacterial spectrum of β-lactams: initial bacteriological characterization. Antimicrob. Agents Chemother. **14**:414–419.
11. **Feldman, W. E., and T. Zweighaft.** 1979. Effect of ampicillin and chloramphenicol against *Streptococcus pneumoniae* and *Neisseria meningitidis*. Antimicrob. Agents Chemother. **15**:240–242.
12. **Fisher, J., R. L. Charnas, S. M. Bradley, and J. R. Knowles.** 1981. Inactivation of the RTEM β-lactamase from *Escherichia coli*. Interaction of penam sulfones with enzyme. Biochemistry **20**:2726–2731.
13. **Fisher, J., R. L. Charnas, and J. R. Knowles.** 1978. Kinetic studies on the inactivation of *Escherichia coli* RTEM β-lactamase by clavulanic acid. Biochemistry **17**:2180–2184.
14. **Grinsted, J., J. R. Saunders, L. C. Ingram, R. B. Sykes, and M. H. Richmond.** 1972. Properties of an R factor which originated in *Pseudomonas aeruginosa* 1822. J. Bacteriol. **110**:529–537.
15. **Kemal, C., and J. R. Knowles.** 1981. Penicillanic acid sulfone: interaction with RTEM β-lactamase from *Escherichia coli* at different pH values. Biochemistry **20**:3688–3694.
16. **Nikaido, H., E. Y. Rosenberg, and J. Foulds.** 1983. Porin channels in *Escherichia coli*: studies with β-lactams in intact cells. J. Bacteriol. **153**:232–240.
17. **Richmond, M. H., D. C. Clark, and S. Wotton.** 1976. Indirect method for assessing the penetration of beta-lactamase-nonsusceptible penicillins and cephalosporins in *Escherichia coli* strains. Antimicrob. Agents Chemother. **10**:215–218.
18. **Richmond, M. H., and S. Wotton.** 1976. Comparative study of seven cephalosporins: susceptibility to β-lactamases and ability to penetrate the surface layers of *Escherichia coli*. Antimicrob. Agents Chemother. **10**:219–222.
19. **Sanders, C. C.** 1983. Novel resistance selected by the new expanded-spectrum cephalosporins: a concern. J. Infect. Dis. **147**:585–589.
20. **Zimmermann, W., and A. Rosselet.** 1977. Function of the outer membrane of *Escherichia coli* as a permeability barrier to beta-lactam antibiotics. Antimicrob. Agents Chemother. **12**:368–372.

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Syntheses of β-Lactams by Ring Contraction of Isothiazolidinones

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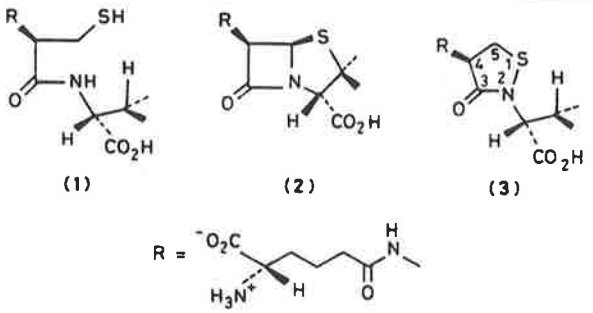
Several methods have been developed for the preparation of β-lactams from isothiazolidinones. Oxidation of 2-t-butyl-4,4-diphenyl- and 2,4,4-trimethyl-isothiazolidin-3-ones (**4a**) and (**4b**) with sulphuryl chloride afforded the 5-chloroisothiazolidinones (**5a**) and (**5b**), which were converted into 1-t-butyl-3,3-diphenyl- and 1,3,3-trimethyl-4-phenylthioazetidin-2-ones (**6a**) and (**6b**) by treatment with phenyl-lithium. Alternatively, reaction of the isothiazolidinones (**4a**) and (**4b**) with phenyl-lithium gave *N*-t-butyl-2,2-diphenyl- and *N*,2,2-trimethyl-3-phenylthiopropionamides (**12a**) and (**12b**), which were transformed into the β-lactams (**6a**) and (**6b**) by halogenation at C-3, followed by treatment with potassium amide. These are examples of methods used to prepare β-lactams from isothiazolidinones. The versatility of these reactions and their relevance to penicillin biosynthesis is discussed.

Studies of the biosynthesis of penicillins have shown that isopenicillin N (**2**) is derived from the tripeptide, [δ-(L-α-aminoadipoyl)]-L-cysteinyl-D-valine (**1**),[1]† but the mechanism of this conversion is unknown. On the basis of the facile *in vitro* oxidation of peptides to isothiazolidinones,[2,3] the isothiazolidinone (**3**) has been proposed as an intermediate in the *in vivo* transformation (**1**)→(**2**).[4] However, attempts to prepare β-lactams from isothiazolidinones, *in vitro*, as models for the conversion (**3**)→(**2**), have been unsuccessful.[4]

In this report full details are given of several preparations of β-lactams from isothiazolidinones.[5] The ease of transformation of isothiazolidinones into β-lactams gives credence to the proposed involvement of similar species in the *in vivo* conversion (**1**)→(**2**) in penicillin biosynthesis.



Results and Discussion

The isothiazolidinones (**4a**) and (**4b**) were chosen for initial studies because the substituents at C-4 limit possible side reactions involving that site. These compounds were prepared as shown in Scheme 1 *via* a procedure similar to that used by Baldwin *et al.*[4] in the preparation of compound (**4a**). The chlorides (**5a**) and (**5b**) were prepared from the isothiazolidinones (**4a**) and (**4b**) by treatment with sulphuryl chloride in carbon tetrachloride. They reacted with phenyl-lithium in ether at −78 °C to give the respective β-lactams (**6a**) and (**6b**) or, when scrupulously dry conditions were not maintained, mixtures of (**6a**) or (**6b**) and the corresponding chloroamide (**7a**) or (**7b**). When mixtures of compound (**5a**) or (**5b**) with 1.5 equivalents of water were treated with 1.5 equivalents of phenyl-lithium, the chloride (**7a**) or (**7b**) was produced in almost quantitative yield. Treatment of the chlorides (**7a**) and (**7b**) with potassium amide at −78 °C gave the corresponding β-lactams (**6a**) and (**6b**). The high yields of the products obtained in these reactions indicate that other modes of reaction are unimportant.

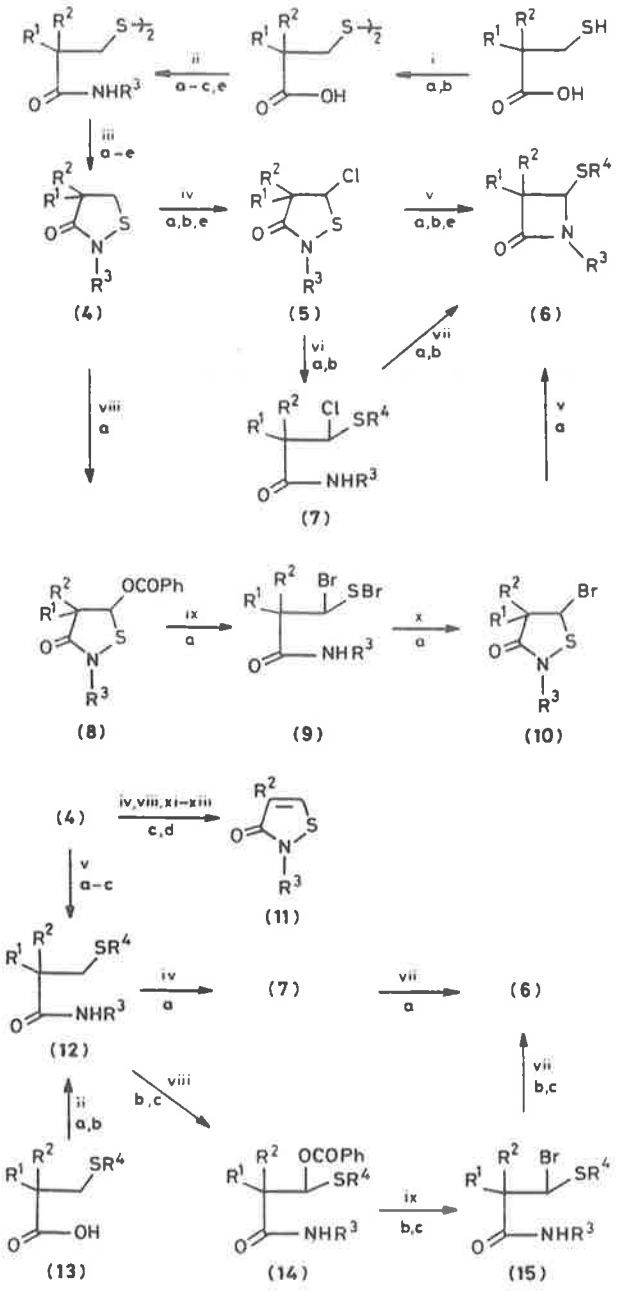
The reaction of the isothiazolidinones (**5a**) and (**5b**) with phenyl-lithium is thought to occur as shown in Scheme 2. Under moist conditions protonation of the intermediate amide anions competes with cyclisation. From the controlled experiment with 1.5 substrate-equivalents of water, it is clear that phenyl-lithium reacts with the isothiazolidinones (**5a**) and (**5b**) faster than it reacts with water, but cyclisation of the intermediate anions is slower than their reaction with water. The high reactivity of isothiazolidinones accounts for the anomalous formation of strained four-membered ring β-lactams by ring contraction of five-membered ring isothiazolidinones.

The ring contraction of isothiazolidinones to β-lactams occurs equally well with displacement of bromide as of chloride. The bromide (**10a**), synthesised from the isothiazolidinone (**4a**), *via* the benzoate (**8a**) and the sulphenyl bromide (**9a**),[4] also reacted with phenyl-lithium to give the β-lactam (**6a**).

An alternative preparation of the β-lactams (**6a**) and (**6b**) was accomplished by ring opening of the isothiazolidinones (**4a**) and (**4b**) prior to halogenation, with subsequent cyclisation. Treatment of the isothiazolidinones (**4a**) and (**4b**) with phenyl-lithium afforded the corresponding amides (**12a**) and (**12b**), identical with samples prepared from the respective carboxylic acids (**13a**) and (**13b**). The synthesis of the β-lactam (**6b**) from the amide (**12b**) *via* the benzoate (**14b**) and the bromide (**15b**) has already been reported.[6] The amide (**12a**) did not react with t-butyl perbenzoate under the conditions used to produce (**14b**), even with 10 molar equivalents of perester, indicating the greater steric hindrance towards hydrogen atom abstraction from the diphenyl-substituted amide (**12a**) than from the dimethyl-substituted analogue (**12b**). However, treatment of compound (**12a**) with sulphuryl chloride afforded the chloride (**7a**), a precursor of the β-lactam (**6a**) (see above).

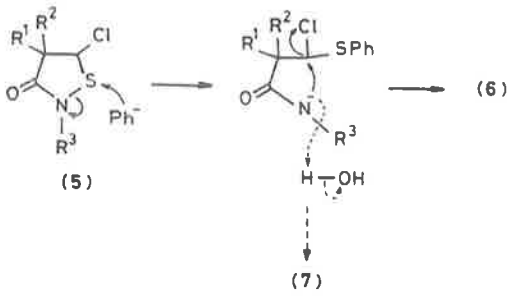
To extend the examination of the versatility of these reactions, the reactions of the isothiazolidinone (**4c**) with no substituents at C-4 were investigated. This compound was prepared from 3,3′-dithiodipropionic acid using the same method as that used to prepare compounds (**4a**) and (**4b**) (Scheme 1). Treatment of compound (**4c**) with sulphuryl chloride, *N*-chlorosuccinimide, chlorine, t-butyl perbenzoate, or bromine, failed to yield any C-5 substituted isothiazolidinone (**5c**), (**8c**), or (**10c**), thus preventing further elaboration to the β-lactam (**6c**). In each reaction the dihydroisothiazolone (**11c**) was produced. The β-lactam (**6c**) was synthesised from the

† δ-(α-Aminoadipoyl) = 5-amino-5-carboxypentanoyl.

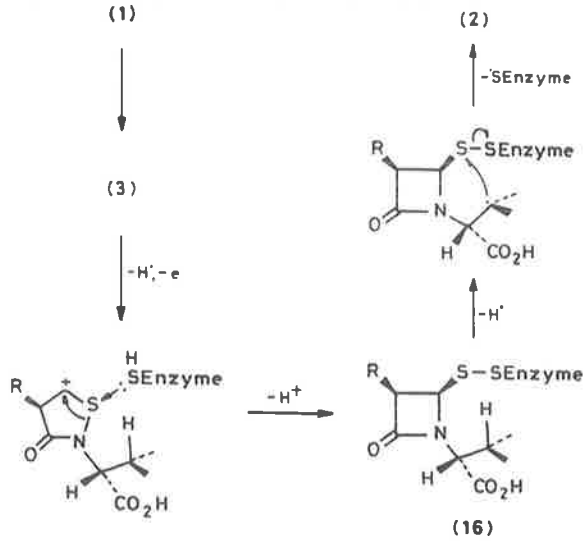


a; $R^1 = R^2 = R^4 = Ph$, $R^3 = Bu^t$
b; $R^1 = R^2 = R^3 = Me$, $R^4 = Ph$
c; $R^1 = R^2 = H$, $R^3 = Me$, $R^4 = Ph$
d; $R^1 = H$, $R^2 = NHCO_2CH_2Ph$, R^3 = 
e; $R^1 = R^2 = Ph$, R^3 =  , $R^4 = Me$

Scheme 1. *Reagents:* i, I_2; ii, (*a*) $SOCl_2$, (*b*) R^3NH_2, Et_3N; iii, Br_2, pyridine; iv, SO_2Cl_2 v, R^4Li; vi, R^4Li, H_2O; vii, KNH_2; viii, $Bu^tOOCOPh$; ix, HBr; x, NaOH; xi, *N*-chlorosuccinimide; xii, Cl_2; xiii, Br_2



Scheme 2.



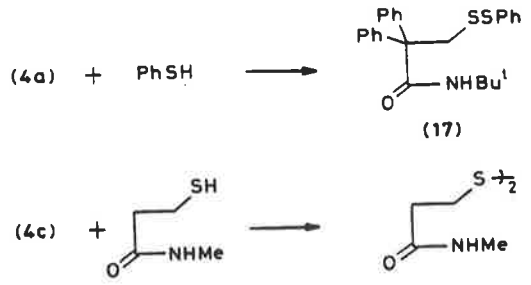
Scheme 3.

isothiazolidinone (**4c**) by the alternative method. Thus, treatment of compound (**4c**) with phenyl-lithium gave the amide (**12c**), from which the synthesis of the β-lactam (**6c**), *via* (**14c**) and (**15c**), has already been reported.[6]

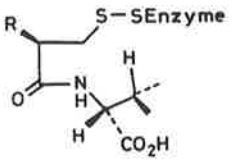
The isothiazolidinone (**4d**) was synthesised[3] since it closely resembles the isothiazolidinone (**3**). Treatment of compound (**4d**) with sulphuryl chloride, *N*-chlorosuccinimide, chlorine, bromine, or t-butyl perbenzoate, gave the dihydroisothiazolone (**11d**). The production of the dihydroisothiazolones (**11c**) and (**11d**) in these reactions of the isothiazolidinones (**4c**) and (**4d**) indicates that for isothiazolidinones not disubstituted at C-4, deprotonation and elimination is a very facile process. The C-5 substituted isothiazolidinones (**5d**), (**8d**), and (**10d**), were not detected in the reactions of compound (**4d**).

The C-4 disubstituted analogue of compound (**4d**), the isothiazolidinone (**4e**), was synthesised as shown in Scheme 1. Treatment of this compound with sulphuryl chloride gave the chloride (**5e**). Subsequent reaction with methyl-lithium afforded a mixture of the diastereoisomers of the β-lactam (**6e**). Thus rearrangement of oxidised isothiazolidinones to β-lactams can be promoted by methyl-lithium or phenyl-lithium, and occurs for a variety of *N*-substituted isothiazolidinones.

The reactions shown in Scheme 1 establish that β-lactams can be prepared from isothiazolidinones. *In vitro*, isothiazolidinones have been oxidised at C-5, and subsequently undergo nucleophile-promoted rearrangement to β-lactams. A similar mechanism could be involved in the *in vivo* transformation



Scheme 4.



(18)

(1)→(2) (Scheme 3). The idea that the nucleophile promoting the biological rearrangement could be a thiol residue of the penicillin synthetase enzyme is particularly appealing, as the formation of the disulphide (16) is fundamental to the mechanism proposed for the formation of the thiazolidine ring in (2).[6,7] This hypothesis is supported by *in vitro* experiments with model compounds. Thus, thiophenol reacted spontaneously with compound (4a) at room temperature to give the disulphide (17) and *N*-methyl-3-mercaptopropionamide reacted with compound (4c) to give *N*,*N*′-dimethyl-3,3′-dithiodipropionamide (Scheme 4). The mechanism shown in Scheme 3 is consistent with the results of all labelling and other biosynthetic studies[8,9] and accounts for the crucial role of the thiol group in compound (1) in binding the substrate to the enzyme during penicillin biosynthesis.[9]

The formation of disulphides by the reaction of isothiazolidinones with thiols provides *in vitro* models for the formation of the disulphide (18) from compound (1), *via* (3). An alternative mechanism proposed for the formation of the β-lactam ring in compound (2),[6,10] proceeding *via* the disulphide (18), did not rationalise the formation of (18) from (1).

Experimental

M.p.s were determined on a Kofler hot-stage apparatus and are uncorrected. I.r. spectra as liquid films, unless otherwise stated, were recorded on a Shimadzu IR-27G spectrometer. ^{1}H N.m.r. spectra were recorded in carbon tetrachloride using Me_4Si as internal standard, unless otherwise stated, on a Varian T60 spectrometer. Mass spectra were recorded on an AEI MS902 spectrometer and a Hewlett Packard 5982A spectrometer. Microanalyses were performed by the microanalytical laboratory, University of Otago. All solvents were purified and dried by standard methods. Ether refers to diethyl ether and light petroleum to the fraction with b.p. 50—70 °C.

2,2,2′,2′-Tetraphenyl-3,3′-dithiodipropionic Acid.—Iodine (2.55 g, 10 mmol) was added in small portions to a mixture of 2,2-diphenyl-3-mercaptopropionic acid[11] (5.2 g, 20 mmol), sodium hydroxide (0.8 g, 20 mmol), and potassium iodide (0.1 g, 0.6 mmol) in methanol (100 ml). After 15 min, sodium sulphite was added to decolourise the solution and the solvent was removed under reduced pressure. The residue was extracted with ethyl acetate and the extracts were evaporated to give crude 2,2,2′,2′-tetraphenyl-3,3′-dithiodipropionic acid (4.9 g, 94%) as a white solid, m.p. 120—124 °C (lit.,[4] 125—128 °C).

2,2,2′,2′-Tetramethyl-3,3′-dithiodipropionic Acid.—This compound was prepared from 2,2-dimethyl-3-mercaptopropionic acid[12] by treatment with iodine as described above. The crude product recrystallised from aqueous ethanol as white plates of the dithiodicarboxylic acid in 72% yield, m.p. 144—147 °C (lit.,[13] 145—146 °C).

2,2,2′,2′-Tetraphenyl-N,N′-di-t-butyl-3,3′-dithiodipropionamide.—A mixture of 2,2,2′,2′-tetraphenyl-3,3′-dithiodipropionic acid (4.6 g, 9 mmol) and thionyl chloride (6.0 g, 51 mmol) in dry benzene (50 ml) was heated under reflux for 4 h. The mixture was concentrated under reduced pressure and the residue was dissolved in dry dichloromethane, then added dropwise with stirring to an ice-cooled solution of t-butylamine (4.0 g, 55 mmol) and triethylamine (5.0 g, 49 mmol) in dichloromethane (50 ml). After 2 h at room temperature the mixture was repeatedly washed with water, then dried ($MgSO_4$) and concentrated to give crude 2,2,2′,2′-tetraphenyl-*N*,*N*′-di-t-butyl-3,3′-dithiodipropionamide (4.7 g, 84%) as a glass with spectral properties consistent with those previously reported.[4]

N,N′,2,2,2′,2′-Hexamethyl-3,3′-dithiodipropionamide.—The crude acid chloride prepared from 2,2,2′,2′-tetramethyl-3,3′-dithiodipropionic acid (5.3 g, 20 mmol) by treatment with thionyl chloride as described above, was dissolved in dry dichloromethane (20 ml) then added dropwise with stirring to an ice-cooled mixture of dichloromethane (50 ml) and 25% aqueous methylamine (50 ml). After 2 h at room temperature the dichloromethane layer was separated, repeatedly washed with water, then dried ($MgSO_4$) and concentrated to give crude *N*,*N*′,2,2,2′,2′-hexamethyl-3,3′-dithiodipropionamide (4.8 g, 82%) as an oil, δ($CDCl_3$) 6.3 (2 H, br), 3.05 (4 H, s), 2.82 (6 H, d, *J* 5 Hz), and 1.27 (12 H, s); $\nu_{max.}$ 3 350, 1 634, and 1 541 cm^{-1}; *m*/*z* 292 (M^+, 5%) and 146 (100).

N,N′-Dimethyl-3,3′-dithiodipropionamide.—Treatment of 3,3′-dithiodipropionic acid (Aldrich) with thionyl chloride and, subsequently, with methylamine as described above gave, after recrystallisation from aqueous ethanol, the required amide in 69% yield, m.p. 104—106 °C (lit.,[14] 105—108 °C).

N,N′-Bis(1-methoxycarbonyl-2-methylpropyl)-2,2,2′,2′-tetraphenyl-3,3′-dithiodipropionamide.—The crude acid chloride prepared from 2,2,2′,2′-tetraphenyl-3,3′-dithiodipropionic acid (4.6 g, 9 mmol) by treatment with thionyl chloride as described above, was dissolved in dry dichloromethane (20 ml) then added dropwise with stirring to an ice-cooled solution of L-valine methyl ester hydrochloride (5.0 g, 30 mmol) and triethylamine (7.5 g, 75 mmol) in dichloromethane (50 ml). After 2 h at room temperature the mixture was washed repeatedly with water, then dried ($MgSO_4$) and concentrated to give the crude amide (4.8 g, 72%) as a glass, δ($CDCl_3$) 7.3—7.0 (20 H, m), 5.9 (2 H, br d, *J* 9 Hz), 4.45 (2 H, dd, *J* 4 and 9 Hz), 3.68 (4 H, s), 3.64 (6 H, s), 2.0 (2 H, m), 0.70 (6 H, d, *J* 7 Hz) and 0.60 (6 H, d, *J* 7 Hz); $\nu_{max.}$ 3 480, 1 726, 1 656, and 1 479 cm^{-1}; *m*/*z* 370 (M^+/2, 2%) and 180 (100); *m*/*z* (C.I.; isobutane) 741 (MH^+).

4,4-Diphenyl-2-t-butylisothiazolidin-3-one (4a).—A solution of 2,2,2′,2′-tetraphenyl-*N*,*N*′-di-t-butyl-3,3′-dithiodipropionamide (4.7 g, 7.5 mmol) and pyridine (1.2 g, 15 mmol) in dichloromethane (100 ml) under N_2, cooled to −78 °C, was treated dropwise with a solution of bromine (1.2 g, 7.5 mmol) in dichloromethane (10 ml). After 15 min the mixture

was warmed to room temperature, concentrated, and the residue chromatographed on silica. Elution with chloroform afforded the isothiazolidinone (**4a**), which recrystallised from light petroleum as needles (3.3 g, 70%), m.p. 88—90 °C (lit.,[4] 89—90 °C).

2,4,4-*Trimethylisothiazolidin*-3-*one* (**4b**).—Treatment of *N,N'*,2,2,2',2'-hexamethyl-3,3'-dithiodipropionamide with bromine as described above afforded the isothiazolidinone (**4b**) as an oil in 57% yield, b.p. 120—130 °C at 0.8 mmHg (GKR); δ 3.27 (2 H, s), 2.85 (3 H, s), and 1.24 (6 H, s); $\nu_{max.}$ 1 652 cm^{-1}; *m/z* 145 (M^+, 78%), 56 (65), and 55 (100); *m/z* 145.0558 (M^+) [Calc. for $C_6H_{11}NOS$ (M^+) *m/z* 145.0561]. This compound was too hygroscopic for satisfactory microanalysis.

2-*Methylisothiazolidin*-3-*one* (**4c**).—Treatment of *N,N'*-dimethyl-3,3'-dithiodipropionamide with bromine as described above afforded the isothiazolidinone (**4c**) as an oil in 47% yield, b.p. 80—85 °C at 0.8 mmHg (lit.,[15] 46—47 °C at 0.05 mmHg).

4-*Benzyloxyformamido*-2-(1-*methoxycarbonyl*-2-*methylpropyl*)*isothiazolidin*-3-*one* (**4d**).—Treatment of benzyloxycarbonylcystinylvaline methyl ester[3] with bromine as described above afforded the isothiazolidinone (**4d**), recrystallised from light petroleum in 38% yield, m.p. 61—63 °C (lit.,[3] 65 °C).

2-(1-*Methoxycarbonyl*-2-*methylpropyl*)-4,4-*diphenylisothiazolidin*-3-*one* (**4e**).—Treatment of *N,N'*-bis(1-methoxycarbonyl-2-methylpropyl)-2,2,2',2'-tetraphenyl-3,3'-dithiodipropionamide with bromine as described above afforded the isothiazolidinone as an oil in 24% yield, b.p. 60—80 °C at 0.1 mmHg (GKR); δ 7.3 (10 H, m), 4.74 (1 H, d, *J* 8 Hz), 4.04 (2 H, s), 3.72 (3 H, s), 2.3 (1 H, m), 1.02 (3 H, d, *J* 7 Hz), and 0.92 (3 H, d, *J* 7 Hz); $\nu_{max.}$ 1 741 and 1 675 cm^{-1}; *m/z* 369 (M^+, 100%), 310 (94), and 254 (57); *m/z* 369.1390 (M^+) [Calc. for $C_{21}H_{23}NO_3S$ (M^+) *m/z* 369.1399].

5-*Chloro*-4,4-*diphenyl*-2-*t*-*butylisothiazolidin*-3-*one* (**5a**).—Sulphuryl chloride (1.35 g, 10 mmol) in carbon tetrachloride (10 ml) was added dropwise at room temperature to a stirred solution of the isothiazolidinone (**4a**) (3.1 g, 10 mmol) in carbon tetrachloride (50 ml). The resultant mixture was treated with $MgSO_4$, then filtered and concentrated to give white crystals of the crude chloride (**5a**) (2.45 g, 71%), m.p. 92—94 °C (decomp.); δ 7.5—7.0 (10 H, m), 6.18 (1 H, s), and 1.49 (9 H, s).

5-*Chloro*-2,4,4-*trimethylisothiazolidin*-3-*one* (**5b**).—Treatment of the isothiazolidinone (**4b**) with sulphuryl chloride as described above gave the crude chloride (**5b**) as an oil in 80% yield, δ 5.46 (1 H, s), 3.00 (3 H, s), and 1.37 (6 H, s).

5-*Chloro*-2-(1-*methoxycarbonyl*-2-*methylpropyl*)-4,4-*diphenylisothiazolidin*-3-*one* (**5e**).—Treatment of the isothiazolidinone (**4e**) with sulphuryl chloride as described above gave the crude chloride (**5e**) as an oil in 65% yield, δ 7.6—7.1 (10 H, m), 6.38 (1 H, s), 4.83 (1 H, d, *J* 8 Hz), 3.56 (3 H, s), 2.3 (1 H, m), and 1.02 (6 H, d, *J* 6 Hz).

3,3-*Diphenyl*-4-*phenylthio*-1-*t*-*butylazetidin*-2-*one* (**6a**).—A solution of the chloride (**5a**) (0.34 g, 1 mmol) in ether (20 ml) under N_2, cooled to −78 °C, was treated with *ca.* 1.6M-phenyllithium (0.7 ml, 1.1 mmol). After 30 min the mixture was warmed to room temperature and treated with water. The ether layer was separated, dried ($MgSO_4$) and concentrated. Distillation of the residue afforded the β-lactam (**6a**) (0.335 g, 86%) as a colourless oil, b.p. 120—130 °C at 1.0 mmHg (GKR), δ 7.3—6.9 (15 H, m), 5.53 (1 H, s), and 1.42 (9 H, s); $\nu_{max.}$ 1 752 cm^{-1}, *m/z* 387 (M^+, 14%), 288 (30), 278 (40), and 194 (100), *m/z* 387.1646 (M^+) [Calc. for $C_{25}H_{25}NOS$ (M^+) *m/z* 387.1657] (Found: C, 77.3; H, 6.7; N, 3.4. Calc. for $C_{25}H_{25}NOS$: C, 77.48; H, 6.50; N, 3.61%).

1,3,3-*Trimethyl*-4-*phenylthioazetidin*-2-*one* (**6b**).—Treatment of the chloride (**5b**) with phenyl-lithium under anhydrous conditions as described above afforded the β-lactam (**6b**) in 89% yield, b.p. 70—80 °C at 1.0 mmHg (GKR), with spectral properties consistent with those previously reported.[6]

1-(1-*Methoxycarbonyl*-2-*methylpropyl*)-4-*methylthio*-3,3-*diphenylazetidin*-2-*one* (**6e**).—Treatment of the chloride (**5e**) with methyl-lithium as described above for the reactions of the chlorides (**5a**) and (**5b**) with phenyl-lithium afforded an oil which crystallised from ethyl acetate–light petroleum as flakes of the β-lactam (**6e**) in 39% yield, m.p. 81—88 °C (lit.[16] 75—87 °C). The diastereoisomers were in the ratio *ca.* 2:1 (^1H n.m.r.)[16]

3-*Chloro*-2,2-*diphenyl*-3-*phenylthio*-N-*t*-*butylpropionamide* (**7a**).—A solution of the chloride (**5a**) (0.34 g, 1 mmol) in ether (20 ml) and water (27 mg, 1.5 mmol) under N_2, cooled at −78 °C, was treated with *ca.* 1.6M-phenyl-lithium (0.95 ml, 1.5 mmol). After 30 min the mixture was warmed to room temperature and treated with water. The ether layer was separated, dried ($MgSO_4$), and concentrated to give the crude chloride (**7a**) as an oil (0.37 g, 88%), δ 7.6—6.9 (15 H, m), 6.22 (1 H, s), 5.3 (1 H,br), and 1.22 (9 H, s).

3-*Chloro*-N,2,2-*trimethyl*-3-*phenylthiopropionamide* (**7b**).—Treatment of the chloride (**5b**) with phenyl-lithium in moist ether as described above gave the chloride (**7b**) as an oil in 84% yield, δ 7.5—7.1 (5 H, m), 6.0 (1 H, br), 5.67 (1 H, s), 2.60 (3 H, d, *J* 5 Hz), and 1.42 (6 H, s).

Reaction of the Chloride (**7a**) *with Potassium Amide.*—The chloride (**7a**) (0.34 g, 0.8 mmol) was dissolved in dichloromethane (10 ml) and cooled to −78 °C, then added dropwise to a solution of potassium amide at −78 °C which had been prepared from ammonia (50 ml), potassium (40 mg, 1 mmol), and ferric nitrate (*ca.* 2 mg). After 30 min at −78 °C ammonia was removed under reduced pressure, dichloromethane (25 ml) and water (25 ml) were added, the organic layer was separated, washed with water, dried ($MgSO_4$) and concentrated, and the residue was distilled to give the β-lactam (**6a**) (0.26 g, 82%), identical in all respects with the sample obtained as described above.

Reaction of the Chloride (**7b**) *with Potassium Amide.*—Treatment of the chloride (**7b**) with potassium amide as described above afforded the β-lactam (**6b**) in 89% yield, identical in all respects with the sample obtained as described above.

Reaction of the Bromide (**10a**) *with Phenyl-lithium.*—Treatment of the bromide (**10a**)[4] with phenyl-lithium under anhydrous conditions as described above afforded the β-lactam (**6a**) in 64% yield, identical in all respects with the sample obtained as described above.

Reactions of the Isothiazolidinones (**4c**) *and* (**4d**) *with Sulphuryl Chloride, Chlorine, Bromine, and* N-*Chlorosuccinimide.*—A solution of the isothiazolidinone (**4c**) or (**4d**) (1 mmol) in carbon tetrachloride (20 ml) was treated at room temperature with sulphuryl chloride, chlorine, bromine, or *N*-chlorosuccinimide (1 mmol), either neat or as a solution in carbon tetrachloride. Analysis by t.l.c. and ^1H n.m.r. spectroscopy indicated virtually complete reaction of the isothiazolinones (**4c**) and (**4d**) and formation of the

corresponding dihydroisothiazolones (**11c**) and (**4d**),[3,14] but not of the respective isothiazolidinones (**5c**) and (**5d**) or (**10c**) and (**10d**). Reactions in other solvents and at lower temperatures produced similar results.

Reactions of the Isothiazolidinones (**4c**) *and* (**4d**) *with t-Butyl Perbenzoate.*—A mixture of the isothiazolidinone (**4c**) or (**4d**) (1 mmol), t-butyl perbenzoate (0.1 g, 2 mmol), and cuprous chloride (*ca.* 5 mg) in benzene (10 ml) under nitrogen, was heated under reflux for 4 h, then cooled. Analysis by t.l.c. and ^{1}H n.m.r. spectroscopy indicated incomplete reaction of the isothiazolidinones (**4c**) and (**4d**) and formation of the corresponding dihydroisothiazolones (**11c**) and (**11d**),[3,14] but not of the isothiazolidinones (**8c**) and (**8d**).

Reaction of the Isothiazolidinone (**4a**) *with Phenyl-lithium.*—Treatment of the isothiazolidinone (**4a**) with phenyl-lithium under anhydrous conditions as described above gave 2,2-diphenyl-3-phenylthio-*N*-t-butylpropionamide (**12a**), which recrystallised from light petroleum as needles in 82% yield, m.p. 80—81 °C, δ 7.4—6.9 (15 H, m), 5.3 (1 H, br), 3.83 (2 H, s), and 1.25 (9 H, s); $\nu_{max.}$ (Nujol) 3 425 and 1 668 cm^{-1}; *m/z* 389 (M^+, 43%), 280 (9), 266 (10), and 180 (100); *m/z* 389.1777 (M^+) [Calc. for $C_{25}H_{27}NOS$ (M^+) *m/z* 389.1813] (Found: C, 76.95; H, 6.9; N, 3.6. Calc. for $C_{25}H_{27}NOS$: C, 77.08; H, 6.99; N, 3.60%).

Reaction of the Isothiazolidinone (**4b**) *with Phenyl-lithium.*—Treatment of the isothiazolidinone (**4b**) with phenyl-lithium under anhydrous conditions as described above afforded *N*,2,2-trimethyl-3-phenylthiopropionamide (**12b**), which recrystallised from ethyl acetate–light petroleum as needles in 92% yield, m.p. 60—61 °C (lit.,[6] 61.5—62.0 °C), identical with a sample synthesised from 2,2-dimethyl-3-phenylthiopropionic acid (**13b**).[6]

Reaction of the Isothiazolidinone (**4c**) *with Phenyl-lithium.*—Treatment of the isothiazolidinone (**4c**) with phenyl-lithium under anhydrous conditions as described above afforded *N*-methyl-3-phenylthiopropionamide (**12c**), which recrystallised from ethyl acetate–light petroleum as needles in 83% yield, m.p. 90—92 °C (lit.,[6] 89—91 °C).

2,2-*Diphenyl*-3-*phenylthio*-N-*t*-*butylpropionamide* (**12a**).—Treatment of 2,2-diphenyl-3-phenylthiopropionic acid (**13a**)[11] with thionyl chloride and, subsequently, with t-butylamine as described above afforded the amide (**12a**) in 90% yield, identical in all respects with the sample obtained as described above.

Attempted Reaction of the Amide (**12a**) *with t-Butyl Perbenzoate.*—A mixture of the amide (0.2 g, 0.5 mmol), t-butyl perbenzoate (0.26 g, 5 mmol), and cuprous chloride (*ca.* 5 mg) in benzene (20 ml) under nitrogen, was heated under reflux for 12 h, then cooled. Analysis by t.l.c. and ^{1}H n.m.r. spectroscopy indicated no reaction of the amide (**12a**), but >95% decomposition of t-butyl perbenzoate.

Reaction of the Amide (**12a**) *with Sulphuryl Chloride.*—Treatment of the amide (**12a**) with sulphuryl chloride as described above afforded the chloride (**7a**) in 60% yield, identical in all respects with the sample obtained as described above.

2,2-*Diphenyl*-3-*phenyldithio*-N-*t*-*butylpropionamide* (**17**).—A mixture of the isothiazolidinone (**4a**) (0.31 g, 1 mmol) and thiophenol (0.11 g, 1 mmol) in carbon tetrachloride (10 ml) was stirred at room temperature for 4 h, then concentrated under reduced pressure. The residue crystallised from light petroleum–dichloromethane to give the disulphide (**17**) (0.38 g, 89%) as a powder, m.p. 96—98 °C; δ($CDCl_3$) 7.4—7.1 (15 H, m), 5.3 (1 H, br), 3.90 (2 H, s), and 1.25 (9 H, s); $\nu_{max.}$ 3 440, 1 670, 1 509, and 1 451 cm^{-1}; *m/z* 421 (M^+, 9%), 312 (26), 280 (24), 194 (57), and 180 (100); *m/z* 421.1542 (M^+) [Calc. for $C_{25}H_{27}NOS_2$ (M^+) *m/z* 421.1534].

Reaction of the Isothiazolidinone (**4c**) *with* 3-*Mercapto*-N-*methylpropionamide.*—A mixture of the isothiazolidinone (**4c**) (0.12 g, 1 mmol) and 3-mercapto-*N*-methylpropionamide[17] (0.12 g, 1 mmol) in chloroform was stirred at room temperature for 24 h, then concentrated under reduced pressure. The residue recrystallised from ethyl acetate–light petroleum to give *N*,*N*′-dimethyl-3,3′-dithiodipropionamide (0.19 g, 81%), identical in all respects with the sample obtained as described above.

Acknowledgements

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References

1 J. O'Sullivan, R. C. Bleaney, J. A. Huddleston, and E. P. Abraham, *Biochem. J.*, 1979, **184**, 421; Y. Sawada, J. E. Baldwin, P. D. Singh, N. A. Solomon, and A. L. Demain, *ibid.*, p. 465; for a review see D. J. Aberhart, *Tetrahedron*, 1977, **33**, 1545.
2 J. E. Baldwin, S. B. Haber, and J. Kitchin, *J. Chem. Soc., Chem. Commun.*, 1973, 790.
3 R. B. Morin, E. M. Gordon, and J. R. Lake, *Tetrahedron Lett.*, 1973, 5213.
4 J. E. Baldwin, A. L. J. Beckwith, A. P. Davis, G. Procter, and K. A. Singleton, *Tetrahedron*, 1981, **37**, 2181.
5 C. J. Easton, *J. Chem. Soc., Chem. Commun.*, 1983, 1349.
6 A. L. J. Beckwith and C. J. Easton, *Tetrahedron*, 1983, **39**, 3995.
7 J. E. Baldwin and T. S. Wan, *J. Chem. Soc., Chem. Commun.*, 1979, 249; C. J. Easton and N. J. Bowman, *J. Chem. Soc., Chem. Commun.*, 1983, 1193.
8 D. J. Morecombe and D. W. Young, *J. Chem. Soc., Chem. Commun.*, 1975, 198; D. J. Aberhart, L. J. Lin, and J. Y. R. Chu, *J. Chem. Soc., Perkin Trans. 1*, 1975, 2157; N. Neuss, C. H. Nash, J. E. Baldwin, P. A. Lemke, and J. B. Grutzner, *J. Am. Chem. Soc.*, 1973, **95**, 3797; H. Kluender, C. H. Bradley, C. J. Sih, P. Fawcett, and E. P. Abraham, *ibid.*, p. 6149; D. J. Aberhart and L. J. Lin, *J. Chem Soc., Perkin Trans. 1*, 1974, 2320.
9 R. D. Adlington, R. T. Aplin, J. E. Baldwin, B. Chakravarti, L. D. Field, E. M. John, E. P. Abraham, and R. L. White, *Tetrahedron*, 1983, **39**, 1061.
10 J. E. Baldwin and A. P. Davis, *J. Chem Soc., Chem. Commun.*, 1981, 1219.
11 H. E. Zaugg, H. J. Glenn, R. J. Michaels, R. U. Schock, and L. R. Swett, *J. Am. Chem. Soc.*, 1957, **79**, 3912.
12 P. Blondeau, R. Gauthier, C. Berse, and D. Gravel, *Can. J. Chem.*, 1971, **49**, 3866.
13 R. M. Acheson, J. A. Barltrop, M. Hichens, and R. E. Hichens, *J. Chem. Soc.*, 1961, **164**, 650.
14 S. N. Lewis, G. A. Miller, M. Hausman, and E. C. Szamborski, *J. Heterocycl. Chem.*, 1971, **8**, 571.
15 A. Luettringhaus and R. Schneider, *Liebigs Ann. Chem.*, 1964, **679**, 123.
16 M. D. Bachi and O. Goldberg, *J. Chem. Soc., Perkin Trans. 1*, 1972, 2332.
17 J. Nyitrai, J. Fetter, G. Hornyak, K. Zauer, and K. Lempert, *Tetrahedron*, 1978, **34**, 1031.

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Regioselectivity in Formations of Amidocarboxy-substituted Free Radicals

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Deuterium isotope effects establish a direct contrast in regioselectivity in reactions of *N*-benzoylvaline methyl ester with sulphuryl chloride and *N*-bromosuccinimide, and are indicative of factors affecting production of amidocarboxy-type capto-dative free radicals.

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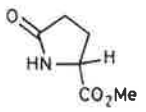
Deuterium isotope effects establish a direct contrast in regioselectivity in reactions of *N*-benzoylvaline methyl ester with sulphuryl chloride and *N*-bromosuccinimide, and are indicative of factors affecting production of amidocarboxy-type capto-dative free radicals.

There have been two reports of regioselective hydrogen-atom transfer reactions affording amidocarboxy-substituted radicals. Irradiation of a mixture of methyl pyroglutamate (**1**) and di-t-butyl peroxide afforded products attributed to dimerization of the radical (**2**).[1] and oxidation of (**3a**) gave the diperoxide (**3b**).[2] We reported recently that reaction of *N*-benzoylvaline methyl ester (**4a**) with sulphuryl chloride gave the β-chlorovaline (**5**) and lesser amounts of diastereoisomers of the γ-chloro derivative (**6**).[3] This result is at variance with the earlier work as it indicates that the radicals (**7a**) and (**8**), intermediates in the reactions to give (**5**) and (**6**) respectively,[4] are formed in preference to the amidocarboxy-



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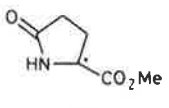

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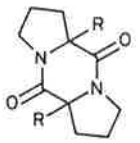


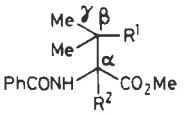
(3)
a; R = H
b; R = OOH

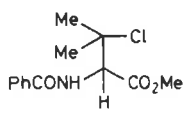
substituted radical (**9a**). This discord prompted our present study of the reaction of (**4a**) and the deuteriated analogues (**4b**) and (**4c**)† with sulphuryl chloride, and of the free-radical reaction of (**4a—c**) with *N*-bromosuccinimide (NBS).[5]

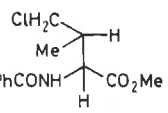
In measuring the relative rates of reaction of (**4a—c**) we exploited their chirality. The enantiomers exhibit identical reactivity, but are physically separable by g.l.c. on a Chrompack XE-60-S-VAL-S-X-PEA column. Thus we were able to measure the relative rates of consumption of (**4a—c**) from mixtures, for example, of the 2*R*-valine (**4a**) and the 2*S*-valine (**4b**). The ratios of the rate constants for the reactions of (**4a—c**) with sulphuryl chloride and NBS were calculated from the relative rates of consumption of (**4a—c**)[6] and these are presented in Table 1.

The relative rate constants for reaction of (**4a—c**) with sulphuryl chloride establish that there is a deuterium isotope effect for β-C–H bond cleavage, but no isotope effect for α-C–H bond cleavage. In direct contrast, the reaction with NBS exhibits no deuterium isotope effect for β-C–H bond cleavage, but there is an effect for α-C–H bond cleavage. These results establish that cleavage of the β-C–H bond is the irreversible rate-determining step in the reaction with sulphuryl chloride, whereas cleavage of the α-C–H bond is the irreversible rate-determining step in the reaction with NBS. We propose that the most reasonable interpretation of these results is that the chlorination involves hydrogen-atom abstraction from the β-position of (**4**) with subsequent chlorine incorporation to give (**5**),‡ whereas the reaction with NBS proceeds *via* hydrogen-atom abstraction from the α-position of (**4**) and yields the dibromide (**10a**), the final product of the reaction with NBS.

Variations of selectivity in hydrogen-atom transfer reactions have been reviewed by Russell.[7] The contrast of regioselectivity observed in the reactions with sulphuryl chloride and NBS may be interpreted in terms of the relative degrees of C–H bond homolysis in the transition states of these reactions. The greater deuterium isotope effect for α-C–H bond cleavage in the reaction with NBS compared to the effect for β-C–H bond homolysis in the chlorination



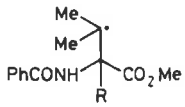


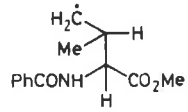


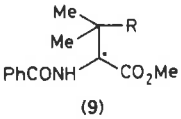
(4)
a; $R^1 = R^2 = H$
b; $R^1 = D$, $R^2 = H$
c; $R^1 = H$, $R^2 = D$

(5)

(6)



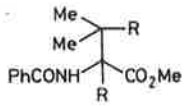




(7)
a; R = H
b; R = Cl

(8)

(9)
a; R = H
b; R = Cl



(10)
a; R = Br
b; R = Cl

Table 1. Relative rates of reaction of the valines (**4a—c**).

	Reagents	
Valine	Sulphuryl chloride[a]	NBS[b]
(**4a**)	1.0[c]	1.0[c]
(**4b**)	1.25 ± 0.05	1.00 ± 0.03
(**4c**)	1.00 ± 0.03	3.70 ± 0.20

[a] Valine and sulphuryl chloride in benzene under nitrogen. Reaction at reflux catalysed by benzoyl peroxide or irradiation. [b] Valine and NBS in CCl_4 under nitrogen. Reaction at reflux catalysed by benzoyl peroxide or irradiation. [c] Assigned as unity for each reagent.

reaction indicates a greater degree of bond homolysis in the transition state of the former. With little development of radical character in the transition state of the chlorination reaction, the regioselectivity in this case is controlled by the inductive electron-withdrawing effect of the amido and carboxy groups acting to retard attack at the adjacent α-position by electrophilic radicals involved in the hydrogen-atom abstraction. The reaction with NBS is more sensitive to radical-stability effects since there is a greater degree of development of radical character in the transition state. Hydrogen-atom transfer from the α-position is favoured, therefore, because the product is the capto-dative radical (**9a**), stabilized by the synergistic effect of resonance electron-donating amido and electron-withdrawing carboxy groups.[8]

In a related system the dichloride (**10b**) reacted with tri-n-butyltin hydride to give the β-chlorovaline (**5**) in almost quantitative yield. Reductions of alkyl halides by organotin hydrides proceed by halogen-atom abstraction with subsequent hydrogen incorporation. In these reactions stability of the free-radical intermediate is the prime factor in determining the rate of halogen-atom abstraction.[9] Thus the production of (**5**) from (**10b**) implies that the capto-dative radical (**9b**) is more stable than the tertiary radical (**7b**). Further, the production of only trace amounts of (**4a**), the product of

† All new compounds were fully characterised.

‡ We assume that the γ-chloro derivative (**6**) is produced by hydrogen-atom transfer from the γ-position of (**4**) with subsequent chlorine incorporation. Because of this alternative reaction pathway the deuterium isotope effect for β-C–H bond homolysis would be moderately higher (*ca.* 30%) than indicated by the relative rates of reaction of (**4a**) and (**4b**).

subsequent reduction of (**5**),[10] in the reaction with tri-n-butyltin hydride establishes that the rate of halogen-atom abstraction from (**10b**) to give (**9b**) is at least three orders of magnitude faster than the rate of halogen-atom transfer from (**5**) to give (**7a**). This implies the greater relative stability of the capto-dative radical (**9b**) compared with (**7a**).

These results support our conclusion that the contrast in regioselectivity in the reactions of *N*-benzoylvaline methyl ester (**4**) with sulphuryl chloride and NBS can be attributed to the respective degrees of C–H bond homolysis in the transition states of these reactions. Extensive bond homolysis and development of radical character in the transition state of the reaction of (**4**) with NBS results in reaction *via* α-C–H bond homolysis to give the stabilized radical (**9a**), whereas the lack of development of radical character in the transition state of the reaction of (**4**) with sulphuryl chloride is manifest in regioselectivity determined by inductive effects and resulting in β-C–H bond homolysis. The amidocarboxy-type capto-dative radicals (**9a**) and (**9b**) are considerably more stable than, for example, the tertiary radicals (**7a**) and (**7b**), but hydrogen-atom transfer reactions may afford less-stable products if electrophilic radicals are involved in the hydrogen-atom abstraction and if there is little development of radical character in the reaction transition state.

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References

1 N. Obata and K. Niimura, *J. Chem. Soc., Chem. Commun.*, 1977, 238.
2 U. Schmidt, J. Hausler, E. Ohler, and H. Poisel, *Prog. Chem. Org. Nat. Prod.*, 1979, **37**, 286.
3 C. J. Easton and N. J. Bowman, *J. Chem. Soc., Chem. Commun.*, 1983, 1193.
4 H. C. Brown and A. B. Ash, *J. Am. Chem. Soc.*, 1955, **77**, 4019; G. A. Russel and H. C. Brown, *ibid.*, p. 4031.
5 M. L. Poutsma, in 'Free Radicals,' ed. J. K. Kochi, Wiley, New York, 1973, vol. 2, p. 211.
6 K. Hayday and R. D. McKelvey, *J. Org. Chem.*, 1976, **41**, 2222; C. Walling and M. J. Gibion, *J. Am. Chem. Soc.*, 1965, **87**, 3361.
7 G. A. Russell, in 'Free Radicals,' ed. J. K. Kochi, Wiley, New York, 1973, vol. 1, p. 275.
8 H. G. Viehe, R. Merenyi, L. Stella, and Z. Janousek, *Angew. Chem., Int. Ed. Engl.*, 1979, **18**, 917.
9 H. G. Kuivila, *Acc. Chem. Res.*, 1968, **1**, 299.
10 N. G. Findlay, unpublished observations.

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SYNTHESIS OF (2R)- AND (2S)-[3-^{2}H]-VALINE

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SUMMARY

(2R)-Valine and the (2S)-isomer were esterified and benzoylated. The derivatives were chlorinated at C-3 by treatment with sulphuryl chloride, reduced to the 3-^{2}H derivatives by reaction with triphenyltin deuteride, then hydrolysed to give (2R)- and (2S)-[3-^{2}H]-valine. The labelling is achieved with complete retention of optical purity.

Key Words: (2R)-[3-^{2}H]-valine, (2S)-[3-^{2}H]-valine, deuterium, triphenyltin deuteride.

INTRODUCTION

In order to study aspects of valine metabolism we required a simple, high yield synthesis of (2R)- and (2S)-[3-^{2}H]-valine. Syntheses of (2R,S)-[3-^{2}H]- and [3-^{3}H]-valines have been reported (1-3). In the most recent work (3) (2R)-[3-^{2}H]- and [3-^{3}H]-valines were obtained by enzymic resolution of (R,S)-mixtures. The synthesis of (2S)-[3-^{2}H]- or [3-^{3}H]-valine has never been reported, although conceivably these compounds could also be prepared by enzymic resolution of (R,S)-mixtures. This paper describes a synthesis of (2R)- and (2S)-[3-^{2}H]-valine that circumvents the need for resolution of (R,S)-mixtures.

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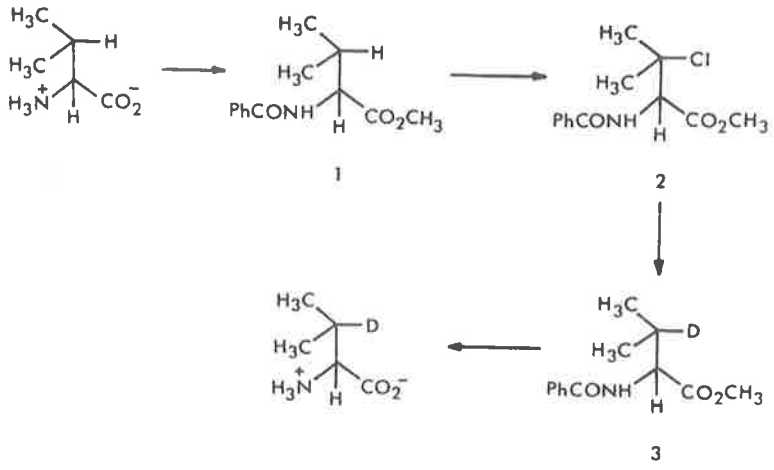
RESULTS AND DISCUSSION

Reaction of (2R)- and (2S)-valine with thionyl chloride in methanol, followed by treatment with benzoyl chloride, afforded (2R)- and (2S)-N-benzoylvaline methyl ester, 1a and 1b. Reaction of these valine derivatives with sulphuryl chloride in benzene (4) afforded the respective 3-chlorovaline derivatives 2a and 2b as the principal products, the yields being solvent dependent as well as dependent on the concentration of the substrates 1a and 1b (5). The chlorides 2a and 2b were purified by HPLC or column chromatography. Reduction of the chlorides 2a and 2b by reaction with triphenyltin deuteride afforded (2R)- and (2S)-[3-^{2}H]-N-benzoylvaline methyl ester, 3a and 3b. Analysis of these compounds by GLC established their chiral integrity. Hydrolysis of the labelled valine derivatives 3a and 3b in refluxing hydrochloric acid afforded (2R)- and (2S)-[3-^{2}H]-valine.



1 2 3

a = (2R), b = (2S)

Thus (2R)- and (2S)-[3-^{2}H]-valine were prepared from (2R)- and (2S)-valine, in 32% and 27% yield, respectively. Because the valine structure is retained throughout the synthesis, the method should be suitable for synthesis of double-labelled valines for use in cotracer experiments.

EXPERIMENTAL

General ^{1}H NMR spectra were recorded on a Varian T60 spectrometer and ^{13}C NMR spectra were recorded on a Varian CFT20 spectrometer. Analytical GLC was carried out using a Chrompack XE-60-S-VAL-S-X-PEA column (50 m x 0.22 mm i.d.), for analysis of chiral compounds.

(2R)-N-Benzoylvaline methyl ester, 1a.(2R)-Valine (3.0 g, 0.025 mole) was added to a stirred solution of thionyl chloride (3.7 ml) in methanol (25 ml). After 3 h the solvent was removed under reduced pressure. The crystalline residue was added to a stirred mixture of potassium carbonate (1.7 g), water (50 ml), and ethyl acetate (50 ml). Benzoyl chloride (10 ml) was then added dropwise with stirring. After 4 h the organic layer was separated, the aqueous layer was extracted with ethyl acetate (2 x 50 ml), and the combined ethyl acetate solutions were washed with 5% aqueous potassium bicarbonate, dried ($MgSO_4$), and concentrated. The residue recrystallised from ethyl acetate - light petroleum to give needles of (2R)-N-benzoylvaline methyl ester, 1a (4.9 g, 83%):m.p. 109-111° [lit.(6) 110.5-111.0°]; ^{1}H NMR (CCl_4) δ1.01 (6H, d, J = 7 Hz), 2.2 (1H, m), 3.73 (3H, s), 4.70 (1H, dd, J = 4 and 9 Hz), 6.54 (1H, broad d, J = 9 Hz), and 7.2-8.0 (5H, m). Chiral purity was established by GLC comparison with an (R,S)- mixture of 1a and 1b.

(2S)-N-Benzoylvaline methyl ester, 1b, was prepared as described above for the (2R)-isomer, 1a : 78% yield; m.p. 108-110° [lit.(7)111°]; ^{1}H NMR as for 1a; optically pure by GLC.

(2R)-N-Benzoyl-3-chlorovaline methyl ester, 2a. (2R)-N-Benzoylvaline methyl ester, 1a (2.0 g, 0.0085 mole), sulphuryl chloride (4 ml), and benzoyl peroxide (30 mg) in benzene (150 ml) were heated under reflux for 1 h. The mixture was concentrated and chromatographed on silica. Elution with ethyl acetate - dichloromethane (1:19) afforded (2R)-N-benzoyl-3-chlorovaline methyl ester, 2a, as an oil (1.4 g, 61%) : ^{1}H NMR (CCl_4) δ1.63 (3H, s), 1.77 (3H, s), 3.77 (3H, s), 4.87 (1H, d, J = 9 Hz), 6.80 (1H, broad d, J = 9 Hz), and 7.1-8.0 (5H, m). Alternatively the chloride 2a was purified by preparative HPLC, performed using a DuPont Zorbax cyanopropyl column (25 cm x 9.4 mm i.d.), using hexane - propan-2-ol(9:1) as eluant, monitoring at 220 nm.

(2S)-N-Benzoyl-3-chlorovaline methyl ester, 2b, was prepared as described above for the chloride 2a : 55% yield; ^{1}H NMR as for 2a.

(2R)-[3-^{2}H]-N-Benzoylvaline methyl ester, 3a. (2R)-N-Benzoyl-3-chlorovaline methyl ester, 2a (0.81 g, 0.003 mole), and triphenyltin deuteride (8) (3.0 ml) in benzene (30 ml) were refluxed under nitrogen for 5 h. The mixture was concentrated and chromatographed on silica. Elution with ethyl acetate - dichloromethane (1:9) afforded (2R)-[3-^{2}H]-N-benzoylvaline methyl ester, 3a, which recrystallised from ethyl acetate - light petroleum (0.55 g, 78%): m.p. 108-109°; ^{1}H NMR (CCl_4) δ0.98 (6H, s), 3.70 (3H, s), 4.64 (1H, d, J = 8 Hz), 6.60 (1H, broad d, J = 8 Hz), and 7.1-7.9 (5H, m). Chiral integrity was established by GLC - none of the (2S)-isomer 3b was detected and the limits of detection were <0.5%.

(2S)-[3-^{2}H]-N-Benzoylvaline methyl ester, 3b, was prepared analogously to the (2R)-[3-^{2}H]-derivative 3a : 82% yield; m.p. 108-110°; ^{1}H NMR as for 3a; single isomer by GLC.

(2R)-[3-^{2}H]-Valine. A solution of (2R)-[3-^{2}H]-N-benzoylvaline methyl ester, 3a (0.4 g, 0.0017 mole), in 2N hydrochloric acid (50 ml) was heated under reflux for 3 h, then cooled, washed with chloroform (3 x 25 ml), and evaporated. The residue was dissolved in ethanol. Addition of pyridine induced precipitation of (2R)-[3-^{2}H]-valine which was isolated by filtration, then recrystallised from aqueous ethanol (0.16 g, 80%); $[\alpha]_D^{25}$= +27.9° (c = 1.5 in 5N HCl); ^{1}H NMR (D_2O) δ0.81 (3H, s), 0.86 (3H, s), and 3.44 (1H, s); ^{13}C NMR (broad-band ^{1}H-decoupled, D_2O) δ16.5 (s), 17.8 (s), 28.6 (t, J_{CD} = 20 Hz), 60.1 (s), and 174.0 (s). Residual protons at C-3 were not detected by ^{1}H NMR. This is consistent with a small signal in the ^{13}C NMR at δ29.0 from non-deuterated valine, indicating ⩽2% residual protons.

(2S)-[3-^{2}H]-Valine, was prepared as described for the (2R)-isomer : 77% yield; $[\alpha]_D^{25}$ = -28.1° (c = 1.5 in 5N HCl); ^{1}H NMR and ^{13}C NMR as for (2R)-[3-^{2}H]-valine.

ACKNOWLEDGMENT

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REFERENCES

1. J.C. Crawhall and D.G. Smyth - Biochem. J. 69 : 280 (1958).
2. P. Adriaens, H. Vanderhaeghe, B. Meesschaert, and H. Eyssen - Antimicrob. Agents Chemother. 8 : 15 (1975).

3. J.E. Baldwin and T.S. Wan - Tetrahedron 37 : 1589 (1981).

4. C.J. Easton and N.J. Bowman - J. Chem. Soc., Chem. Commun. : 1193 (1983).

5. N.J. Bowman and M.P. Hay, unpublished results.

6. T.H. Applewhite, H. Waite, and C. Niemann - J. Amer. Chem. Soc. 80 : 1465 (1958).

7. J.B. Jones, C. Niemann, and G.E. Hein - Biochem. 4 : 1735 (1965).

8. A.L.J. Beckwith, C.J. Easton, T. Lawrence, and A.K. Serelis - Aust. J. Chem. 36 : 545 (1983).

Preferential Reactivity of Glycine Residues in Free Radical Reactions of Amino Acid Derivatives

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Reactions of amino acid derivatives, including a novel synthetic procedure for direct and selective functionalisation of glycine derivatives, indicate that the particular reactivity of glycine residues in free radical reactions is due to the stability of the radicals produced by their atom transfer reactions.

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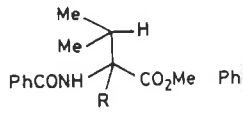
Reactions of amino acid derivatives, including a novel synthetic procedure for direct and selective functionalisation of glycine derivatives, indicate that the particular reactivity of glycine residues in free radical reactions is due to the stability of the radicals produced by their atom transfer reactions.

Preferential reactivity of glycine residues in free radical reactions of proteins, peptides, and other amino acid derivatives has been attributed to selective hydrogen atom abstraction from the α-carbon of the glycine moieties.[1] This selectivity is contrary to the expectation that tertiary radicals should be formed in preference to secondary ones.[2] Glycine residues afford secondary radicals by α-C–H bond homolysis, whereas analogous reactions of derivatives of other amino acids produce tertiary radicals. We have studied reactions of derivatives of glycine, alanine, and valine to examine this hitherto unexplained reactivity of glycine derivatives.

Relative rates of reaction of (**1a**), (**2a**), and (**3a**), and of the



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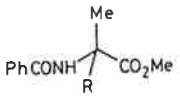

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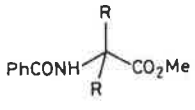

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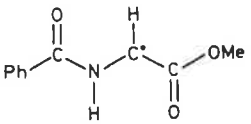
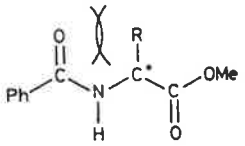
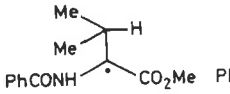


(6a)



(5) R = Me
(10) R = Br



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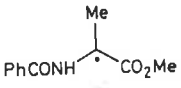

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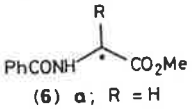

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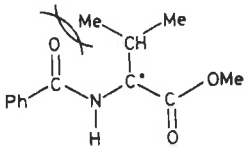
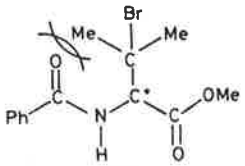
b; R = D

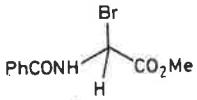


(4)



(11)

Figure 1



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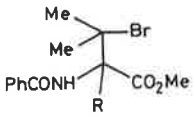
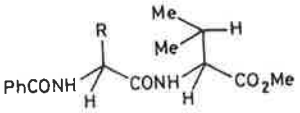


(8) a; R = Br
b; R = H



(9) a; R = H
b; R = Br
c; R = D

deuteriated analogues (**1b**), (**2b**), and (**3b**),† with *N*-bromosuccinimide (NBS) were determined using methods described previously[3] and these are presented in Table 1. Based on our earlier studies[3] of atom transfer reactions of (**1**) and the deuterium isotope effects observed in these reactions of (**2**) and (**3**), we propose that the relative rates of reaction of (**1a**)—(**3a**) reflect the ease of hydrogen atom abstraction from the α-positions of these compounds. Thus our results show that hydrogen atom transfer from the glycine derivative (**3a**) to give the secondary radical (**6a**) is faster than production of the tertiary radicals (**4**) and (**5**) in similar reactions of (**1a**) and (**2a**) respectively.

Since hydrogen atom abstractions in reactions with NBS are selective for production of the most stable product radical,[4] our results indicate that, in direct contrast to expectation, the secondary radical (**6a**) is marginally more stable than the tertiary radical (**5**), and both (**6a**) and (**5**) are considerably more stable than (**4**). We attribute this peculiar stability of the radical (**6a**) to a particularly favourable geometry. Stabilization of the captodative[5] radicals (**4**)—(**6**) will result from overlap of the semi-occupied p orbitals with the π orbitals of the amido and methoxycarbonyl substituents. There will be maximum overlap of these orbitals in planar conformations of the radicals (**4**)—(**6**), Figure 1. The radical (**5**) will be destabilized compared to (**6a**) by non-bonding interactions associated with planar conformations of (**5**), and (**4**) will be even less stable owing to more severe non-bonding interactions. These destabilizing influences outweigh the normal thermodynamic preference for the production of tertiary radicals.

Table 1. Relative rates of reaction of the amino acid derivatives (**1**)—(**3**) with NBS.[a]

Valine (**1a**)	1[b]	(**1b**)	0.27 ± 0.02[c]
Alanine (**2a**)	7.7 ± 1.1	(**2b**)	4.1 ± 0.40
Glycine (**3a**)	23 ± 3.5	(**3b**)	7.3 ± 1.5

[a] Reaction under nitrogen in refluxing carbon tetrachloride, catalysed by irradiation. [b] Assigned as unity. [c] In ref. 3, the relative rate constants were reported incorrectly as their reciprocals.

The reaction of the glycine derivative (**3a**) with NBS gave the monobromide (**7**) in high yield, presumably by bromine atom transfer to (**6a**). The lack of subsequent reaction of (**7**) is consistent with our rationale for the reactivity of (**3**). The radical (**10**) (Figure 1) produced by hydrogen atom abstraction from (**7**) would be less stable than (**6a**) because of non-bonding interactions.

In a related system we have examined reactions of the monobromoglycine derivative (**7**) and the dibromovaline derivative (**8a**) with tri-n-butyltin hydride. The dibromovaline derivative (**8a**) is the final product of reaction of (**1a**) with NBS. Reaction of (**8a**) with tri-n-butyltin hydride affords the monobromovaline derivative (**8b**)[6] and (**3a**) is produced from (**7**). These reactions are expected to proceed by halogen atom abstraction with subsequent hydrogen incorporation, and the stability of the free radical intermediate is the prime factor in determining the rate of halogen atom abstraction.[7] From mixtures of (**7**) and (**8a**) we achieved selective reduction of (**7**) to (**3a**), which we take to indicate the greater relative stability of the radical (**6a**) compared to (**11**). This is not surprising when the non-bonding interactions in (**6a**) and (**11**) are compared (Figure 1).

We conclude that the selective reaction of glycine residues

† All new compounds were fully characterised.

in these and other[1] free radical reactions of amino acid derivatives is due to the stability of the radicals produced by atom transfer reactions. Radicals produced by similar reactions of other amino acid derivatives are relatively unstable because of non-bonding interactions. Reactions with NBS provide a viable synthetic procedure for direct and selective functionalisation of glycine derivatives. Accordingly, treatment of the dipeptide (**9a**) gave the bromide (**9b**) in high yield. Subsequent reaction with triphenyltin deuteride produced the regioselectively-labelled dipeptide (**9c**).

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References

1 J. Sperling and D. Elad, *J. Am. Chem. Soc.*, 1971, **93**, 967; 3839; M. Schwarzberg, J. Sperling, and D. Elad, *ibid.*, 1973, **95**, 6418; T. Henriksen, T. B. Melø, and G. Saxebøl, in 'Free Radicals in Biology,' ed. W. A. Pryor, Academic Press, New York, 1976, vol. 2, p. 213.
2 G. A. Russell, in 'Free Radicals,' ed. J. K. Kochi, Wiley, New York, 1973, vol. 1, p. 275.
3 C. J. Easton and M. P. Hay, *J. Chem. Soc., Chem. Commun.*, 1985, 425.
4 M. L. Poutsma, in 'Free Radicals,' ed. J. K. Kochi, Wiley, New York, 1973, vol. 2, p. 211.
5 H. G. Viehe, Z. Janousek, and R. Merenyi, *Acc. Chem. Res.*, 1985, **18**, 148.
6 S. Love and C. Easton, unpublished observations.
7 H. G. Kuivila, *Acc. Chem. Res.*, 1968, **1**, 299.

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DIRECT INTRODUCTION OF A BENZOYLOXY SUBSTITUENT AT THE C-4 POSITION OF β-LACTAMS

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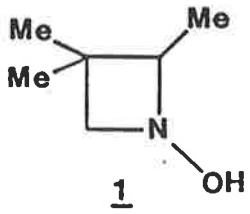
SUMMARY: The copper-promoted reaction of β-lactams with t-butyl perbenzoate results in benzoyloxylation of the azetidin-2-one ring at the C-4 position. There is no competing reaction at the C-3 position, but reaction at exocyclic carbon α to nitrogen competes with ring substitution.

One limit to the use of azetidinones in syntheses of β-lactam antibiotics[1] is a lack of methods for introducing a substituent directly at the C-4 position of an azetidinone ring. Oxidation of the hydroxyazetidine (1) with lead tetraacetate gave the 4-acetoxyazetidinone (2a), but this reaction is thought to occur by 1,3-addition of the oxidizing agent to the nitrone (3), not by direct substitution of the β-lactam (2b).[2] It has been



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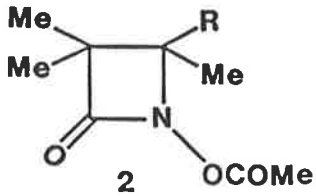

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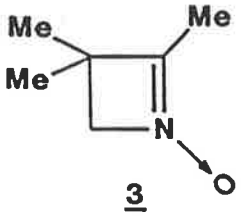


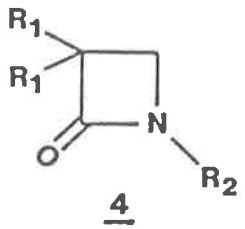
3

a, R=OCOMe

b, R=H

established that further substitution occurs at the azetidinone C-4 position when the site is already activated by adjacent sulphur.[3] In this communication we describe a method for functionalization of β-lactams at C-4 through the introduction of a benzoyloxy substituent. Our work is based on the knowledge that free-radical oxidation of γ- and δ-lactams occurs readily at the ring carbon α to nitrogen.[4]

The β-lactams (4a-g)[5] were prepared from corresponding 3-halopropionamides by treatment with sodium hydride.[6] To limit competing acrylamide formation in the preparations of 4a and 4b, the propionamides were added slowly, in dilute solution, to a thin suspension of sodium hydride.[7] The corresponding 3-iodopropionamide was used to prepare 4a.



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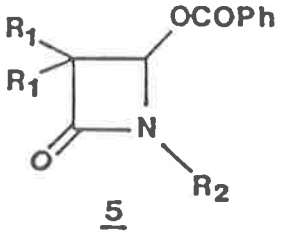
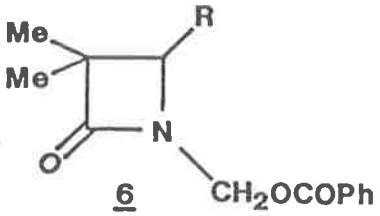


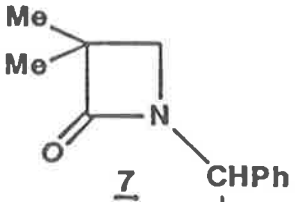
5

a, $R_1 = H$, $R_2 = t\underline{Bu}$
b, $R_1 = H$, $R_2 = Ph$
c, $R_1 = R_2 = Me$
d, $R_1 = Me$, $R_2 = CH_2Ph$
e, $R_1 = Me$, $R_2 = t\underline{Bu}$
f, $R_1 = Me$, $R_2 = Ph$
g, $R_1 = Ph$, $R_2 = t\underline{Bu}$



6

a, R = H
b, R = OCOPh



7

a, R = OH
b, R = OCOPh

Reaction of 4a (100mg, 0.8 mmol) with t-butyl perbenzoate (2.4 mmol) in the presence of cupric octanoate (0.02 mmol) in benzene (5ml) under nitrogen at reflux for 6 hr afforded, after chromatography on a Chromatotron silica gel plate, the 4-benzoyloxy substituted β-lactam (5a) [59%,[8] ^1H n.m.r. ($CDCl_3$) δ 1.40 (9H, s, tBu), 2.81 (1H, dd, J 1,14Hz, C3-H*cis*), 3.33 (1H, dd, J 4,14Hz, C3-H*trans*), 6.34 (1H, dd, J 1,4Hz, C4-H), and 7.2-8.3 (5H, m, Ar-H)]. Similar treatment of 4b gave 5b [53%, ^1H n.m.r. ($CDCl_3$), δ 3.17 (1H, dd, J 2,16Hz, C3-H*cis*), 3.66 (1H, dd, J 4,16Hz, C3-H *trans*), 6.74 (1H, dd, J 2,4Hz, C4-H), and 7.1-8.3 (10H, m, Ar-H)]. The ^1H n.m.r. spectra of 5a and 5b show unambiguously that the benzoyloxy substituent has been incorporated at the C-4 position.[9] The geminal coupling constants indicate that the methylene group is adjacent to the amide carbonyl group.

The major features of the mechanism of reactions involving t-butyl perbenzoate have been elucidated.[10] Formation of 5a and 5b can be attributed to hydrogen-atom transfer from the corresponding β-lactams (4a) and (4b) to t-butoxy radical, followed by benzoate incorporation at the site of hydrogen abstraction. Clearly the C-4 methylene is more reactive than the C-3 position, presumably because of the activating effect of adjacent nitrogen.[4]

In order to examine the relative reactivity of an exocyclic carbon α to nitrogen as compared to an endocyclic carbon, the reaction of the β-lactam (4c) was investigated. The primary products of reaction of 4c with t-butyl perbenzoate were the endocyclic substitution product (5c) [^{1}H n.m.r. ($CDCl_3$), δ 1.33 (3H, s, CMe*cis*), 1.40 (3H, s, CMe*trans*), 2.91 (3H, s, NMe), 5.88 (1H, s, CH), and 7.3-8.3 (5H, m, Ar-H)] and the exocyclic substitution product (6a) [^{1}H n.m.r. ($CDCl_3$), δ 1.33 (6H, s, 2xCMe), 3.32 (2H, s, C-CH_2-N), 5.45 (2H, s, O-CH_2-N), and 7.3-8.3 (5H, m, Ar-H)] in the ratio *ca*.1:2. As the extent of reaction increased the disubstitution product (6b) [^{1}H n.m.r. ($CDCl_3$), δ 1.33 (3H, s, CMe*cis*), 1.45 (3H, s, CMe*trans*), 5.58 (2H, s,CH_2), 6.13 (1H, s, CH), and 7.3-8.3 (10H, m, Ar-H)] was also formed. Analysis of product ratios at varying extents of reaction showed that 6b is a secondary product formed by subsequent reaction of 5c and 6a. Formation of 5c, 6a, and 6b in the reaction of 4c demonstrates that reaction at exocyclic carbon α to nitrogen competes with ring substitution.

In the β-lactam (4f) the exocyclic carbon α to nitrogen is activated further by a phenyl substituent. The final product of reaction of 4f with t-butyl perbenzoate, isolated after chromatography, was the alcohol (7a) [12%, ^{1}H n.m.r. ($CDCl_3$), δ 1.26 (3H, s, Me), δ 1.33 (3H, s, Me), 2.68 (1H, d, *J* 6Hz, CH), 3.14 (1H, d, *J* 6Hz, CH), 4.30 (1H, b.s, OH), 6.36 (1H, s, CH), and 7.2-7.6 (5H, m, Ar-H)]. We attribute formation of this product to hydrolysis of the benzoate (7b) during chromatography. No ring substitution product was detected.

Finally we examined reactions of the β-lactams (4e-g) having substituents at C-3. Reactions of 4e and 4f afforded 5e [28%, ^{1}H n.m.r. (CCl_4), δ 1.10 (3H, s, Me*cis*), 1.34 (3H, s, Me*trans*), 1.39 (9H, s, tBu), 5.88 (1H, s, CH), and 7.3-8.2 (5H, m, Ar-H)] and 5f [19%, ^{1}H n.m.r. ($CDCl_3$), δ 1.34 (3H, s, Me*cis*), 1.53 (3H, s, Me*trans*), 6.42 (1H, s, CH), and 7.1-8.3 (10H, m, Ar-H)], respectively. Relative yields of the benzoates (5a) and (5b) compared to (5e) and (5f) from reactions having the same molar ratio of perester indicate that substituents at C-3 reduce the reactivity. This steric effect accounts for the observation that the β-lactam (4g) was completely unreactive.

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REFERENCES AND NOTES

1. See for example K. Fujimoto, Y. Iwano and K. Hirai, Tetrahedron Lett., 1985, 26, 89; W.F. Huffman, K.G. Holden, T.F. Buckley, J.G. Gleason and L. Wu, J. Am. Chem. Soc., 1977, 99, 2352; T.N. Salzmann, R.W. Ratcliffe, B.G. Christensen and F.A. Bouffard, J. Am. Chem. Soc., 1980, 102, 6161; M.M. Campbell and B.P. Connarty, Heterocycles, 1982, 19, 1853; M.D. Bachi and A. Gross, J. Chem. Soc. Perkin Trans. 1, 1983, 1157; M. Ihara, A. Nakayama and K. Fukumoto, Tetrahedron, 1982, 38, 2489.

2. M.L.M. Pennings and D.N. Reinhoudt, J. Org. Chem., 1983, 48, 4043.

3. H. Matsumura, T. Yano, M. Ueyama, K. Tori and W. Nagata, J. Chem.Soc. Chem. Commun., 1979, 485.

4. M. Okita, T. Wakamatsu and Y. Ban, J. Chem. Soc. Chem .Commun., 1979 749; M. Okita, T. Wakamatsu, M. Mori and Y. Ban, Heterocycles, 1980, 14, 1089; J.W. Pavlik and S. Tantayanon, J. Am. Chem. Soc., 1981, 103, 6755; J.C. Gramain, R. Remuson and Y. Troin, J. Chem. Soc. Chem. Commun., 1976, 194; for a review see T. Shono, Tetrahedron, 1984, 40, 811.

5. All new compounds were fully characterised.

6. I.L. Knunyants and N.P. Gambaryan, Izv. Akad. Nauk. SSSR, Otdel. Khim. Nauk, 1955, 1037.

7. H.H. Wasserman, D.J. Hlasta, A.W. Tremper and J.S. Wu, Tetrahedron Lett., 1979, 549.

8. Yields based on recovered starting material were virtually quantitative. Quoted yields are based on reaction with 3 molar equivalents of perbenzoate. Higher yields were obtained using larger excesses of perester.

9. K.D. Barrow and T.M. Spotswood, Tetrahedron Lett., 1965, 3325.

10. D.J. Rawlinson and G. Sosnovsky, Synthesis, 1972, 1.

(Received in UK 13 March 1986)

Tetrahedron Letters,Vol.28,No.24,pp 2747-2750,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

SELECTIVE γ-HYDROGEN ATOM ABSTRACTION IN REACTIONS OF N-ACETYLAMINO ACIDS AND N-ALKYLACETAMIDES WITH TITANOUS ION AND HYDROGEN PEROXIDE

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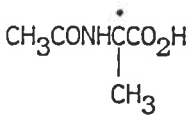
SUMMARY: Whereas reactions of N-acetylglycine, N-acetylalanine, and pyroglutamic acid, with $TiCl_3/H_2O_2$ in a flow system give e.p.r. spectra arising from the corresponding α-centred radicals (1-3), reactions of N-acetylvaline, 2-acetamidobutyric acid, N-propylacetamide, and N-(2-methylpropyl)-acetamide, give spectra arising from the respective γ-centred radicals (4-7). Production of the γ-centred radicals (4-7) is attributed to complexation of titanium to the amide group of the substrates.

As part of an investigation of free radical reactions of amino acid derivatives, we undertook an e.p.r. study of radicals formed in a flow system by the interaction of N-acetylamino acids with titanous chloride-hydrogen peroxide generated hydroxyl radical, using the method of Dixon and Norman.[1] The spectra were recorded at room temperature on a Varian E9 e.p.r. spectrometer. Spectral parameters are listed in the Table.

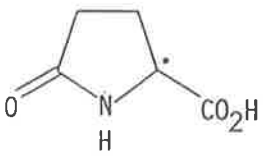
As expected on the basis of previous work,[2] N-acetylglycine and N-acetylalanine gave spectra consistent with formation of the corresponding α-centred radicals (1) and (2).

(1)

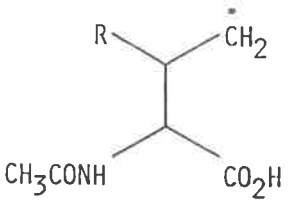


(2)

No other signals were detected in the spectrum from N-acetylalanine. In the spectrum from N-acetylglycine, however, relatively low intensity signals consistent with the radical resulting from decarboxylation, i.e., $CH_3CONH\dot{C}H_2$,[3,4] were also observed. Decarboxylation of dicarboxylic acids with $TiCl_3/H_2O_2$ has been reported previously,[5] and formation of $CH_3CONH\dot{C}H_2$ by irradiation of N-acetylglycine in the presence of transition metal ions has already been noted.[3]

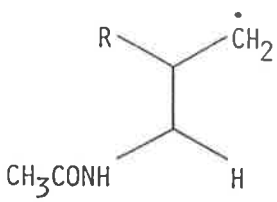


(3)



(4) R=Me

(5) R=H



(6) R=H

(7) R=Me

The e.p.r. spectrum obtained from reaction of pyroglutamic acid is consistent with formation of the corresponding α-centred radical (3). No other signals were detected in the spectrum.

N-Acetylvaline and 2-acetamidobutyric acid gave e.p.r. spectra indicating formation of the corresponding γ-centred radicals (4) and (5). No other signals were detected in the spectrum from N-acetylvaline. With 2-acetamidobutyric acid, other partially obscured, relatively low intensity signals were observed. These signals may be attributed to the radical resulting from decarboxylation. The e.p.r. spectrum obtained from a 1:1 (W/W) mixture of N-acetylglycine and N-acetylvaline showed signals for the γ-centred radical (4) derived from N-acetylvaline and the α-centred radical (1) derived from N-acetylglycine in the ratio ca. 10:1.

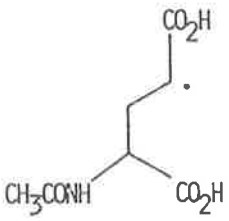
It is unlikely that observation of the γ-centred radicals (4) and (5) is facilitated by preferential reaction of other radicals with titanous ion or hydrogen peroxide,[6,7] as only the α-centred radicals (2) and (3) were observed with N-acetylalanine and pyroglutamic acid, respectively. It appears, therefore, that N-acetylvaline and 2-acetamidobutyric acid react by selective γ-hydrogen atom abstraction.

There is much evidence that the free hydroxyl radical is the active species in oxidations with $TiCl_3/H_2O_2$.[8] It is difficult, however, to attribute selective formation of the γ-centred radicals (4) and (5) to reactions involving free hydroxyl radicals. The selectivity may be attributed to reaction through complexation of titanium to the amide group of substrates. Moderately selective β-hydrogen transfer in reactions of carboxylic acids with $TiCl_3/H_2O_2$ has been attributed to reaction through a complexation of titanium to the carboxyl group.[9] We postulate complexation to the amide group of the substrates, rather than the carboxyl group, in light of our observations that N-propylacetamide and N-(2-methylpropyl)-acetamide give e.p.r. spectra consistent with formation of the γ-centred radicals (6) and (7), respectively.

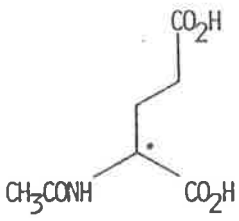
Table. ESR Parameters for Radicals (1-9)

Substrate	Radical	Hyperfine Coupling Constants
N-Acetylglycine	(1)[2]	16.5G, a(α-H) (d)
		2.5G, a(N) (t)
N-Acetylalanine	(2)[2]	16.5G, a(β-H) (q)
		2.0G, a(N) (t)
Pyroglutamic acid	(3)[10]	22.5G, a(β-H) (t)
		3.0G, a(γ-H) (t)
		2.5G, a(N-H) (d)
N-Acetylvaline	(4)	27.5G, a(β-H) (d)
		22.0G, a(α-H) (t)
2-Acetamidobutyric acid	(5)	26.0G, a(β-H) (t)
		22.5G, a(α-H) (t)
N-Propylacetamide	(6)	27.5G, a(β-H) (t)
		22.0G, a(α-H) (t)
N-(2-Methylpropyl)-acetamide	(7)	27.5G, a(β-H) (d)
		21.5G, a(α-H) (t)
N-Acetylglutamic acid	(8)	21.0G, a(β-H) (t)
		21.0G, a(α-H) (d)
	(9)	22.5G, a(β-H) (t)
		4.0G, a(N) (t)

N-Acetylglutamic acid gave a spectrum consistent with formation of the γ-centred radical (8) and the α-centred radical (9) in the ratio ca. 1:1. The corresponding β-centred radical was not detected.



(8)



(9)

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References.

1. W.T. Dixon and R.O.C. Norman, J. Chem. Soc., 1963, 3119.
2. H. Taniguchi, H. Hatano, H. Hasegawa and T. Maruyama, J. Phys. Chem., 1970, 74, 3063.
3. I. Rosenthal, R. Poupko and D. Elad, J. Phys. Chem., 1973, 77, 1944.
4. T. Yonezawa, I. Noda and T. Kawamura, Bull. Chem. Soc. Jpn., 1969, 42, 650; R. Livingston and Z. Zeldes, J. Chem. Phys., 1967, 47, 4173.
5. W.T. Dixon, R.O.C. Norman and A.L. Buley, J. Chem. Soc., 1964, 3625.
6. D. Behar, A. Samuni and R.W. Fessenden, J. Phys. Chem., 1973, 77, 2055.
7. R.O.C. Norman and P.R. West, J. Chem. Soc. B., 1969, 389.
8. B.C. Gilbert and A.J. Dobbs in 'Organic Peroxides' (Ed. D. Swern), Vol. 3, p. 271 (Interscience: New York, 1972).
9. F.R. Hewgill and G.M. Proudfoot, Aust. J. Chem., 1981, 34, 335.
10. H. Taniguchi and Y. Kirino, J. Am. Chem. Soc., 1977, 99, 3625.

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Occurrence of unusual molecular species of sphingomyelin containing 28–34-carbon polyenoic fatty acids in ram spermatozoa

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The high levels of very long chain fatty acids found in ram spermatozoa are located almost exclusively in one of two separable species of sphingomyelin. Mass spectral analysis, including fast atom bombardment of the purified sphingomyelin, has shown the fatty acids to have a carbon chain length of between 28 and 34, with between four and six double bonds, and to belong predominantly to the $n-3$ series.

INTRODUCTION

Polyenoic long chain fatty acids with carbon chain lengths greater than 22 (polyenoic VLCFA) have been detected in a number of mammalian tissues including brain (Poulos *et al.*, 1986*a*), retina (Aveldano, 1987; Aveldano & Sprecher, 1987) and endocytes (Rosenthal & Hill, 1984). In bovine retina these fatty acids, which include both $n-3$ and $n-6$ series derivatives, are located mostly in unusual di-polyunsaturated molecular species of phosphatidylcholine (Aveldano & Sprecher, 1987). However, in human brain, the polyenoic VLCFA are almost exclusively $n-6$ series acids and are found mostly in cholesterol esters although the ultra-long chain polyenoic fatty acids, i.e. those with carbon chain lengths greater than 32, are confined exclusively to a minor unidentified phospholipid (Sharp *et al.*, 1987).

We have recently reported that mammalian spermatozoa contain significant amounts of polyenoic VLCFA (Poulos *et al.*, 1986*b*). In particular, ram and bull spermatozoa contain high proportions of $n-3$ fatty acids with 32 and 34 carbon atoms.

In view of the reported differences in lipid distribution of polyenoic VLCFA we undertook an investigation into the distribution of the polyenoic VLCFA in ram spermatozoa. It was hoped that these studies could provide a clue as to their possible function.

We report that the $n-3$ polyenoic VLCFA with 30–34 carbon atoms are located exclusively in sphingomyelin.

MATERIALS AND METHODS

Ram semen was collected by electrical stimulation with a bipolar rectal electrode (Blackshaw, 1954). Spermatozoa were isolated from semen by centrifugation at 1000 *g* for 20 min at room temperature. The spermatozoa from four ejaculates were pooled and then extracted by the technique of Folch *et al.* (1957). The total lipids were separated into neutral lipid, glycolipid and phospholipid fractions by silicic acid column chromatography, and the phospholipids were separated into neutral and acidic components as described by Sharp *et al.* (1987). The total non-acidic lipids were applied as 15 cm bands to two (20 cm × 20 cm) silica-gel 60 (Merck) plates and chromatograms were developed in chloroform/methanol/water (70:30:4, by vol.). The various lipid zones were located by spraying the plates with 0.2% (w/v) dichlorofluorescein in 95% (v/v) ethanol and eluted with 10 ml of chloroform/methanol/water (5:5:1, by vol.). After partitioning to remove the dichlorofluorescein, portions of the eluates were *trans*-esterified (Poulos *et al.*, 1986*a*). The resulting fatty acid methyl esters were subjected to combined g.c.–m.s. as described earlier (Poulos *et al.*, 1986*a*). Determination of the *n*-series number of polyunsaturated fatty acids was carried out using the mass spectrometric technique described earlier (Fellenberg *et al.*, 1987). Phosphorus analyses were performed on the eluates as described by Owens (1966). Identification of the various lipids was based on a comparison of their t.l.c. mobility with authentic standards, using chloroform/methanol/water (70:30:4, by vol.) as developing solvent. In the case of sphingomyelin, identification was based on collision-activation mass-analysed ion kinetic energy spectroscopy (CAMIKES) of ions produced by fast atom bombardment as described by Easton *et al.* (1988). For these studies, mass spectra were measured on a Vacuum Generator ZAB 2 HF mass spectrometer operating in the positive ion fast atom bombardment mode. Additional proof of structure was obtained by g.l.c.–m.s. identification of sphingosine released after acid hydrolysis (Polito *et al.*, 1968).

RESULTS AND DISCUSSION

Polyenoic VLCFA ($n-3$ series) with 30–34 carbon atoms are the major VLCFA in ram spermatozoa (Poulos *et al.*, 1986*b*). Silicic acid column chromatography of ram sperm lipids confirmed that most of these fatty acids were phospholipid components. Further fractionation of the sperm phospholipids by preparative t.l.c. indicated that virtually all of the polyenoic VLCFA were present in one phosphate-containing lipid (zone 2). Acid hydrolysis released two lipid products identified by g.c.–m.s. as sphingosine and a mixture of long chain fatty

Abbreviations used: VLCFA, very long chain fatty acids; CAMIKES, collision-activation mass-analysed ion kinetic energy spectroscopy.
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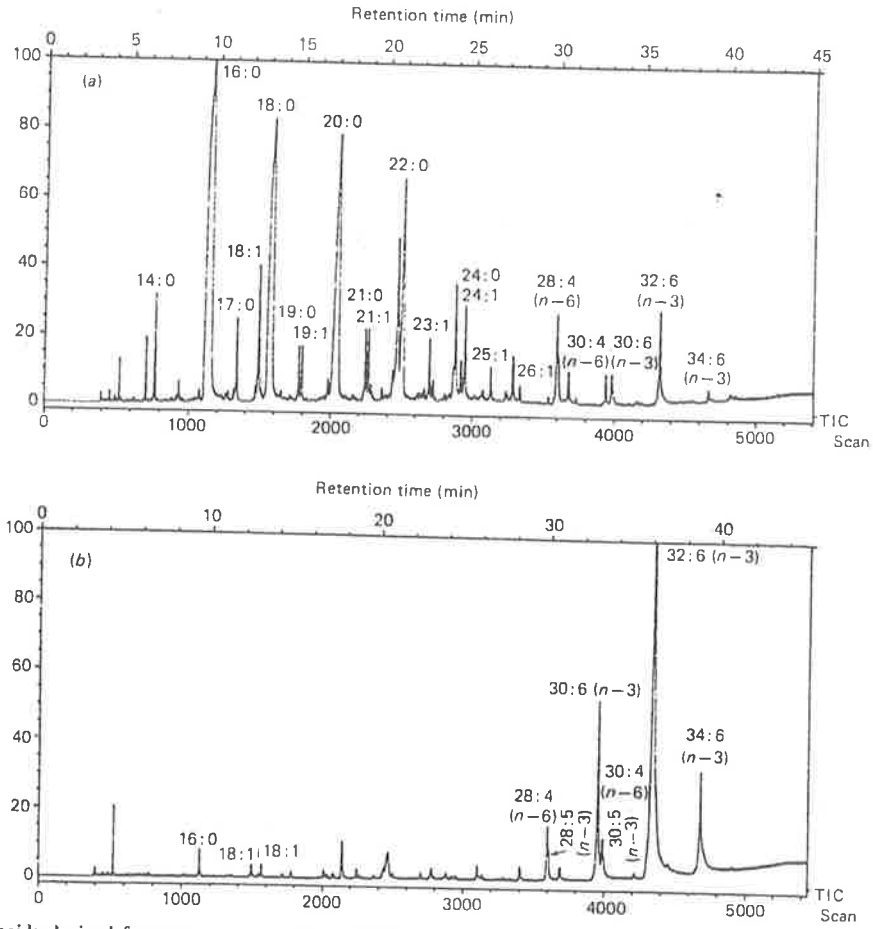

Fig. 1. G.c. of fatty acids derived from ram sperm sphingomyelin

Total ion chromatograms of the fatty acids of ram sperm sphingomyelin were obtained as described in the Materials and methods section. (*a*) Zone 1 sphingomyelin. (*b*) Zone 2 sphingomyelin.

acids, indicating that the lipid was sphingomyelin. The fatty acids released were almost exclusively (> 90%) 28–34 carbon polyenoic fatty acids with a predominance of 30:6($n-3$) and 32:6($n-3$) fatty acids (Fig. 1*a*). The latter comprised nearly 50% of the total fatty acids released from this particular lipid. In contrast, the slower-moving sphingomyelin (zone 1) contained predominantly shorter chain fatty acids, i.e. 16:0, 18:0, 18:1, 20:0 fatty acids with smaller amounts of polyenoic VLCFA (Fig. 1*b*). Accurate quantification of the fatty acids was not possible because polyenoic fatty acid standards are not available.

The spectrum obtained by fast atom bombardment mass spectrometric analysis (Fig. 2*a*) of the faster-moving sphingomyelin (zone 2) contains strong signals corresponding to ions at m/z 184 and 465, as well as bands of signals corresponding to ions between m/z 864 and m/z 944. The ion with m/z 184 afforded a CAMIKES mass spectrum containing ions at m/z 60, 86, 125 and 166 which is consistent with the structure of phosphorylcholine (Fig. 2*b*). The ion at m/z 465 can be attributed to sphingosylphosphorylcholine formed by deacylation of the parent lipid. The presence of an ion at m/z 184 in the CAMIKES spectrum generated from the m/z 465 fragment, presumably due to the phosphorylated base, provides strong supporting evidence for its identity (Fig. 2*c*). The ions at m/z 864, 888, 916 and 944 are ($M+1$) ions of individual molecular species of sphingomyelin containing 28:4, 30:6, 32:6 and 34:6 fatty acids (Fig. 2*a*).

To our knowledge, the presence of such unusual molecular species has not been previously reported although sphingomyelin from some tissues, notably brain (Stallberg-Stenhagen & Svennerholm, 1965), does contain high proportions of saturated and mono-unsaturated VLCFA while shorter chain polyenoic fatty acids, such as arachidonic acid, have been detected in human and rat tissue (Kokatnur *et al.*, 1985).

It should be emphasized that these lipids are not minor components of sperm. Thus, approx. 9% of the total lipid phosphorus in the lipids isolated from ram spermatozoa is found in the faster-moving sphingomyelin band (Darin-Bennett, 1975) and, therefore, the major molecular species which contains a 32:6 fatty acid

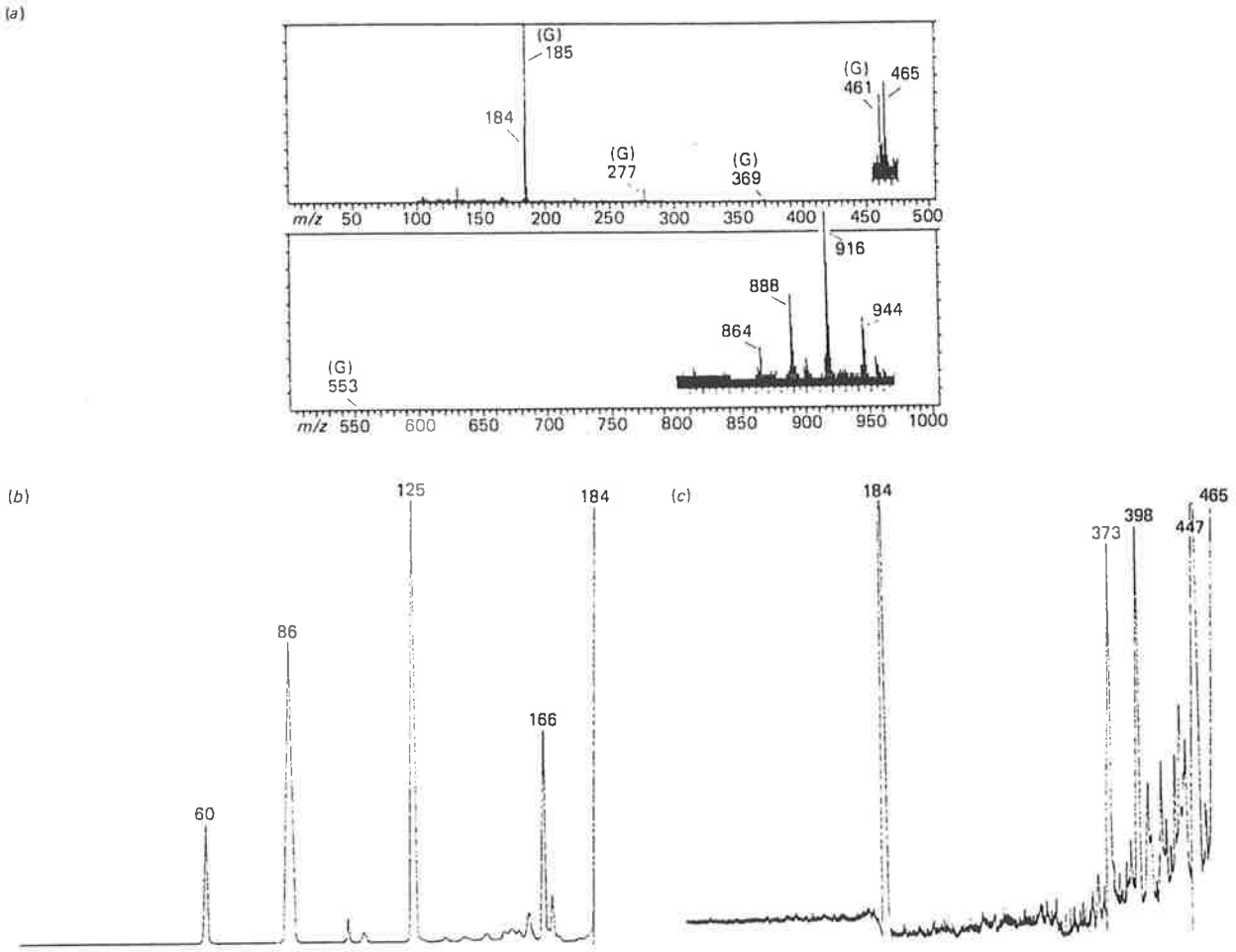

Fig. 2. M.s. of sphingomyelin isolated from ram spermatozoa

(*a*) Positive-ion fast atom bombardment mass spectrum of sphingomyelin. The peaks labelled (G) are protonated adducts of the solvent glycerol. (*b*) CAMIKES spectrum of the fragment of *m/z* 184 in the fast atom bombardment mass spectrum of sphingomyelin (*a*). (*c*) CAMIKES spectrum of the fragment of *m/z* 465 in the fast atom bombardment mass spectrum of sphingomyelin (*a*).

comprises nearly 4% of the total lipid phosphorus of ram spermatozoa. The occurrence of these unusual lipids raises a number of important questions concerning their mode of synthesis, degradation and their sub-cellular location.

The lipid distribution of the $n-3$ polyenoic VLCFA in ram sperm differs quite markedly from the distribution of the corresponding fatty acids in bovine retina where they occur largely in photoreceptor membranes as di-polyunsaturated phosphatidylcholines (Aveldano & Sprecher, 1987). Although we are unable to discount the possibility that the latter are constituents of ram spermatozoa, clearly there are fundamental differences in lipid composition between the two tissues because 30–34-carbon polyenoic fatty acids are virtually undetectable in sperm phosphatidylcholine. It has been speculated that retina $n-3$ polyenoic fatty acids may be required for the normal functioning of photoreceptor membrane proteins and therefore play a significant role in the visual process (Aveldano & Sprecher, 1987). Because of the major structural and physiological differences between retina and spermatozoa it is not unreasonable to speculate that the function of polyenoic VLCFA in these two tissues also differs. Some supporting evidence is provided by the differences in lipid distribution. Thus, while glycerolipid-bound fatty acids are metabolically active through the action of various phospholipases, the release of the corresponding amide-linked fatty acids is thought to involve the sequential action of sphingomyelinase and ceramidase (Mooibroek *et al.*, 1985) and is therefore probably slower. Whether this difference reflects a difference in function is possible, but remains to be determined.

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REFERENCES

Aveldano, M. I. (1987) J. Biol. Chem. **262**, 1172–1179

Aveldano, M. & Sprecher, H. (1987) J. Biol. Chem. **262**, 1180–1186

Blackshaw, A. W. (1954) Aust. Vet. J. **30**, 249–250

Darin-Bennett, A. (1975) Ph.D thesis, Sydney University, Sydney, Australia

Easton, C., Johnson, D. W. & Poulos, A. (1988) J. Lipid Res., in the press

Fellenberg, A. J., Johnson, D. W., Poulos, A. & Sharp, P. (1987) Biomed. Environ. Mass Spectrom. **14**, 127–129

Folch, J., Lees, M. & Sloane-Stanely, J. (1957) J. Biol. Chem. **226**, 497–509

Kokatnur, M., Brooks, V. & Plauche, W. C. (1985) Lipids **20**, 449–453

Mooibroek, M. J., Cook, H. W., Clarke, J. T. R. & Spence, M. W. (1985) J. Neurochem. **44**, 1551–1558

Owens, K. (1966) Biochem. J. **100**, 354–360

Polito, A. J., Akita, J. & Sweeley, C. C. (1968) Biochemistry **7**, 2609–2614

Poulos, A., Sharp, P., Singh, H., Johnson, D. W., Fellenberg, A. J. & Pollard, A. C. (1986*a*) Biochem. J. **235**, 607–610

Poulos, A., Sharp, P., Johnson, D. W., White, I. G. & Fellenberg, A. J. (1986*b*) Biochem. J. **240**, 891–895

Rosenthal, M. D. & Hill, J. R. (1984) Biochim. Biophys. Acta **795**, 171–178

Sharp, P., Poulos, A., Fellenberg, A. J. & Johnson, D. W. (1987) Biochem. J. **248**, 61–67

Stallberg-Stenhagen, S. & Svennerholm, L. (1965) J. Lipid Res. **6**, 146–156

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Determination of phospholipid base structure by CA MIKES mass spectrometry

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Summary The fast atom bombardment spectra of three phospholipids containing a secondary, tertiary, and quaternary nitrogenous base attached to a 1,2-dipalmitoyl-*sn*-glycero-3-phosphate skeleton were shown to each contain an ion derived from the intact nitrogenous base. The collisional activation mass analyzed ion kinetic energy spectroscopy (CA MIKES) spectrum produced from each of these ions was shown to be unique for the particular base. The technique thus provides a non-destructive rapid identification of the phosphorylated base of phospholipids.—**Easton, C., D. W. Johnson, and A. Poulos.** Determination of phospholipid base structure by CA MIKES mass spectrometry. *J. Lipid Res.* 1988. **29**: 109–112.

Supplementary key words phosphocholine • phosphomethylethanolamine • phospho-N,N-dimethylethanolamine • sphingomyelin • Niemann-Pick disease

Phospholipids are major membrane components of all eukaryotic and prokaryotic cells. Individual cells contain a great number of molecular species that vary according to the nature of the bound fatty acids and the hydrophilic group. The most common hydrophilic groups are primary and quaternary nitrogenous bases, generally ethanolamine and choline, respectively, although smaller amounts of the corresponding secondary and tertiary bases (N-monomethylethanolamine and N,N-dimethylethanolamine, respectively) (1, 2), as well as neutral hydrophilic groups, such as inositol and glycerol, and amphipathic groups such as serine are also present (3).

The confirmation of the structure of an isolated phospholipid is based primarily on chromatographic identification of the hydrophilic group released from the parent lipid by either acid or alkaline hydrolysis (1, 2, 4). More recently, however, mass spectrometric analysis of the undegraded lipids has been employed with varying success. Electron impact ionization can provide useful information on phospholipid structure (5, 6), but the complexity of the spectra produced from the large numbers of molecular species present, even in a single phospholipid class derived from natural sources, makes the interpretation of data quite difficult.

The softer techniques of chemical ionization (7, 8), field desorption (9–12), and more recently positive (13, 14) and negative ion (15) fast atom bombardment produce less complex spectra. However, it should be emphasized that the assignment of base structure by these techniques is largely dependent on the identification of fragments produced from individual bases. In complex mixtures there may be many fragments in the region of interest in the mass spectrum and therefore accurate structural assignment is not always possible.

In this study we report on the results of our investigations on the determination of phospholipid structure using collisional activation mass analyzed ion kinetic energy spectroscopy (CA MIKES) (16, 17). This technique produces a mass spectrum that is characteristic for the particular phosphorylated base of an individual phospholipid. It permits the unambiguous identification of the hydrophilic moiety and as a test it was used to confirm the structure of the major phospholipid stored in the liver of a patient with Niemann-Pick disease (sphingomyelin abnormality) (18).

MATERIALS

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2 dipalmitoyl-*sn*-glycero-3-phospho-N-monomethylethanolamine (DPMMPE), 1,2 dipalmitoyl-*sn*-glycero-3-phospho-N,N-dimethylethanolamine (DPDMPE), and bovine brain sphingomyelin were obtained from Sigma Chemical Co., St. Louis, MO. Sphingomyelin (SM) was also isolated from the liver of a patient with Niemann-Pick Type A disease (19).

METHODS

A chloroform solution (2 mg/ml) of each of the phospholipids (DPPC, DPDMPE, DPMMPE, and SM) was evaporated on the fast-atom bombardment probe tip of the mass spectrometer and then covered with a drop of glycerol. Mass spectra were measured on a Vacuum Generators ZAB 2F mass spectrometer operating in the positive ion fast-atom bombardment mode. Argon gas was used in the source with a primary beam energy of 8 kV.

Abbreviations: CA MIKES, collisional activation mass analyzed ion kinetic energy spectroscopy; DPPC, phosphatidylcholine; 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DPMMPE, 1,2-dipalmitoyl-*sn*-glycero-3-phospho-N-monomethylethanolamine; DPDMPE, 1,2-dipalmitoyl-*sn*-glycerol-3-phospho-N,N-dimethylethanolamine; SM, sphingomyelin.

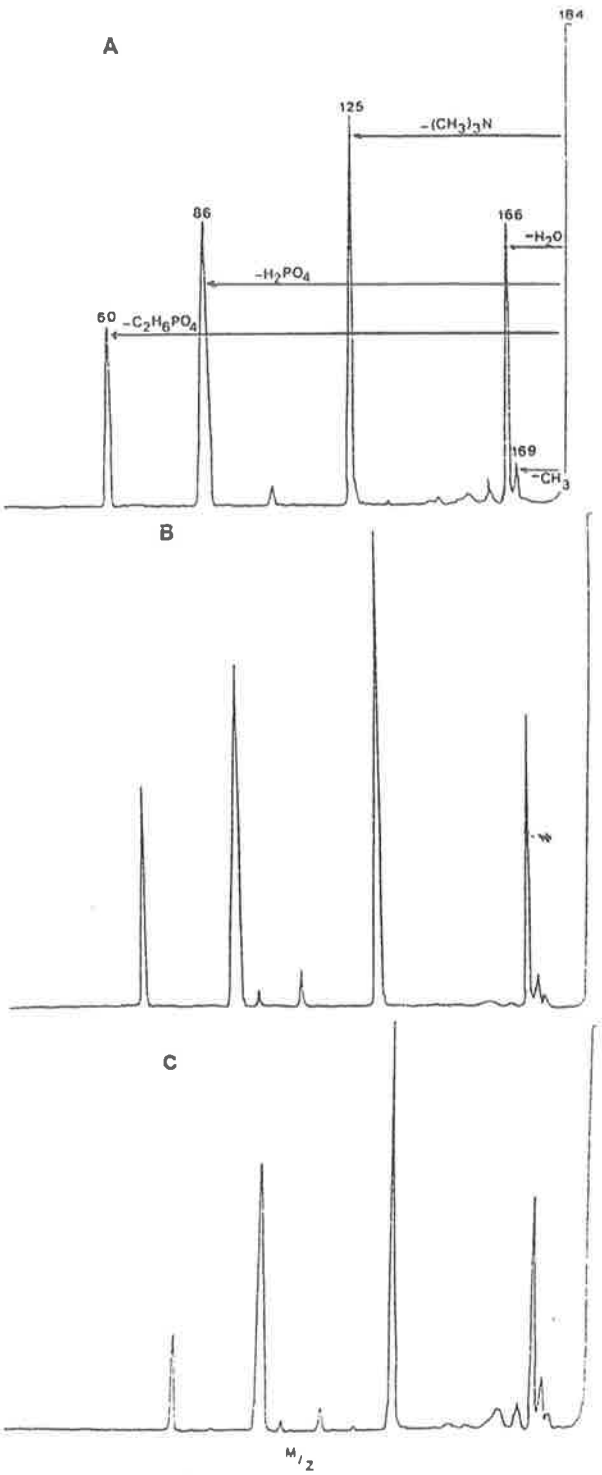

Fig. 1. CA MIKES mass spectrum of the ion with m/z 184 in the positive ion fast-atom bombardment mass spectrum of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) (Fig. 1A), bovine brain sphingomyelin (Fig. 1B), and sphingomyelin from the liver of a patient with Niemann-Pick disease (Fig. 1C).

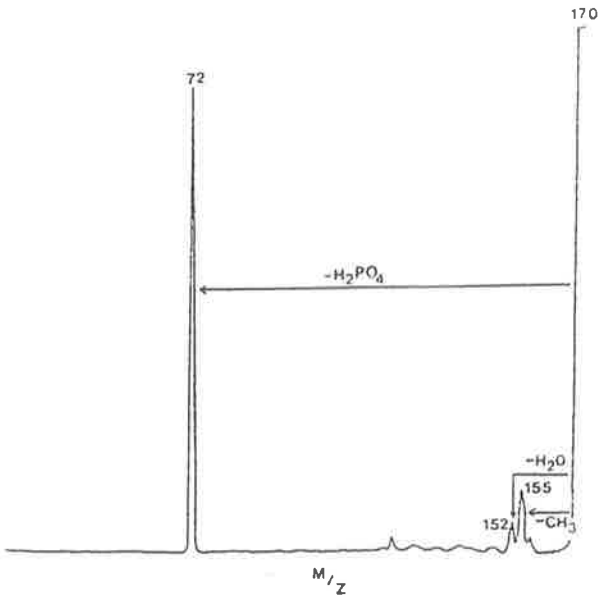

Fig. 2. CA MIKES mass spectrum of the ion with m/z 170 in the mass spectrum of 1,2-dipalmitoyl-*sn*-glycero-3-phospho-N,N-dimethylethanolamine (DPDMPE).

RESULTS AND DISCUSSION

Although each phospholipid gave a complex spectrum, the spectra of DPPC, DPDMPE, and DPMMPE showed ions at m/z 184, 170, and 156, respectively, attributable to the corresponding nitrogenous bases. These ions were analyzed by the CA MIKES technique. In this technique the spectrometer magnet allows only the transmission of the ion to be investigated into a collision cell containing neutral helium at a pressure of 2×10^{-7} torr. Under these conditions the ion undergoes a single collision with helium. Product ions formed by fragmentation of the collisionally activated ions are analyzed.

The CA MIKES spectrum of the ion at m/z 184 in the mass spectrum of DPPC is shown in **Fig. 1A**. It contains a peak at m/z 184 (M^+) and the following peaks which can be rationalized as representing charged fragments that result from the loss, from DPPC, of the groups in parentheses, at m/z 169 ($-CH_3$), 166 ($-H_2O$), 125 ($-(CH_3)_3N$), 86 ($-H_2PO_4$) and 60 ($-C_2H_6PO_4$).

The CA MIKES spectrum of the ion with m/z 170 in the mass spectrum of DPDMPE (**Fig. 2**) is consistent with the structure of phospho-N,N-dimethylethanolamine, with peaks at m/z 155 ($-CH_3$), 152 ($-H_2O$) and the major peak at 72 ($-H_2PO_4$). With DPMMPE the peaks at 141 ($-CH_3$), 138 ($-H_2O$), and 58 ($-H_2PO_4$) in the CA MIKES spectrum of the iion with m/z 156 (**Fig. 3**) are again consistent with an ion generated from phospho-N-methyl-ethanolamine.

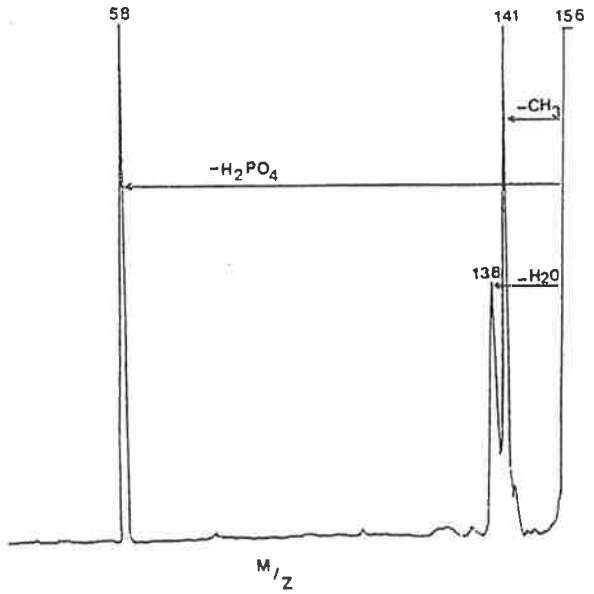

Fig. 3. CA MIKES mass spectrum of the ion with m/z 156 in the mass spectrum of 1,2-dipalmitoyl-*sn*-glycero-3-phospho-N-monomethylethanolamine (DPMMPE).

Positive ion fast-atom bombardment mass spectra of bovine brain sphingomyelin and of sphingomyelin isolated from a patient with Niemann-Pick Type A disease showed an ion with m/z 184 in each case. The respective CA MIKES spectra, Fig. 1B and 1C, confirm the identity of the nitrogenous base as phosphocholine. Indeed, the similarities between Fig. 1A-C show the extent to which this technique produces mass spectra characteristic of the phosphorylated base of the phospholipid and the strength of the technique in the unambiguous identification of the hydrophilic moiety.

In the practical application of this methodology, amounts of material on the order of several hundred micrograms are sufficient, the majority of which can be recovered after separation from the glycerol matrix. It is expected that prior purification by either thin-layer or high pressure liquid chromatography of the phospholipid would be performed; although CA MIKES mass spectrometry exhibits excellent discrimination, the presence of other components in the glycerol matrix can affect ionization and hence detectability of the phospholipid. The technique is thus unsuitable for the quantitation of components of mixtures of phospholipids. The extension of the technique to the identification of other glycerophospholipids with different hydrophilic groups would only be possible if a protonated species of the hydrophilic group could be generated in the source of the mass spectrometer.

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REFERENCES

1. Lester, R. L., and D. C. White. 1967. Quantitative gas-liquid chromatographic analysis of ethanolamine, monomethyl ethanolamine, and dimethyl ethanolamine from lipids. *J. Lipid Res.* **8**: 565-568.
2. Katyal, S. L., and B. Lombardi. 1974. Quantitation of phosphatidyl N-methyl and N,N-dimethyl aminoethanol in liver and lung of N-methylaminoethanol-fed rats. *Lipids.* **9**: 81-85.
3. Skipski, V. P., and M. Barclay. 1969. Thin-layer chromatography of lipids. *Methods Enzymol.* **14**: 531-598.
4. Dittmer, J. C., and M. A. Wells. 1969. Quantitative and qualitative analysis of lipids and lipid components. *Methods Enzymol.* **14**: 482-530.
5. Klein, R. A. 1971. Mass spectrometry of the phosphatidylcholines: dipalmitoyl, dioleoyl and stearoyl-oleoyl glycerylphorylcholines. *J. Lipid Res.* **12**: 123-131.
6. Saito, K., and K. Satouchi. 1982. Mass spectrometric analysis of natural glycerophospholipids. *Shitsuryo Bunseki.* **30**: 17-29.
7. Crawford, C. G., and R. D. Plattner. 1983. Ammonia chemical ionization mass spectrometry of intact diacyl phosphatidylcholine. *J. Lipid Res.* **24**: 456-460.
8. Dessort, D., M. Mersel, P. Lepage, and A. V. Dorsselaer. 1984. Fast-heating mass spectrometry of phosphatidylcholine, lysophosphatidylcholine, phosphatidylethanolamine and sphingomyelin. *Anal. Biochem.* **142**: 43-52.
9. Wood, G. W., P. Y. Lau, G. Morrow, G. N. S. Rao, D. E. Schmidt, and J. Tuebner. 1977. Field desorption mass spectrometry of phospholipids. Survey of structural types. *Chem. Phys. Lipids.* **18**: 316-333.
10. Sugatani, J., M. Kino, K. Saito, T. Matsuo, H. Matsuda, and I. Katakuse. 1982. Analysis of molecular species of phospholipids by field desorption mass spectrometry. *Biomed. Mass Spectrom.* **9**: 293-301.
11. Lehmann, W. D., and M. Kessler. 1983. Characterization and quantification of human plasma lipids from crude lipid extracts by field desorption mass spectrometry. *Biomed. Mass Spectrom.* **10**: 220-226.
12. Lehmann, W. D., and M. Kessler. 1983. Fatty acid profiling of phospholipids by field desorption and fast atom bombardment mass spectrometry. *Chem. Phys. Lipids.* **32**: 123-135.
13. Fenwick, G. R., J. Eagles, and R. Self. 1983. The fast atom bombardment mass spectra of glucosinolates and glucosinolate mixtures. *Biomed. Mass Spectrom.* **10**: 382-386.
14. Ohashi, Y. 1984. Structure determination of phospholipids by secondary ion mass spectrometric techniques: differentiation of isomeric esters. *Biomed. Mass Spectrom.* **11**: 383-385.
15. Münster, H., J. Stein, and H. Budzikiewitz. 1986. Structure analysis of underivatized phospholipids by negative ion fast atom bombardment mass spectrometry. *Biomed. Environ. Mass Spectrom.* **13**: 423-427.

16. Porter, C. J., J. H. Beynon, and T. Ast. 1981. The modern mass spectrometer—a complete chemical laboratory. *Org. Mass Spectrom.* **16**: 101–114.

17. Beynon, J. H., R. G. Cooks, J. W. Amy, W. E. Baitinger, and T. Y. Ridley. 1973. Design and performance of a mass-analysed ion kinetic energy (MIKE) spectrometer. *Anal. Chem.* **45**: 1023A–1031A.

18. Frederickson, D. S., and H. R. Sloan. 1972. Sphingomyelin lipidosis: Niemann-Pick disease. *In* The Metabolic Basis for Inherited Disease. J. B. Standbury, J. B. Wyngaarden, and D. S. Fredrickson, editors. McGraw-Hill Book Co., New York.

19. Vanier, M. 1983. Biochemical studies in Niemann-Pick disease. I. Major sphingolipids of liver and spleen. *Biochim. Biophys. Acta.* **750**: 178–184.

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Regioselective Chlorination of Valine Derivatives

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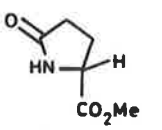
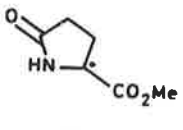
Reaction of *N*-benzoylvaline methyl ester (**5a**) with sulphuryl chloride gave the β-chlorovaline derivative (**6a**) and lesser amounts of diastereoisomers of the γ-chlorovaline derivative (**7a**). A similar mixture of products was obtained through photolysis of the *N*-chloroamide (**13**). The reactions of the valine derivatives (**5a**) and (**13**) involve regioselective intermolecular transfer of the β-valinyl hydrogen. There is no evidence for reaction at the α-position. The β-chloroalanine derivative (**23**) was produced through reaction of *N*-benzoylalanine methyl ester (**17a**) with sulphuryl chloride and by photolysis of the *N*-chloroamide (**22**). Chlorination of the azetidinone (**16a**) gave (**16b**) in modest yield. These reactions establish the chemical validity of a regiospecific hydrogen-atom abstraction proposed in penicillin biosynthesis.

There have been several reports of regioselective hydrogen-atom transfer reactions affording amidocarboxy-substituted radicals. Irradiation of a mixture of methyl pyroglutamate (**1**) and di-t-butyl peroxide afforded products attributed to dimerization of the radical (**2**),[1] and oxidation of (**3a**) gave the diperoxide (**3b**), presumably *via* (**4a**) and (**4b**).[2] The radicals (**2**), (**4a**), and (**4b**) are stabilized by the combined action of an electron-releasing amido substituent and an electron-withdrawing carboxy substituent. They may be classified as captodative,[3] mero-stabilized,[4] or push-pull stabilized[5] radicals. Although synergistic stabilization by electron-donating and electron-withdrawing groups has not been demonstrated,[6] nevertheless these types of radicals are relatively stable and comparatively easy to form.

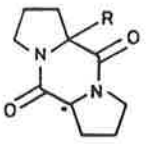


(**1**)



(**2**)

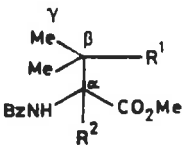
(**3**)
a; R = H
b; R = OOH



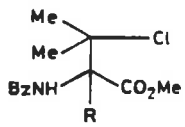
(**4**)
a; R = H
b; R = OOH

We reported recently that reaction of *N*-benzoylvaline methyl ester (**5a**) with sulphuryl chloride gave the β-chlorovaline derivative (**6a**) and lesser amounts of diastereoisomers of the γ-chlorovaline derivative (**7a**).[7] On the assumption that the chlorination proceeds by intermolecular hydrogen-atom transfer from (**5a**) with subsequent chlorine incorporation at the site of hydrogen abstraction,[8] this result is at variance with the earlier work as it indicates that the radicals (**8**) and (**9**), intermediates in the reactions to give (**6a**) and (**7a**) respectively, are formed in preference to the amidocarboxy-substituted radical (**10**).

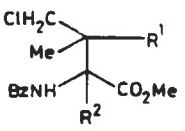
There are alternative explanations for the regioselective



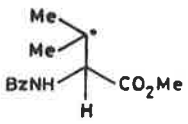
(**5**)
a; $R^1 = R^2 = H$
b; $R^1 = D, R^2 = H$
c; $R^1 = H, R^2 = D$



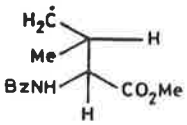
(**6**)
a; R = H
b; R = D



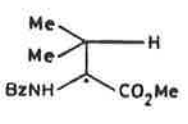
(**7**)
a; $R^1 = R^2 = H$
b; $R^1 = D, R^2 = H$
c; $R^1 = H, R^2 = D$



(**8**)

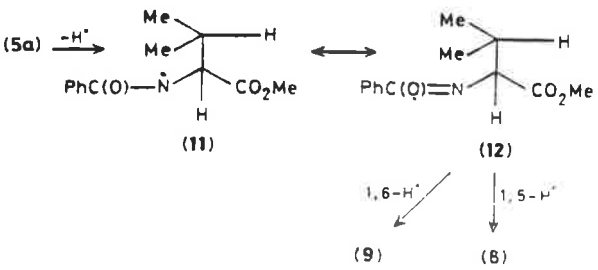


(**9**)



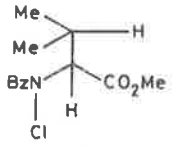
(**10**)

chlorination. For example, the reaction could involve the amido radical (**11**) as an intermediate (Scheme 1). Intramolecular hydrogen-atom transfer to the nitrogen-centred radical (**11**) is unlikely to occur because of geometrical constraints.[9,10] Although hydrogen transfer to amide oxygen has not been observed previously,[10] it is conceivable that intramolecular 1,5-hydrogen-atom transfer to the oxygen-centred radical (**12**) could occur. This would involve the same size cyclic transition state as that preferred by alkoxy radicals[11] and would account for the regioselective formation of (**8**). Reaction at the γ-position could be the result of less facile 1,6-hydrogen transfer to amide oxygen.



Scheme 1

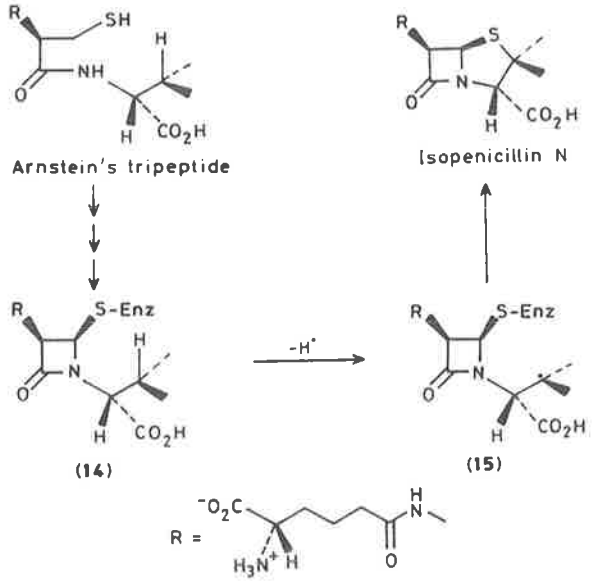
The work described here examines the mechanism of the chlorination of the valine derivative (**5a**). We have studied the reaction to determine if it involves intermolecular β-hydrogen transfer from (**5a**). We have studied photolyses of the *N*-chloroamide (**13**) to investigate reactions of the amido radical (**11**) and



(**13**)

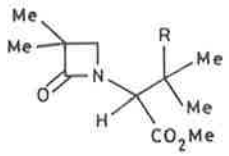
to probe for intramolecular hydrogen transfer to the oxygen-centred radical (**12**).

Initially we set out to examine hydrogen-atom transfer reactions of valine derivatives in order to assess the chemical validity of the regiospecific hydrogen-atom transfer (**14**)→(**15**) proposed in penicillin biosynthesis (Scheme 2).[12] Our original



Scheme 2.

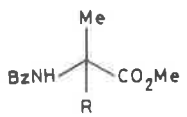
work with (**5a**) has been extended to study reaction of the azetidinone (**16a**), a closer analogue of (**14**). In addition we have studied chlorination of the alanine derivative (**17a**).



(**16**)

a; R = H

b; R = Cl



(**17**)

a; R = H

b; R = D

Results and Discussion

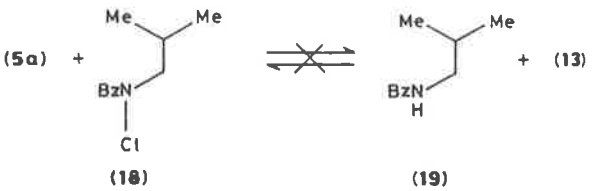
Treatment of *N*-benzoylvaline methyl ester (**5a**) with sulphuryl chloride in carbon tetrachloride or benzene at reflux under nitrogen afforded mixtures of the β-chlorovaline derivative (**6a**) and diastereoisomers of the γ-chlorovaline derivative (**7a**). The components were separated by h.p.l.c. The structure of the principal product, the β-chlorovaline derivative (**6a**), was confirmed by comparison with an authentic sample.[13] The combined yield of the chlorinated products (**6a**) and (**7a**) was high provided the extent of reaction of (**5a**) was restricted to less than 80%. More extensive reaction resulted in decomposition of the primary products.

Reaction of the β-chlorovaline derivative (**6a**) with triphenyltin hydride gave (**5a**). Use of enantiomerically pure (2*S*)-(**5a**) in the reaction with sulphuryl chloride gave the β-chlorovaline derivative (**6a**) which upon reduction with triphenyltin hydride, afforded the pure enantiomer (2*S*)-(**5a**). This sequence of reactions has been exploited in the synthesis of (2*R*)- and (2*S*)-[3-^{2}H]-valine.[14]

Photolysis of the *N*-chloroamide (**13**), obtained from the reaction of (**5a**) with t-butyl hypochlorite, afforded (**6a**) and (**7a**). Considerable quantities of (**5a**) were also produced. When the (2*S*)-valine derivative (**5a**) was used to prepare the *N*-chloroamide (**13**), photolysis of (**13**) and reduction of the product (**6a**) with triphenyltin hydride afforded (2*S*)-(**5a**). The reactions occur without racemisation at the α-position.

Photolysis of the *N*-chloroamide (**13**), prepared from the (2*S*)-valine derivative (**5a**), in the presence of the (2*R*)-valine derivative (**5a**), gave (**6a**) which reacted with triphenyltin hydride to give a mixture of the (2*R*)- and (2*S*)-valine derivative (**5a**). Since the production of (**6a**) from (2*S*)-(**5a**) *via* the *N*-chloroamide (**13**) occurs without racemisation in the absence of (2*R*)-(**5a**), the racemisation in the presence of the (2*R*)-valine derivative (**5a**) must result from interaction with this compound.

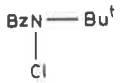
It is unlikely that the racemisation is due to reaction of the (2*R*)-valine derivative (**5a**) with the *N*-chloroamide (**13**) to give (2*S*)-(**5a**) and the enantiomer of (**13**). No interconversion between the valine derivative (**5a**) and the *N*-chloroamide (**18**) to give the amide (**19**) and the *N*-chlorovaline derivative (**13**), or *vice versa* (Scheme 3), was observed under the reaction



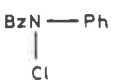
Scheme 3.

conditions. Presumably the radical (**11**), formed by photolysis of (**13**), reacts by intermolecular hydrogen abstraction. Reaction of the radical (**12**) by intramolecular hydrogen transfer (Scheme 1) would not result in racemisation. Although the possibility of some intramolecular reaction cannot be excluded, it is clear that intermolecular hydrogen transfer to the amidyl radical (**11**) competes effectively with intramolecular hydrogen transfer to the oxygen-centred radical (**12**), even in dilute solution. The radical (**11**) reacts by intermolecular hydrogen-atom abstraction to give (**8**) and (**9**), the precursors of (**6a**) and (**7a**) respectively.

Photolysis of mixtures of the valine derivative (**5a**) and *N*-chloro-*N*-t-butylbenzamide (**20**) or *N*-chloro-*N*-phenylbenzamide (**21**) in benzene afforded the chlorinated products



(**20**)



(**21**)

(**6a**) and (**7a**). Since (**13**) was not detected under the reaction conditions, it appears that the amidyl radicals produced by irradiation of the *N*-chloroamides (**20**) and (**21**), react by intermolecular hydrogen-atom abstraction from (**5a**) to give (**8**) and (**9**), which react to give (**6a**) and (**7a**) respectively.

To investigate the mechanism of the reaction of the valine derivative (**5a**) with sulphuryl chloride we examined reactions of the deuteriated analogues (**5b**) and (**5c**). (2*S*)-[3-^{2}H]Valine was prepared using the method of Baldwin and Wan,[15] and converted into (**5b**) using standard procedures.[16] Alternatively (**5b**) was prepared from the (2*S*)-valine derivative (**5a**) as outlined above, by reaction with sulphuryl chloride to give (**6a**), followed by reduction with triphenyltin deuteride.[14] (2*S*)-[2-^{2}H]Valine was prepared using the method of Greenstein and Winitz[17] and converted into (**5c**) using standard procedures.[16]

Whereas reaction of the valine derivative (**5a**) (0.0085M) with sulphuryl chloride in benzene afforded (**6a**) and (**7a**) in the ratio *ca.* 1.75:1.0, reaction of (**5b**) under identical conditions gave (**6a**) and (**7b**) in the ratio *ca.* 1.10:1.0. Reaction of (**5c**) gave (**6b**) and (**7c**) in the ratio *ca.* 1.75:1.0. These results indicate that there is a deuterium isotope effect of β-C–H bond cleavage.

In measuring the relative rates of reaction of the valine derivatives (**5a**—**c**) with sulphuryl chloride we exploited their chirality. The enantiomers exhibit identical reactivity in reactions with sulphuryl chloride, but they are physically separable for analysis by g.l.c. on a Chrompack XE-60-S-VAL-S-A-PEA column. Thus we were able to measure the relative rates of reaction of (**5a**—**c**) using a mixture of the (2*S*)-valine derivative (**5a**) and the (2*S*)-valine derivative (**5b**), and a mixture of (2*R*)-(**5a**) and (2*S*)-(**5c**). The ratios of the rate constants for the reactions of (**5a**—**c**) with sulphuryl chloride were calculated from the relative rates of consumption.[18] Whereas the β-deuteriated compound (**5b**) reacts with sulphuryl chloride *ca.* 0.80 times as fast as the unlabelled compound (**5a**) reacts, (**5a**) and the α-deuteriated derivative (**5c**) react at the same rate. The relative rate constants for reaction of (**5a**—**c**) show a deuterium isotope effect for β-C–H bond cleavage but no isotope effect for α-C–H bond cleavage.

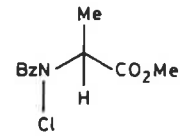
The deuterium isotope effects reflected in the relative rates of reaction of (**5a**) and (**5b**) and in the relative ratios of the products obtained from the reactions of those compounds, indicate that reaction of (**5a**) with sulphuryl chloride involves direct intermolecular hydrogen transfer from (**5a**) leading to (**6a**) and (**7a**). Assuming that abstraction of the β-hydrogen from (**5a**) or (**5b**) results in the production of (**6a**), and that abstraction of a γ-hydrogen from (**5a**) and (**5b**) leads to (**7a**) and (**7b**) respectively, on the basis of the product ratios (**5a**) would be expected to react *ca.* 1.31 times faster than (**5b**) [equation (1)] if

$$\frac{k(\mathbf{5a})}{k(\mathbf{5b})} = \frac{(\mathbf{6a}) + (\mathbf{7a})}{(\mathbf{6a}) + (\mathbf{7b})} = \frac{1.75 + 1.0}{1.10 + 1.0} = 1.31 \quad (1)$$

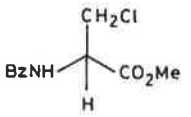
hydrogen abstraction from (**5a**) and (**5b**) is the irreversible rate-determining step. This value is, within experimental error, the same as the observed value of *ca.* 1.25.

We can not exclude the possibility that the amidyl radical (**11**) may be an intermediate in the reaction of (**5a**) with sulphuryl chloride. It does appear that reaction of the amidyl radical (**11**), generated by photolysis of the *N*-chloroamide (**13**), and the reaction of (**5a**) with sulphuryl chloride both involve intermolecular transfer of β- and γ-valinyl hydrogens. We believe the most reasonable rationalisation of the chlorination of (**5a**) with sulphuryl chloride is that intermolecular hydrogen-atom transfer from (**5a**) affords (**8**) and (**9**), which react by chlorine incorporation to give (**6a**) and (**7a**) respectively. There was no deuterium isotope effect for α-C–H bond cleavage and no evidence for products resulting from reaction at the α-position. High yields of (**6a**) and (**7a**) were obtained and the chlorination to give (**6a**) occurred without racemisation at the α-position. These points indicate that the radicals (**8**) and (**9**) are formed, by intermolecular hydrogen-atom transfer, in preference to the captodative radical (**10**).

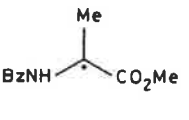
Treatment of *N*-benzoylalanine methyl ester (**17a**) with sulphuryl chloride afforded moderate amounts of the β-chloroalanine derivative (**23**), identical with an authentic sample.[19] Photolysis of the *N*-chloroalanine derivative (**22**) also gave the chlorinated product (**23**) in moderate yield. To probe for α-C–H bond cleavage in the reaction of (**17a**) with sulphuryl chloride, we studied the relative rates of reaction of the (2*R*)-alanine derivative (**17a**) and the (2*S*)-α-deuteriated analogue (**17b**) using the procedures described above. (2*S*)-[2-^{2}H]Alanine was prepared using the same procedure as that used to prepare (2*S*)-[2-^{2}H]valine.[17] The small deuterium isotope effect that was observed in the relative rates of reaction of (**17a**) and (**17b**) [k(**17a**)/k(**17b**) = 1.16], indicates that some reaction does occur at the α-position. It appears that formation of the tertiary captodative radical (**24**) occurs in competition with hydrogen-atom transfer from (**17a**) to give the primary radical (**25**).



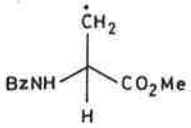
(**22**)



(**23**)



(**24**)



(**25**)

The original aim of this work was to examine hydrogen-atom transfer reactions of valine derivatives in order to assess the chemical validity of the regiospecific hydrogen-atom transfer (**14**)⟶(**15**) proposed in penicillin biosynthesis (Scheme 2).[12] To the extent that (**5a**) may be considered as a model of (**14**), the chlorinations of (**5a**) proceeding *via* regioselective β-C–H bond homolysis establish the chemical validity of the hydrogen-atom abstraction (**14**)⟶(**15**) and support the proposed mechanism for carbon–sulphur bond formation in penicillin biosynthesis shown in Scheme 2. In a more closely related system we found that reaction of the azetidinone (**16a**) with sulphuryl chloride, and photolysis of a mixture of the azetidinone (**16a**) and *N*-chloro-*N*-t-butylbenzamide (**20**), resulted in formation of the chlorinated azetidinone (**16b**) in each case.

During the course of this work Baldwin *et al.*[20] reported studies of the interaction of penicillin synthetase enzyme with modified substrates, supporting the previous contention[12] that abstraction of the β-valinyl hydrogen in penicillin biosynthesis is a homolytic process. Our work has shown that abstraction of the β-valinyl hydrogen from species analogous to (**14**) does occur despite the predicted relative stabilities of β-centred radicals such as (**15**) compared to the corresponding α-centred radicals. A rationale for the regioselectivity of these hydrogen transfer reactions is presented in the accompanying paper.[21]

Experimental

M.p.s were determined on a Kofler hot-stage apparatus and are uncorrected. I.r. spectra as liquid films, unless otherwise stated, were recorded on either a Shimadzu IR-27G or a Pye-Unicam

SP3-300 spectrophotometer. ^{1}H N.m.r. spectra were recorded in carbon tetrachloride using Me_4Si as internal standard, unless otherwise stated, on either a Varian T-60, a Varian CFT-20, or a Varian XL-300 spectrometer. Mass spectra were recorded on an AEI MS902 spectrometer and a Hewlett-Packard 5982A spectrometer. Microanalyses were performed by the microanalytical laboratory, University of Otago. G.l.c. analyses were performed on a Varian 3700 gas chromatograph fitted with a Chrompack XE-60-S-VAL-S-A-PEA column. Unless otherwise stated, preparative chromatography was carried out on a Chromatotron (a preparative, centifugally accelerated, radial thin layer chromatograph, Model 7924, Harrison Research Inc.) equipped with rotors coated with silica gel PF-254 (type 60 for t.l.c. Merck 7749) of varying thickness (generally 1 or 2 mm). H.p.l.c. analyses were performed with a Shimadzu LC-4A chromatograph on a Brownlee Laboratories OH-10A Diol column (26 cm × 4.6 mm i.d.) and a DuPont Zorbax cyanopropyl column (25 cm × 9.4 mm i.d.), using hexane–propan-2-ol (9:1) as eluant, monitoring at 220 nm. Preparative h.p.l.c. was carried out using the Zorbax column.

All solvents were purified and dried by standard methods. Light petroleum refers to the fraction with b.p. 50—70 °C. Valine, (2*R*)- and (2*S*)-valine, alanine, and (2*R*)-alanine were purchased from Sigma Chemical Co. (2*S*)-[3-^{2}H]Valine,[15] (2*S*)-[2-^{2}H]valine,[17] (2*S*)-[2-^{2}H]alanine,[17] t-butyl hypochlorite,[22] and triphenyltin hydride[23] were all prepared and purified by literature procedures. The chloroamides (**20**) and (**21**) were prepared by the reaction of benzoyl chloride with t-butylamine and aniline to give the respective amides, which were subsequently treated with t-butyl hypochlorite. The valine derivatives (**5a**), (2*S*)- and (2*R*)-(**5a**), (2*S*)-(**5b**), and (2*S*)-(**5c**), and the alanine derivatives (**17a**), (2*R*)-(**17a**), and (2*S*)-(**17b**), were prepared and purified using standard procedures.[16] Where appropriate, chiral purity was shown to be greater than 99% by g.l.c. analysis. By mass spectrometery, the deuterium content of (2*S*)-(**5b**), (2*S*)-(**5c**), and (2*S*)-(**17b**) was found to be 92, 85, and 83%, respectively.

Reaction of N-*Benzoylvaline Methyl Ester* (**5a**) *and the Deuteriated Analogues* (**5b**) *and* (**5c**) *with Sulphuryl Chloride.*—A mixture of *N*-benzoylvaline methyl ester (**5a**) (0.2 g, 0.85 mmol), sulphuryl chloride (0.1 ml, 1.2 mmol), and benzoyl peroxide (*ca.* 2 mg), in benzene (15 ml), was heated at reflux under nitrogen for 1 h and then cooled. H.p.l.c. of the mixture gave *N*-benzoyl-3-chlorovaline methyl ester (**6a**) as an oil (92 mg, 40%); ^{1}H n.m.r. δ 1.60 (s, 3 H), 1.74 (s, 3 H), 3.77 (s, 3 H), 4.90 (d, *J* 9 Hz, 1-H), 6.50 (br d, *J* 9 Hz, 1-H), and 7.10—8.00 (m, 5-H), identical with a sample synthesized using methods described previously.[13] Also obtained were diastereoisomers of *N*-benzoyl-4-chlorovaline methyl ester (**7a**), each of which crystallised from ethyl acetate–light petroleum as colourless needles. One isomer (28 mg, 12%) had m.p. 72—74 °C; δ 1.09 (d, *J* 7 Hz, 3-H), 2.50 (m, 1-H), 3.50 (m, 2-H), 3.80 (s, 3-H), 4.95 (dd, *J* 4 and 8 Hz, 1-H), 6.65 (br d, *J* 8 Hz, 1-H), and 7.30—7.90 (m, 5-H); $\nu_{max.}$(Nujol) 692, 1 641, and 1 750 cm^{-1}; *m/z* 271 and 269 (M^+, 1 and 3%, respectively), 234 (63), 212 (23), 210 (60), and 105 (100); *m/z* 269.0817 (M^+) [Calc. for $C_{13}H_{16}ClNO_3$ (M^+) *m/z* 269.0819] (Found: C, 57.64; H, 6.01. Calc. for $C_{13}H_{16}ClNO_3$: C, 57.89; H, 5.98%). The other isomer (33 mg, 14%) had m.p. 108—110 °C; δ 1.14 (d, *J* 7 Hz, 3-H), 2.50 (m, 1-H), 3.60 (m, 2-H), 3.84 (s, 3-H), 5.00 (dd, *J* 5 and 9 Hz, 1-H), 6.80 (br d, *J* 9 Hz, 1-H), and 7.30—8.00 (m, 5-H); $\nu_{max.}$(Nujol) 697, 1 642, and 1 747 cm^{-1}; *m/z* 271 and 269 (M^+, 1 and 4% respectively), 234 (55), 212 (17), 210 (42), and 105 (100); *m/z* 269.0799 (M^+) [Calc. for $C_{13}H_{16}ClNO_3$ (M^+) *m/z* 269.0819] (Found: C, 57.7; H, 5.85. Calc. for $C_{13}H_{16}ClNO_3$: C, 57.89; H, 5.98%).

The products were separated on a larger scale, but in lower percentage yield, when separation was carried out using the Chromatotron or by column chromatography on silica. Analysis of crude reaction mixtures by h.p.l.c. showed that the ratio of products (**6a**):(**7a**)(i):(**7a**)(ii) was *ca.* 3.2:1:1. This ratio was dependent on the solvent used in the reaction. In carbon tetrachloride the ratio of products (**6a**):(**7a**)(i):(**7a**)(ii) was *ca.* 2:1:1. The product ratio varied slightly with solvent concentration when benzene was used as a solvent.

Exactly analogous behaviour to that described above, was observed when the (2*S*)-valine derivative (**5a**) was treated with sulphuryl chloride.

To compare the reactions of (**5a**) and the deuteriated analogues (**5b**) and (**5c**) with sulphuryl chloride, each of the valine derivatives (8.5mM) was treated with sulphuryl chloride in benzene as described above. Product ratios for experiments performed in triplicate were determined by h.p.l.c. analyses carried out in triplicate. Reaction of (**5a**) gave (**6a**) and (**7a**) in the ratio 1.75 ± 0.1 : 1.0, reaction of (**5b**) gave (**6a**) and (**7b**) in the ratio 1.10 ± 0.10:1.0, and reaction of (**5c**) gave (**6b**) and (**7c**) in the ratio 1.75 ± 0.10:1.0.

To determine the relative rates of reaction of (**5a**), (**5b**), and (**5c**) with sulphuryl chloride, mixtures of (2*R*)-(**5a**) (4.25mM) and (2*S*)-(**5b**) (4.25mM), and of (2*R*)-(**5a**) (4.25mM) and (2*S*)-(**5c**) (4.25mM) in benzene were treated with sulphuryl chloride as described above. The extent of reaction of the valine derivatives (**5a—c**) was determined for experiments carried out in triplicate, by g.l.c. analyses performed in triplicate, and used to calculate the relative rates of reaction[18] of (**5a**), (**5b**), and (**5c**) as 1.0:0.80 ± 0.04:1.0 ± 0.03.

Reaction of N-*Benzoyl-3-chlorovaline Methyl Ester* (**6a**) *with Triphenyltin Hydride.*—A mixture of *N*-benzoyl-3-chlorovaline methyl ester (**6a**) (0.15 g, 0.56 mmol) and triphenyltin hydride (0.50 g, 1.42 mmol) in benzene (10 ml), was heated at reflux under nitrogen for 5 h, then cooled, concentrated, and chromatographed on silica. Elution with ethyl acetate–dichloromethane (1:9) afforded *N*-benzoylvaline methyl ester (**5a**) (89 mg, 68%).

When a sample of (**6a**), obtained by treatment of (2*S*)-(**5a**) with sulphuryl chloride, was treated with triphenyltin hydride, the product was the pure enantiomer (2*S*)-(**5a**) as determined by g.l.c. analysis.

N-*Benzoyl*-N-*chlorovaline Methyl Ester* (**13**).—A solution of *N*-benzoylvaline methyl ester (**5a**) (1.0 g, 4.3 mmol) and t-butyl hypochlorite (3 ml, 26.5 mmol) in toluene (20 ml) was left for 16 h in the dark. Excess of t-butyl hypochlorite was destroyed by the addition of potassium t-butoxide and the solvent was removed. The residue was dissolved in chloroform (25 ml) and the solution washed with water (4 × 60 ml), dried ($MgSO_4$), and concentrated to give crude *N*-benzoyl-*N*-chlorovaline methyl ester (**13**) as a red oil; δ($CDCl_3$) 1.00 (d, *J* 7 Hz, 3-H), 1.04 (d, *J* 7 Hz, 3-H), 2.47 (m, 1-H), 3.76 (s, 3-H), 4.55 (d, *J* 10 Hz, 1-H), and 7.20—7.90 (m, 5-H).

A sample of (**13**) was prepared from (2*S*)-(**5a**) in a similar manner.

Photolysis of N-*Benzoyl*-N-*chlorovaline Methyl Ester* (**13**).—Irradiation of a solution of the crude *N*-chlorovaline derivative (**13**) (0.2 g, 0.74 mmol) in benzene (20 ml), in a Rayonet photochemical reactor equipped with 16 RPR 3000 lamps, for 14 h, gave a mixture of (**5a**), (**6a**), and (**7a**) in the ratio *ca.* 45:3:1, as determined by h.p.l.c. analysis. In more concentrated solution relatively more (**6a**) and (**7a**) were produced, but the ratio of (**6a**) to (**7a**) remained constant. The products were separated by chromatography.

Photolysis of a sample of (**13**), obtained by treatment of (2*S*)-(**5a**) with t-butyl hypochlorite, afforded similar mixtures of products. When a sample of the β-chlorovaline derivative (**6a**),

prepared from (2*S*)-(**5a**) *via* the *N*-chlorovaline derivative (**13**), was treated with triphenyltin hydride, the product was the pure enantiomer (2*S*)-(**5a**) as determined by g.l.c. analysis.

Photolysis of a 1:1 mixture of the *N*-chlorovaline derivative (**13**), prepared from (2*S*)-(**5a**), and the (2*R*)-valine derivative (**5a**), gave a mixture of products from which the β-chlorovaline derivative (**6a**) was separated and treated with triphenyltin hydride to give a mixture of the (2*R*)- and (2*S*)-valine derivative (**5a**) in the ratio 1.0:1.20 ± 0.05 as determined by g.l.c. analysis.

Photolysis of N-*Chloro*-N-*t-butylbenzamide* (**20**) *in the Presence of* N-*Benzoylvaline Methyl Ester* (**5a**).—Irradiation of a mixture of *N*-chloro-*N*-t-butylbenzamide (**20**) (4.0 g, 18.9 mmol) and *N*-benzoylvaline methyl ester (**5a**) (0.5 g, 2.1 mmol) in benzene (50 ml) as described above afforded, after chromatography, the 3-chlorovaline derivative (**6a**) (0.29 g, 51%) and a mixture of diastereoisomers of the 4-chlorovaline derivative (**7a**) (42 mg, 7%).

Photolysis of N-*Chloro*-N-*phenylbenzamide* (**21**) *in the Presence of* N-*Benzoylvaline Methyl Ester* (**5a**).—Irradiation of a mixture of *N*-chloro-*N*-phenylbenzamide (**21**) (5.0 g, 21.6 mmol) and *N*-benzoylvaline methyl ester (**5a**) (0.5 g, 2.1 mmol) in benzene (50 ml) as described above afforded, after chromatography, the 3-chlorovaline derivative (**6a**) (114 mg, 20%) and a mixture of diastereoisomers of the 4-chlorovaline derivative (**7a**) (13 mg, 2%).

N-*Benzoyl*-3-*chloroaniline Methyl Ester* (**23**).—*Method A.* Treatment of *N*-benzoylalanine methyl ester (**17a**) with sulphuryl chloride in benzene as described above gave, after chromatography, *N*-benzoyl-3-chloroalanine methyl ester (**22**) in 36% yield, identical with a sample synthesized using methods described previously.[19]

Method B. Treatment of *N*-benzoylalanine methyl ester (**17a**) with t-butyl hypochlorite as described above gave *N*-benzoyl-*N*-chloroalanine methyl ester (**22**). Photolysis of the *N*-chloroalanine derivative (**22**) as described above gave, after chromatography, the 3-chloroalanine derivative (**23**) in 21% yield.

Reaction of the Alanine Derivatives (**17a**) *and* (**17b**) *with Sulphuryl Chloride.*—The relative rates of reaction of the alanine derivatives (**17a**) and (**17b**) with sulphuryl chloride were determined as described above for the valine derivatives (**5a**—**c**).

1-(1-*Methoxycarbonyl*-2-*methylpropyl*)-3,3-*dimethylazetidin*-2-*one* (**16a**).—Thionyl chloride (18.3 ml, 256 mmol) was added dropwise to methanol. Valine (10.0 g, 85 mmol) was then added and the solution was stirred at room temperature for 3 h after which it was concentrated to give crude valine methyl ester hydrochloride. A solution of 3-chloro-2,2-dimethylpropionic acid (10.4 g, 76 mmol) in thionyl chloride (11 ml, 152 mmol) was heated under reflux for 3 h and then concentrated. The residual oil was dissolved in dichloromethane (100 ml) and added dropwise to a solution of the crude valine methyl ester hydrochloride in dichloromethane (50 ml) and water (50 ml), to which potassium hydrogen carbonate was added as required to keep the solution basic. The mixture was stirred for 4 h and then the dichloromethane layer was separated, washed with water, dried ($MgSO_4$), and concentrated to give a solid which recrystallised from light petroleum as colourless crystals of *N*-(3-chloro-2,2-dimethylpropionyl)valine methyl ester (3.1 g, 16%), m.p. 62—63 °C; δ($CDCl_3$) 0.92 (d, *J* 7 Hz, 3-H), 0.94 (d, *J* 7 Hz, 3-H), 1.33 (s, 6-H), 2.16 (m, 1-H), 3.61 (s, 2-H), 3.75 (s, 3-H), 4.55 (dd, *J* 4 and 9 Hz, 1-H), and 6.26 (br d, *J* 9 Hz, 1-H); $\nu_{max.}$ 1 630 and 1 760 cm^{-1} (Found: C, 52.95; H, 8.15; N, 5.55. Calc. for $C_{11}H_{20}ClNO_3$: C, 52.90; H, 8.07; N, 5.61%).

Sodium hydride (50% in oil; 383 mg, 8 mmol) pre-washed with light petroleum, was suspended in a mixture of dichloromethane and dimethylformamide (3:1; 80 ml). To this suspension a solution of *N*-(3-chloro-2,2-dimethylpropionyl)-valine methyl ester (1.26 g, 5.1 mmol) in dichloromethane and dimethylformamide (3:1; 20 ml) was added dropwise. The solution was stirred under nitrogen for 6 h and then diluted with water (10 ml). The dichloromethane layer was separated, washed with water, dried ($MgSO_4$), and concentrated to give a residual oil which distilled to give the title azetidin-2-one (**16a**) as a colourless oil (0.39 g, 36%), b.p. 150—152 °C/18 mmHg block; δ 0.96 (d, *J* 6 Hz, 6-H), 1.28 (s, 6-H), 2.15 (m, 1-H), 3.10 (d, *J* 6 Hz, 1-H), 3.31 (d, *J* 6 Hz, 1-H), 3.73 (s, 3-H), and 4.06 (d, *J* 8 Hz, 1-H); $\nu_{max.}$ 1 722 and 1 740 cm^{-1}; *m/z* 213 (M^+, 72%) and 154 (100); *m/z* 213.1361 (M^+) [Calc. for $C_{11}H_{19}NO_3$ (M^+) *m/z* 213.1364].

1-(2-*Chloro*-1-*methoxycarbonyl*-2-*methylpropyl*)-3,3-*dimethylazetidin*-2-*one* (**16b**).—*Method A.* Treatment of the azetidin-2-one (**16a**) with sulphuryl chloride in benzene as described above gave, after chromatography, the title azetidin-2-one (**16b**) as a colourless oil in 6% yield; δ($CDCl_3$) 1.32 (s, 3-H), 1.35 (s, 3-H), 1.68 (s, 3-H), 1.75 (s, 3-H), 3.50 (d, *J* 6 Hz, 1-H), 3.63 (d, *J* 6 Hz, 1-H), 3.78 (s, 3-H), and 4.61 (s, 1-H); $\nu_{max.}$ 1 740 and 1 755 cm^{-1}; *m/z* 249 and 247 (M^+, 8 and 28%, respectively), 190 (38), and 188 (100); *m/z* 247.0970 (M^+) [Calc. for $C_{11}H_{18}ClNO_3$ (M^+) *m/z* 247.0975].

Method B. Irradiation of a mixture of *N*-chloro-*N*-t-butylbenzamide (**20**) (2.0 g, 9.5 mmol) and the azetidin-2-one (**16a**) (0.3 g, 1.4 mmol) in benzene (20 ml) as described above afforded, after chromatography, the title azetidin-2-one (**16b**) (0.13 g, 38%).

Acknowledgements

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References

1 N. Obata and K. Niimura, *J. Chem. Soc., Chem. Commun.*, 1977, 238.
2 U. Schmidt, J. Hausler, E. Ohler, and H. Poisel, *Prog. Chem. Org. Nat. Prod.*, 1979, **37**, 286.
3 H. G. Viehe, R. Merenyi, L. Stella, and Z. Janousek, *Angew. Chem., Int. Ed. Engl.*, 1979, **18**, 917; H. G. Viehe, Z. Janousek, and R. Merenyi, *Acc. Chem. Res.*, 1985, **18**, 148.
4 R. W. Baldock, P. Hudson, A. R. Katritzky, and F. Soti, *J. Chem. Soc., Perkin Trans. 1*, 1974, 1422.
5 A. T. Balaban, *Rev. Roum. Chim.*, 1971, **16**, 725.
6 D. Griller, *Annu. Rep. Prog. Chem., Sect. B*, 1984, **81**, 69.
7 C. J. Easton and N. J. Bowman, *J. Chem. Soc., Chem. Commun.*, 1983, 1193.
8 H. C. Brown and A. B. Ash, *J. Am. Chem. Soc.*, 1955, **77**, 4019; G. A. Russell and H. C. Brown, *ibid.*, p. 4031.
9 A. L. J. Beckwith and A. Goodrich, *Aust. J. Chem.*, 1965, **18**, 747; D. H. R. Barton, A. L. J. Beckwith, and A. Goosen, *J. Chem. Soc.*, 1965, 181; R. S. Neale, N. L. Marcus, and R. G. Schepers, *J. Am. Chem. Soc.*, 1966, **88**, 3051.
10 Y. L. Chow and T. C. Joseph, *J. Chem. Soc., Chem. Commun.*, 1969, 490; R. A. Johnson and F. D. Greene, *J. Org. Chem.*, 1975, **40**, 2186; T. C. Joseph, J. N. S. Tam, M. Kitadani, and Y. L. Chow, *Can. J. Chem.*, 1976, **54**, 3517.
11 C. Walling and A. Padwa, *J. Am. Chem. Soc.*, 1963, **85**, 1597.
12 J. E. Baldwin and T. S. Wan, *J. Chem. Soc., Chem. Commun.*, 1979, 249.
13 J. Oh-Hashi and K. Harada, *Bull. Chem. Soc. Jpn.*, 1966, **39**, 2287.
14 C. J. Easton and N. G. Findlay, *J. Labelled Compds. Radiopharm.*, 1985, **22**, 667.
15 J. E. Baldwin and T. S. Wan, *Tetrahedron*, 1981, **37**, 1589.
16 T. H. Applewhite, H. Waite, and C. Niemann, *J. Am. Chem. Soc.*, 1958, **80**, 1465.

17 J. P. Greenstein and M. Winitz, 'Chemistry of the Amino Acids,' Wiley and Sons, London 1961, p. 2375.
18 K. Hayday and R. D. McKelvey, *J. Org. Chem.*, 1976, **41**, 2222; C. Walling and M. J. Gibion, *J. Am. Chem. Soc.*, 1965, **87**, 3361.
19 P. Hughes, M. Martin, and J. Clardy, *Tetrahedron Lett.*, 1980, **21**, 4579.
20 J. E. Baldwin, R. M. Adlington, N. J. Turner, B. P. Domayne-Hayman, H. H. Ting, A. E. Derome, and J. A. Murphy, *J. Chem. Soc., Chem. Commun.*, 1984, 1167; J. E. Baldwin, R. M. Adlington, A. E. Derome, H. H. Ting, and N. J. Turner, *ibid.*, p. 1211.
21 C. J. Easton, M. P. Hay, and S. G. Love, *J. Chem. Soc., Perkin Trans. 1*, 1988, following paper.
22 H. M. Teeter and E. W. Bell, *Org. Synth.*, Coll. Vol. IV, 1963, 125.
23 H. G. Kuivila and O. F. Beumel, *J. Am. Chem. Soc.*, 1961, **83**, 1246.

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Regioselective Formation of Amidocarboxy-substituted Free Radicals

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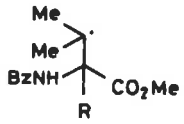
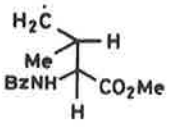
Factors affecting the production of amidocarboxy-substituted free radicals have been investigated by examining reactions of derivatives of valine and sarcosine. Variations in the regioselectivity of reactions of these compounds are exemplified by the reactions of *N*-benzoylvaline methyl ester (**4a**) and *N*-benzoylsarcosine methyl ester (**12a**) with sulphuryl chloride and *N*-bromosuccinimide. Whereas the reaction of (**4a**) with sulphuryl chloride involves hydrogen-atom transfer from the β-position of (**4a**) with subsequent chlorine incorporation to give (**4g**), in direct contrast the reaction with *N*-bromosuccinimide proceeds *via* hydrogen-atom abstraction from the α-position of (**4a**) and yields the dibromide (**4d**). *N*-Benzoylsarcosine methyl ester (**12a**) reacts with *N*-bromosuccinimide to give the α-bromosarcosine derivative (**12b**), whereas with sulphuryl chloride the product is the *N*-chloromethylglycine derivative (**12c**). These studies indicate that amidocarboxy-substituted radicals such as (**3a**) and (**13**) are considerably more stable than the tertiary alkyl radical (**1a**) and the amido-substituted radical (**14**), respectively, but hydrogen-atom transfer reactions may afford the less stable products if electrophilic radicals are involved in the hydrogen-atom abstraction and if there is little development of radical character in the reaction transition state.

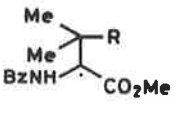
In the accompanying paper[1] we reported the regioselective chlorination of valine derivatives. Studies of the mechanism of the chlorination indicated that radicals such as (**1a**) and (**2**) are formed by direct intermolecular hydrogen-atom transfer. There was no evidence for the formation of the corresponding α-centred radical (**3a**), despite the expected greater stability of (**3a**) compared to (**1a**) and (**2**). The α-centred radical (**3a**) is stabilized



(**1**)
a ; R = H
b ; R = Br
c ; R = Cl



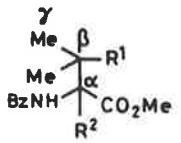
(**2**)



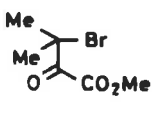
(**3**)
a ; R = H
b ; R = Br
c ; R = Cl

by the combined action of resonance electron-donating amido and electron-withdrawing carboxy substituents.

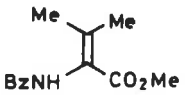
In the work described in this report[2] we have studied a variety of free-radical reactions of amino acid derivatives to investigate this anomaly.



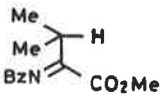
(**4**)
a ; $R^1 = R^2 = H$
b ; $R^1 = D, R^2 = H$
c ; $R^1 = H, R^2 = D$
d ; $R^1 = R^2 = Br$
e ; $R^1 = R^2 = Cl$
f ; $R^1 = Br, R^2 = H$
g ; $R^1 = Cl, R^2 = H$



(**5**)



(**6**)



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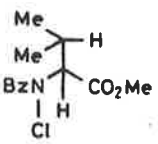


(**8**)

Results and Discussion

The benzoyl peroxide or photochemically initiated reaction of *N*-benzoylvaline methyl ester (**4a**) with 3 equivalents of *N*-bromosuccinimide (NBS) in carbon tetrachloride at reflux under nitrogen, afforded the dibromovaline derivative (**4d**) in high yield. Reaction with 2 equivalents of NBS gave the β-bromo-α-keto ester (**5**). Hydrogenolysis of the dibromovaline derivative (**4d**) over palladium on carbon produced a mixture of the acylenamine (**6**) and the valine derivative (**4a**). The acylenamine (**6**) was identified by comparison with an authentic sample, produced by condensation of *N*-benzoylglycine with acetone, and treatment of the product with sodium methoxide in methanol.[3] The acylenamine (**6**) was also produced by reaction of the dibromovaline derivative (**4d**) with pyridine. Treatment of (**6**) with NBS in carbon tetrachloride resulted in the formation of (**4d**).

In the reactions of (**4a**) with NBS, ^{1}H n.m.r. spectra of reaction mixtures, where less than 20% of the valine derivative (**4a**) had reacted, showed a doublet resonance at δ 1.3 p.p.m. (*J* 7 Hz) which could not be resolved further. This is consistent with formation of the acylimine (**7**) as a reaction intermediate.[4] All attempts to isolate the intermediate failed, and an attempt to synthesize an authentic sample of the acylimine (**7**) for comparison, by dehydrochlorination of the *N*-chloroamide (**8**),[4] was not successful. Trace amounts of the acylenamine (**6**) were detected in the reactions of (**4a**) with NBS, by ^{1}H n.m.r. spectroscopy and h.p.l.c. analysis.

The relative rates of reaction of (**4a**), and the deuteriated analogues (**4b**) and (**4c**), with NBS were determined using

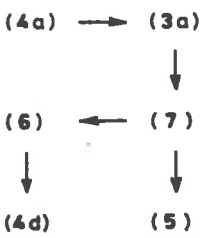
Table. Relative rates of reaction of the valine derivatives (**4a—c**)[a]

	Reagents		
	Sulphuryl chloride	Di-t-butyl peroxide	NBS
(**4a**)	1.0[b]	1.0[b]	1.0[b]
(**4b**)	0.80 ± 0.04	0.53 ± 0.04	1.00 ± 0.03
(**4c**)	1.00 ± 0.03	0.67 ± 0.03	0.27 ± 0.05

[a] Valine derivative and reagent in benzene under nitrogen. Reaction at reflux with irradiation by a 250-W mercury lamp. [b] Assigned as unity for each reagent.

methods described in the previous paper[1] and these are presented in the Table. Whereas the β-deuteriated valine derivative (**4b**) reacted at the same rate as the unlabelled compound (**4a**), the α-deuteriated analogue (**4c**) reacted at a reduced rate. There is a deuterium isotope effect for α-C–H bond cleavage, but no isotope effect for β-C–H bond cleavage.

Based on these results, production of (**4d**) and (**5**) in the reactions of (**4a**) with NBS can be rationalised as outlined in Scheme 1. From the deuterium isotope effect it appears that the



Scheme 1.

valine derivative (**4a**) reacts by regiospecific hydrogen-atom transfer to give the α-centred radical (**3a**). Subsequent reaction of the radical (**3a**) affords the acylimine (**7**), which hydrolyses in the presence of hydrogen bromide to give, after subsequent reaction with bromine or NBS, the β-bromo-α-keto ester (**5**). When an excess of NBS is present to remove the hydrogen bromide,[5] the acylimine (**7**) undergoes tautomerism to give the acylenamine (**6**), which reacts by the addition of bromine to give the dibromovaline derivative (**4d**).

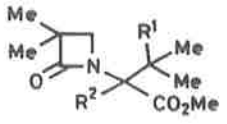
The relative rates of the photochemically induced reactions of (**4a**), and the deuteriated analogues (**4b**) and (**4c**), with di-t-butyl peroxide were measured and these are presented in Table 1, together with the relative rates of reaction of (**4a—c**) with sulphuryl chloride,[1] and with NBS. In direct contrast to the reactions of (**4a—c**) with NBS, the relative rates of reaction of (**4a—c**) with sulphuryl chloride exhibit a deuterium isotope effect for β-C–H bond cleavage, but no isotope effect for α-C–H bond cleavage. For reaction with di-t-butyl peroxide there is a deuterium isotope effect for both α- and β-C–H bond cleavage. It appears that whereas the reaction of (**4a**) with sulphuryl chloride involves selective hydrogen-atom transfer from the β-position to give (**1a**), the reaction with NBS proceeds *via* hydrogen-atom abstraction from the α-position of (**4a**) to give (**3a**). With di-t-butyl peroxide, reaction occurs at either position to give (**1a**) or (**3a**).

The variations in regioselectivity observed in these reactions may be interpreted in terms of the relative degrees of C–H bond homolysis in the reaction transition states.[6] With little development of radical character in the transition state of the chlorination reaction, the regioselectivity in this case is controlled by the inductive electron-withdrawing effect of the amido and carboxy groups acting to retard attack at the adjacent α-position by electrophilic radicals involved in the hydrogen-atom abstraction, thus favouring reaction at the β-position. The reaction with NBS is more sensitive to radical-stability effects since there is a greater degree of development of radical character in the transition state. Hydrogen-atom transfer from the α-position is favoured, therefore, because the product radical (**3a**) is stabilized by the combined effect of resonance electron-donating amido and electron-withdrawing carboxy groups. In the reaction with di-t-butyl peroxide, polar effects retarding attack at the α-position balance resonance effects facilitating reaction at that position. Thus, reaction occurs at the β-position in competition with reaction at the α-position.

In a related system we examined reactions of the dibromide (**4d**) and the corresponding dichloride (**4e**) with tributyltin hydride. The dichloride (**4e**) was obtained by treatment of the acylenamine (**6**) with sulphuryl chloride in carbon tetrachloride at room temperature. Reductions of alkyl halides by organotin hydrides proceed by halogen-atom abstraction with subsequent hydrogen incorporation. In these reactions stability of the intermediate free radical is a prime factor in determining the rate of halogen-atom abstraction.[7] In the unlikely event that halogen-atom abstraction from a vicinal dihalide affords the less stable of the possible product radicals, a facile 1,2-halogen migration to give the thermodynamically more stable radical would be expected.[8] The dihalogenated compounds (**4d**) and (**4e**) reacted with tributyltin hydride to give the corresponding β-halogenovaline derivatives (**4f**) and (**4g**). This indicates that the amidocarboxy-substituted radicals (**3b**) and (**3c**) are more stable than the corresponding β-centred radicals (**1b**) and (**1c**). Further, the production of only trace amounts of (**4a**), the product of subsequent reduction of (**4f**) and (**4g**), in the reactions with tributyltin hydride indicates that the radicals (**3b**) and (**3c**) are more stable than (**1a**).

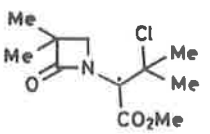
Treatment of the dichloroazetidinone (**9a**) with tributyltin hydride gave the monochloroazetidinone (**9b**), indicating that the radical (**10**) is more stable than the radical (**11a**). On this basis, production of the chlorinated azetidinone (**9b**) upon treatment of the azetidinone (**9c**) with sulphuryl chloride[1] must be attributed to polar effects, resulting in the regioselective formation of (**11b**) from (**9c**).

Reaction of *N*-benzoylsarcosine methyl ester (**12a**) with NBS afforded the α-bromosarcosine derivative (**12b**). In contrast,

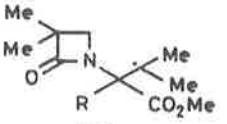


(**9**)

a ; $R^1 = R^2 = Cl$
b ; $R^1 = Cl$, $R^2 = H$
c ; $R^1 = R^2 = H$

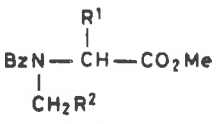


(**10**)



(**11**)

a ; R = Cl
b ; R = H



(**12**)

a ; $R^1 = R^2 = H$
b ; $R^1 = Br$, $R^2 = H$
c ; $R^1 = H$, $R^2 = Cl$

the reaction of (**12a**) with sulphuryl chloride gave the *N*-chloromethylglycine derivative (**12c**). This contrast in regioselectivity in the reactions of (**12a**) with NBS and sulphuryl chloride can also be attributed to the respective degrees of C–H bond homolysis in the transition states of the reactions. Extensive bond homolysis and development of radical character in the transition state of the reaction of (**12a**) with NBS results in reaction *via* the amidocarboxy-substituted radical (**13**), whereas the lack of development of radical character in the transition state of the reaction of (**12a**) with sulphuryl chloride is manifest in regioselectivity determined by polar effects and resulting in reaction *via* the radical (**14**).

(**13**) (**14**)

These studies indicate that amidocarboxy-substituted radicals such as (**3a**) and (**13**) are considerably more stable than the tertiary alkyl radical (**1a**) and the amido-substituted radical (**14**), respectively, but hydrogen-atom transfer reactions may afford the less stable products if electrophilic radicals are involved in the hydrogen-atom abstraction and if there is little development of radical character in the reaction transition state.

Experimental

General experimental details have been given in the accompanying paper.[1] Methyl 2-benzamido-3-methylbut-2-enoate (**6**)[3] and *N*-benzoylsarcosine methyl ester (**12a**)[9] were prepared and purified by literature procedures.

N-*Benzoyl*-2,3-*dibromovaline Methyl Ester* (**4d**).—A mixture of *N*-benzoylvaline methyl ester (**4a**) (0.5 g, 2.1 mmol) and NBS (1.2 g, 6.7 mmol) in carbon tetrachloride (30 ml), was heated at reflux under nitrogen, while irradiated with a 250-W mercury lamp, for 0.5 h. The cooled solution was filtered and concentrated to give the title ester (**4d**) as a pale yellow oil (0.73 g, 88%); δ 2.18 (s, 6-H), 3.67 (s, 3-H), and 7.3—8.0 (m, 6-H); $\nu_{max.}$ 1 686 and 1 745 cm^{-1}; *m/z* 395, 393, and 391 (M^+, 1, 2, and 1%, respectively), 314 (10), 313 (9), 312 (10), 311 (9), and 105 (100); *m/z* 392.9387 (M^+) [Calc. for $C_{13}H_{15}Br_2NO_3$ (M^+) *m/z* 392.9400].

Methyl 3-*Bromo*-3-*methyl*-2-*oxobutanoate* (**5**).—A mixture of *N*-benzoylvaline methyl ester (**4a**) (1.0 g, 4.3 mmol) and NBS (1.6 g, 9.0 mmol) in carbon tetrachloride (20 ml), was heated at reflux under nitrogen, while irradiated with a 250-W mercury lamp, for 1 h. The cooled solution was filtered and concentrated to give an oil, which was purified by column chromatography on silica. Elution with a gradient of ethyl acetate–light petroleum gives the title ester (**5**) (0.71 g, 79%); δ ($CDCl_3$) 1.98 (s, 6-H) and 3.90 (s, 3-H); $\nu_{max.}$ 1 720 and 1 740 cm^{-1}; *m/z* 210 and 208 (M^+, 11 and 10%, respectively), 151 (49), 149 (56), 123 (100), and 121 (96); *m/z* 207.9726 (M^+) [Calc. for $C_6H_9BrO_3$ (M^+) 207.9735].

Hydrogenolysis of N-*Benzoyl*-2,3-*dibromovaline Methyl Ester* (**4d**).—A mixture of the dibromovaline derivative (**4d**) (0.2 g, 0.51 mmol), sodium acetate (0.4 g), acetic acid (0.4 g), and 5% palladium on carbon (0.1 g), in methanol–water (4:1; 10 ml), was shaken under hydrogen (1 atm) for 2 h. Celite was added, and the solution was filtered and concentrated. The residue was dissolved in ethyl acetate and the solution washed with water, dried ($MgSO_4$), and concentrated to give an oil, which was chromatographed on silica to give *N*-benzoylvaline methyl ester (**4a**) (16 mg, 13%) and methyl 2-benzamido-3-methylbut-2-enoate (**6**)[3] (47 mg, 40%).

Reaction of N-*Benzoyl*-2,3-*dibromovaline Methyl Ester* (**4d**) *with Pyridine*.—A solution of the dibromovaline derivative (**4d**) (0.2 g, 0.51 mmol) in pyridine (6 ml), was heated at reflux for 0.5 h. The cooled solution was dissolved in ethyl acetate and the solution washed with water, 0.1 M hydrochloric acid, again with water, and then dried ($MgSO_4$) and concentrated. The residue recrystallised from ethyl acetate–light petroleum to give methyl 2-benzamido-3-methylbut-2-enoate (**6**)[3] (76 mg, 64%).

Reaction of Methyl 2-*Benzamido*-3-*methylbut*-2-*enoate* (**6**) *with NBS*.—A mixture of methyl 2-benzamido-3-methylbut-2-enoate (**6**) (0.3 g, 1.3 mmol) and NBS (0.5 g, 2.8 mmol) in carbon tetrachloride (15 ml), was heated at reflux under nitrogen, while irradiated with a 250-W mercury lamp, for 0.5 h. The cooled solution was filtered and concentrated to give *N*-benzoyl-2,3-dibromovaline methyl ester (**4d**) (0.36 g, 70%), identical with a sample obtained as described above.

N-*Benzoyl*-2,3-*dichlorovaline Methyl Ester* (**4e**).—A mixture of methyl 2-benzamido-3-methylbut-2-enoate (**6**) (0.5 g, 2.1 mmol) and sulphuryl chloride (0.4 ml, 5.0 mmol) in carbon tetrachloride (50 ml), was kept at room temperature for 0.5 h and then concentrated to give the title ester (**4e**) as a pale yellow oil (0.53 g, 83%); δ 2.00 (s, 6-H), 3.66 (s, 3-H), and 7.3—8.0 (m, 6-H); $\nu_{max.}$ 1 646 and 1 742 cm^{-1}; *m/z* 303 (M^+, 1%), 270 (2), 269 (8), 268 (5), 267 (21), and 105 (100); *m/z* 267.0663 (M^+ − HCl) [Calc. for $C_{13}H_{14}ClNO_3$ (M^+ − HCl) *m/z* 267.0662].

Reaction of N-*Benzoyl*-2,3-*dibromovaline Methyl Ester* (**4d**) *with Tributyltin Hydride*.—A solution of *N*-benzoyl-2,3-dibromovaline methyl ester (**4d**) (220 mg, 0.56 mmol) and tributyltin hydride (150 mg, 0.52 mmol) in benzene (3 ml), was kept at room temperature under nitrogen for 0.5 h and then concentrated and chromatographed on silica. Elution with a gradient of ethyl acetate–light petroleum gave *N*-benzoyl-3-bromovaline methyl ester (**4f**) as a low melting solid (83 mg, 51%), m.p. 50—52 °C; δ 1.83 (s, 3-H), 2.02 (s, 3-H), 3.80 (s, 3-H), 4.78 (d, *J* 9 Hz, 1-H), 6.85 (br d, *J* 9 Hz, 1-H), and 7.2—7.8 (m, 5-H); $\nu_{max.}$ 1 650 and 1 747 cm^{-1}; *m/z* 315 and 313 (M^+, 2 and 2%, respectively), 254 (48), 256 (49), 234 (46), and 105 (100); *m/z* 315.0279 (M^+) [Calc. for $C_{13}H_{16}BrNO_3$ (M^+) *m/z* 315.0294].

Analysis of crude reaction mixtures by h.p.l.c. showed that the ratio of (**4f**) to (**4a**) produced in these reactions was greater than 100:1.

Reaction of N-*Benzoyl*-2,3-*dichlorovaline Methyl Ester* (**4e**) *with Tributyltin Hydride*.—A solution of *N*-benzoyl-2,3-dichlorovaline methyl ester (**4e**) (100 mg, 0.33 mmol) and tributyltin hydride (90 mg, 0.31 mmol) in benzene (3 ml), was kept at room temperature under nitrogen for 0.5 h and then concentrated and chromatographed on silica. Elution with a gradient of ethyl acetate–light petroleum gave *N*-benzoyl-3-chlorovaline methyl ester (**4g**) as an oil[1] (65 mg, 73%).

Analysis of crude reaction mixtures by h.p.l.c. showed that the ratio of (**4g**) and (**4a**) produced in these reactions was greater than 100:1.

N-(3-*Bromo*-2,2-*dimethylpropionyl*)*valine Methyl Ester*.—Thionyl chloride (9.4 ml, 129 mmol) was added dropwise to methanol (150 ml). Valine (7.6 g, 65 mmol) was then added and the solution was stirred at room temperature for 3 h, then concentrated to give crude valine methyl ester hydrochloride. A solution of 3-bromo-2,2-dimethylpropionic acid (7.8 g, 43 mmol) in thionyl chloride (6.3 ml, 86 mmol) was heated under reflux for 3 h and then concentrated. The residual oil was

dissolved in dichloromethane (100 ml) and the solution added dropwise to a solution of the crude valine methyl ester hydrochloride in dichloromethane (50 ml) and water (50 ml), to which potassium hydrogen carbonate was added as required to keep the solution basic. The mixture was stirred for 4 h after which the dichloromethane layer was separated, washed with water, dried ($MgSO_4$), and concentrated to give a solid which recrystallised from light petroleum to give the title ester (6.2 g, 49%), m.p. 55—56 °C; δ($CDCl_3$) 0.92 (d, *J* 6 Hz, 3-H), 0.95 (d, *J* 6 Hz, 3-H), 1.35 (s, 6-H), 2.15 (m, 1-H), 3.50 (s, 1-H), 3.53 (s, 1-H), 3.75 (s, 3-H), 4.56 (dd, *J* 4 and 8 Hz, 1-H), and 6.2 (br d, *J* 8 Hz, 1-H); $\nu_{max.}$ 1 632 and 1 750 cm^{-1} (Found: C, 45.05; H, 6.95; N, 4.67. Calc. for $C_{11}H_{20}BrNO_3$: C, 44.91; H, 6.85; N, 4.76).

1,(1-*Methoxycarbonyl*-2-*methylprop*-1-*enyl*)-3,3-*dimethylazetidin*-2-*one*.—A solution of *N*-(3-bromo-2,2-dimethylpropionyl)valine methyl ester (6.1 g, 21 mmol), sulphuryl chloride (10.7 ml, 133 mmol), and benzoyl peroxide (*ca.* 100 mg), in benzene (20 ml), was heated at reflux under nitrogen for 8 h. The cooled solution was concentrated and the residue chromatographed on silica to give crude *N*-(3-bromo-2,2-dimethylpropionyl)-3-chlorovaline methyl ester as a pale yellow oil; δ($CDCl_3$) 1.42 (s, 6-H), 1.65 (s, 3-H), 1.76 (s, 3-H), 3.78 (s, 2-H), 3.81 (s, 3-H), 4.78 (d, *J* 9 Hz, 1-H), and 6.60 (br d, *J* 9 Hz, 1-H); $\nu_{max.}$ 1 638 and 1 745 cm^{-1}; *m/z* 331, 329, and 327 (M^+, 1, 6, and 8%, respectively), 165 (100) and 163 (98); *m/z* 327.0241 (M^+), [Calc. for $C_{11}H_{19}BrClNO_3$ (M^+) *m/z* 327.0236].

The oil was dissolved in a mixture of dichloromethane and dimethylformamide (4:1, 20 ml) and added dropwise to a suspension of sodium hydride (50% in oil; 1.48 g, 30.8 mmol), pre-washed with light petroleum, in a mixture of dichloromethane and dimethylformamide (4:1; 100 ml). The solution was stirred under nitrogen for 4 h and then diluted with water (10 ml). The dichloromethane layer was separated, washed with water, dried ($MgSO_4$), and concentrated. The residual oil was chromatographed on silica. Elution with a gradient of ethyl acetate–light petroleum gave the title ketone as an oil (1.7 g, 38%), b.p. 96—98 °C/16 mmHg, block; δ 1.30 (s, 6-H), 1.88 (s, 3-H), 2.17 (s, 3-H), 3.19 (s, 2-H), and 3.73 (s, 3-H); $\nu_{max.}$ 1 631, 1 720, and 1 758 cm^{-1}; *m/z* 211 (M^+, 45%), 183 (23), 180 (26), 155 (100), and 152 (64); *m/z* 211.1208 (M^+) [Calc. for $C_{11}H_{17}NO_3$ (M^+) *m/z* 211.1208].

Preparation of 1-(1,2-*Dichloro*-1-*methoxycarbonyl*-2-*methylpropyl*)-3,3-*dimethylazetidin*-2-*one* (**9a**) *and Reaction of* (**9a**) *with Tributyltin Hydride*.—A mixture of 1-(1-methoxycarbonyl-2-methylprop-1-enyl)-3,3-dimethylazetidin-2-one (0.1 g, 0.47 mmol), and sulphuryl chloride (0.2 g, 1.5 mmol), in carbon tetrachloride (2 ml), was stirred at room temperature for 15 min and then concentrated to give the crude title ketone (**9a**) as a pale yellow oil; δ 1.24 (s, 6-H), 1.77 (s, 3-H), 1.82 (s, 3-H), 3.39 (s, 2-H), and 3.75 (s, 3-H); $\nu_{max.}$ 1 723 and 1 745 cm^{-1}; *m/z* 285, 283, and 281 (M^+, 1, 3, and 4%, respectively), 248 (3), 246 (10), and 211 (100); *m/z* 246.0891 (M^+ − Cl) [Calc. for $C_{11}H_{17}ClNO_3$ (M^+ − Cl) *m/z* 246.0897].

The oil was dissolved in benzene (2 ml) and tributyltin hydride (0.4 g, 1.4 mmol) was added. The mixture was stirred at room temperature under nitrogen for 2 h after which it was concentrated and the residue chromatographed on silica. Elution with a gradient of ethyl acetate–light petroleum gave 1-(2-chloro-1-methoxycarbonyl-2-methylpropyl)-3,3-dimethylazetidin-2-one (**9b**) as an oil[1] (38 mg, 32%).

N-*Benzoyl*-2-*bromosarcosine Methyl Ester* (**12b**).—A mixture of *N*-benzoylsarcosine methyl ester (**12a**) (0.5 g, 2.4 mmol) and NBS (0.43 g, 2.4 mmol) in carbon tetrachloride (10 ml), was heated at reflux under nitrogen, while irradiated with a 250-W mercury lamp, for 15 min. The cooled solution was filtered and concentrated to give the title ester (**12b**) as an oil (0.59 g, 86%); δ 3.12 (s, 3-H), 3.82 (s, 3-H), 6.84 (s, 1-H), and 7.3—7.6 (m, 5-H); $\nu_{max.}$ 1 662 and 1 753 cm^{-1}; *m/z* 287 and 285 (M^+, 4 and 4%, respectively), and 206 (100); *m/z* 206.0819 (M^+ − Br) [Calc. for $C_{11}H_{12}NO_3$ (M^+ − Br) *m/z* 206.0817].

N-*Benzoyl*-N-*chloromethylglycine Methyl Ester* (**12c**).—A mixture of *N*-benzoylsarcosine methyl ester (**12a**) (0.5 g, 2.4 mmol) and sulphuryl chloride (0.2 ml, 2.5 mmol) in carbon tetrachloride (10 ml), was heated at reflux under nitrogen, while irradiated with a 250-W mercury lamp, for 0.5 h. The cooled solution was filtered and concentrated to give the title ester (**12c**) as an oil (0.52 g, 89%); δ 3.78 (s, 3-H), 4.25 (s, 2-H), 5.35 (s, 2-H), and 7.3—7.7 (m, 5-H); $\nu_{max.}$ 1 659 and 1 744 cm^{-1}; *m/z* 243 and 241 (M^+, 3 and 9%, respectively), 206 (7), 205 (18), 192 (8), and 105 (100); *m/z* 205.0739 (M^+ − HCl) [Calc. for $C_{11}H_{11}NO_3$ (M^+ − HCl) *m/z* 205.0739].

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References

1 N. J. Bowman, C. J. Easton, M. P. Hay, and S. G. Love, *J. Chem. Soc., Perkin Trans. 1*, preceding paper.
2 C. J. Easton and M. P. Hay, *J. Chem. Soc., Chem. Commun.*, 1985, 425.
3 G. R. Ramage and J. L. Simonsen, *J. Chem. Soc.*, 1935, 532.
4 H. Poisel and U. Schmidt, *Chem. Ber.*, 1975, **108**, 2547.
5 P. S. Skell and J. G. Traynham, *Acc. Chem. Res.*, 1984, **17**, 160.
6 G. A. Russell in 'Free Radicals,' ed. J. K. Kochi, Wiley, New York, 1973, Vol. 1, p. 275.
7 H. G. Kuivila, *Acc. Chem. Res.*, 1968, **1**, 299.
8 A. L. J. Beckwith and K. U. Ingold in 'Rearrangements in Ground and Excited States,' ed. P. de Mayo, Academic Press, New York, 1980, Vol. 1, p. 162.
9 A. H. Cook and I. M. Heilbron in 'The Chemistry of Penicillins,' eds. H. T. Clarke, J. R. Johnson, and R. Robinson, Princeton University Press, Princeton, 1949, p. 921.

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SELECTIVE MODIFICATION OF GLYCINE RESIDUES IN DIPEPTIDES

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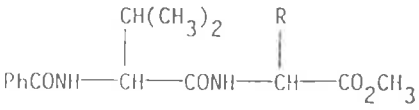
Summary: Reaction of dipeptides with N-bromosuccinimide results in selective α-bromination of glycine residues. Subsequent reactions of brominated peptides with diethyl malonate and allyltributyltin afford the diethyl malonyl- and allyl-substituted glycine derivatives, respectively.

A number of methods for the synthesis of amino acids involve ionic reactions in the elaboration of α-halogenated glycine derivatives.[1-4] With peptides these methods have been applied only to the modification of C-terminal glycine residues in dipeptides.[2,3] This restriction may be attributed, at least in part, to a lack of methods for the synthesis of peptides with an α-halogenated glycine residue at other than the C-terminal position. In the present work, which is based on our recent study of the preferential reaction of glycine residues in reactions of amino acid derivatives with N-bromosuccinimide,[5] we describe the direct and selective α-bromination of N- and C-terminal glycine residues in dipeptides. We report on the application of this method to the modification of glycine residues in dipeptides, including the introduction of the functionally versatile allyl group through the free-radical reaction of brominated peptides with allyltributyltin.[6]

The dipeptides (1a - 5a) reacted with N-bromosuccinimide (1 eq.) in dichloromethane, on photolysis at reflux under nitrogen, to give the corresponding brominated peptides (1b - 5b). Although 1b - 5b were not sufficiently stable for isolation and purification, their formation was detected directly by ^{1}H n.m.r. spectroscopic analysis of crude reaction mixtures after removal of solvent. The spectra of 1b - 5b each showed a doublet resonance attributable to the α-proton of the brominated glycine residue, at δ 6.60 (J 10Hz) and 6.62 (J 10Hz) for the diastereoisomers of 1b, δ 7.04 (J 9Hz) for 2b, δ 6.95 (J 9Hz) for 3b, δ 6.90 (J 10Hz) for 4b, and δ 6.95 (J 10Hz) and 6.98 (J 10Hz) for the diastereoisomers of 5b.

Yields of the brominated peptides (1b - 5b) and the selectivity for bromination of glycine residues in 1a - 5a were gauged by preparing derivatives of 1b - 5b. The bromides (1b) and (2b) were converted to the corresponding methoxy-substituted peptides (1c) and (2c), *in situ*, through addition of methanol directly to crude reaction mixtures after cooling to room temperature. The peptides (1c) and (2c) were isolated in yields of 65 and 73%, based on 1a and 2a, respectively, and were fully characterised.[7,8] The bromides (2b - 5b) were reduced to the corresponding deuterated peptides (2d - 5d), *in situ*, through addition of tributyltin deuteride to crude reaction mixtures cooled to room temperature. The deuterated dipeptides (2d - 5d) were isolated in yields ranging from 37-54%, based on 2a - 5a, and their deuterium content was determined using mass spectrometric analysis to range



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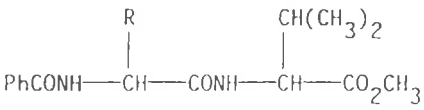

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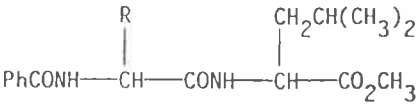

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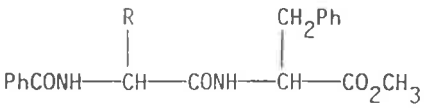

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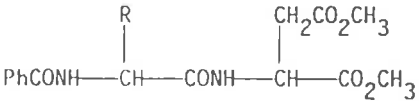

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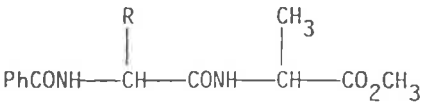

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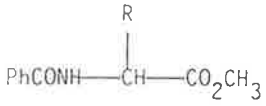

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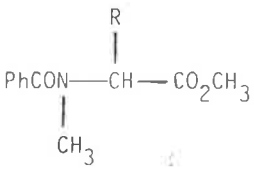

(8)

a) R=H
b) R=Br
c) R=OCH_3
d) R=D
e) R=$CH(CO_2Et)_2$
f) R=$CH_2CH{=}CH_2$

from 75-79% D_1. It was determined that the deuterium was incorporated regiospecifically at the α-position of glycine residues in 2d - 5d, by mass spectrometric analysis of molecular ions and ions with m/z 105 ($PhCO^{+\cdot}$), 134 ($PhCONHCH_2^{+\cdot}$) and 135 ($PhCONHCHD^{+\cdot}$).

Reaction of the glycylalanine derivative (6a) with N-bromosuccinimide followed by reduction with tributyltin deuteride gave 6d in only 11% yield, regiospecifically deuterated at the α-position of the glycine residue, but with only 40% deuterium incorporation. Presumably the low yield of 6d reflects the low selectivity in reactions of derivatives of glycine and alanine with N-bromosuccinimide.[5]

Within the scope indicated by the reactions of 1a - 6a, reaction of peptides with N-bromosuccinimide affords α-bromoglycine derivatives suitable for elaboration using methods previously described. Reactions of halogenated glycine derivatives with malonate anion have been utilised in syntheses of aspartic acid derivatives.[2,4] Accordingly, treatment of the brominated peptides (1b) and (2b), *in situ*, with diethyl malonate anion (2 eq.) at -10°C gave the corresponding adducts (1e) and (2e), which were fully characterised.[9,10]

Reactions of the α-bromoglycine derivative (7b) and the α-bromosarcosine derivative (8b), with allyltributyltin illustrate a complementary method for the elaboration of α-halogenated glycine derivatives. Treatment of 7b with allyltributyltin (2 eq.), *in situ*, at room temperature gave, after chromatography on silica, the allylglycine derivative (7f) in 62% yield.[11] Similar treatment of the sarcosine derivative (8b) gave 8f in 58% yield. The method proved suitable for the allylation of brominated glycine residues in peptides. Reaction of 1b and 2b afforded the corresponding allylated dipeptides (1f) and (2f), isolated in 33 and 35% yield, respectively. The allylglycine derivatives (1f, 2f, 7f and 8f) were fully characterised.[12-15]

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References and Notes

1. U. Zoller and D. Ben-Ishai, Tetrahedron, 1975, 31, 863; Z. Bernstein and D. Ben-Ishai, Tetrahedron, 1977, 33, 881; R. Kober, K. Papadopoulos, W. Miltz, D. Enders and W. Steglich, Tetrahedron, 1985, 41, 1693; P.J. Sinclair, D. Zhai, J. Reibenspies and R.M. Williams, J.Am.Chem.Soc., 1986, 108, 1103; R.M. Williams, D. Zhai and P.J. Sinclair J.Org.Chem., 1986, 51, 5021; Z. Lidert and S. Gronowitz, Synthesis, 1980, 322; R. Kober and W. Steglich, Liebigs Ann.Chem., 1983, 599.
2. S. Shiono and K. Harada, Bull.Chem.Soc.Jpn., 1985, 58, 1061.

3. A.L. Castelhano, S. Horne, R. Billedeau and A. Krantz, Tetrahedron Lett., 1986, 27, 2435.

4. D.H. Rich and M.K. Dhaon, Tetrahedron Lett., 1983, 24, 1671.

5. C.J. Easton and M.P. Hay, J.Chem.Soc.,Chem.Commun., 1986, 55.

6. For reviews on reactions of alkyl halides with allylstannanes see: G.E. Keck, E.J. Enholm, J.B. Yates and M.R. Wiley, Tetrahedron, 1985, 41, 4079; B. Giese in "Radicals in Organic Synthesis: Formation of Carbon-Carbon Bonds" (Pergamon Press: Oxford, 1986).

7. 1:1 mixture of diastereoisomers of 1c, m.p. 127-132°C; ^{1}H n.m.r. ($CDCl_3$) δ 1.00 (d, J 8Hz, 6H), 2.15 (m, 1H), 3.34 and 3.44 (s and s, total 3H), 3.63 and 3.73 (s and s, total 3H), 4.79 (t, J 9Hz, 1H), 5.50 and 5.57 (d and d, J 9Hz and J 9Hz, total 1H) and 7.20-8.20 (m, 7H).

8. 1:1 mixture of diastereoisomers of 2c, oil; ^{1}H n.m.r. ($CDCl_3$) δ 0.95 (d, J 7Hz, 6H), 2.20 (m, 1H), 3.53 (s, 3H), 3.97 (s, 3H), 4.60 (dd, J 5,9Hz, 1H), 5.80 and 5.90 (d and d, J 8Hz and J 8Hz, total 1H), and 7.20-8.20 (m, 7H).

9. 3:1 mixture of diastereoisomers of 1e, m.p. 147-156°C, 18% yield based on 1a, ^{1}H n.m.r. ($CDCl_3$) δ 1.00 (m, 6H), 1.19 (t, J 7Hz, 3H), 1.30 (t, J 7Hz, 3H), 2.25 (m, 1H), 3.73 and 3.77 (s and s, total 3H), 3.75 (m, 1H), 4.20 (m, 4H), 4.59 (t, J 8Hz, 1H), 5.36 (dd, J 4,9Hz, 1H), 6.84 (br.d, J 8Hz, 1H), 7.02 (br.d, J 9Hz, 1H) and 7.50-8.00 (m, 5H).

10. 1:1 mixture of diastereoisomers of 2e, m.p. 119-127°C, 18% yield based on 2a, ^{1}H n.m.r. ($CDCl_3$) δ 0.95 (m, 6H), 1.22 (t, J 7Hz, 3H), 1.33 (m, 3H), 2.17 (m, 1H), 3.66 and 3.75 (s and s, total 3H), 3.70 (m, 1H), 4.18 (m, 4H), 4.44 (m, 1H), 5.40 (m, 1H), 7.35 (br.d, J 8Hz, 1H), 7.50-7.90 (m, 5H) and 8.03 and 8.20 (br.d and br.d, J 8Hz and J 8Hz, total 1H).

11. Yields given for the allylated products (1f, 2f, 7f and 8f) are based on the quantity of the corresponding parent glycine derivatives (1a, 2a, 7a and 8a) used to prepare the bromides (1b, 2b, 7b and 8b).

12. 1f, m.p. 78-79°C; ^{1}H n.m.r. (CCl_4) δ 2.66 (m, 2H), 3.75 (s, 3H), 4.88 (m, 1H), 5.15 (m, 2H), 5.75 (m, 1H), 6.94 (br.d, J 7Hz, 1H), 7.42 (m, 3H) and 7.78 (m, 2H).

13. 2f, oil; ^{1}H n.m.r. (CCl_4) δ 2.61 (m, 2H), 2.88 (s, 3H), 3.70 (s, 3H), 5.20 (m, 2H), 5.31 (m, 1H), 5.82 (m, 1H) and 7.50 (m, 5H).

14. 3:1 mixture of diastereoisomers of 1f, m.p. 159-163°C; ^{1}H n.m.r. ($CDCl_3$) δ 0.95 (m, 6H), 2.20 (m, 1H), 2.55 (m, 2H), 3.70 and 3.76 (s and s, total 3H), 4.55 (m, 2H), 5.10 (m, 2H), 5.70 (m, 1H), 6.82 (br.d, J 10Hz, 1H), 7.02 (br.d, J 9Hz, 1H), and 7.40-7.90 (m, 5H).

15. 1:1 mixture of diastereoisomers of 2f, m.p. 138-141°C; ^{1}H n.m.r. ($CDCl_3$) δ 0.93 (d, J 6Hz, 3H), 0.98 (d, J 6Hz, 3H), 2.20 (m, 1H), 2.65 (m, 2H), 3.71 and 3.76 (s and s, total 3H), 4.60 (m, 1H), 4.75 (m, 1H), 5.25 (m, 2H), 5.85 (m, 1H) and 7.10-8.10 (m, 7H).

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Dimerization of Glycine Derivatives

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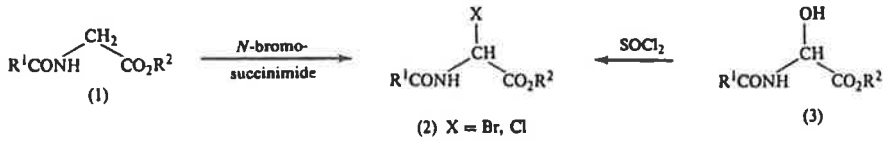
Abstract

The bromide (5), prepared by treatment of the glycine derivative (4) with *N*-bromosuccinimide, reacted with hexabutylditin to give diastereoisomers of the glycine dimer (7), only when moisture was rigorously excluded from the reaction. Otherwise the major products were the diastereoisomers of the ether (11). The structure of the racemic diastereoisomer (11) was determined by X-ray crystallography. Photolysis of mixtures of the glycine derivative (4) and di-t-butyl peroxide gave the alanine derivative (10) in addition to the diastereoisomeric dimers (7).

Introduction

A number of methods for the synthesis of amino acid derivatives involve elaboration of α-halogenated glycine derivatives (2).[1–7] The halogenated glycine derivatives (2) have been prepared either by treatment of the corresponding alcohols (3) with thionyl chloride,[1] or by reaction of the glycine derivatives (1) with *N*-bromosuccinimide.[2,3] In the present work, we sought to develop a method to produce the diastereoisomers of the glycine dimer (7), by coupling of the radical (9) produced by bromine-atom transfer from the bromide (5). We foresee that such a reaction could provide a



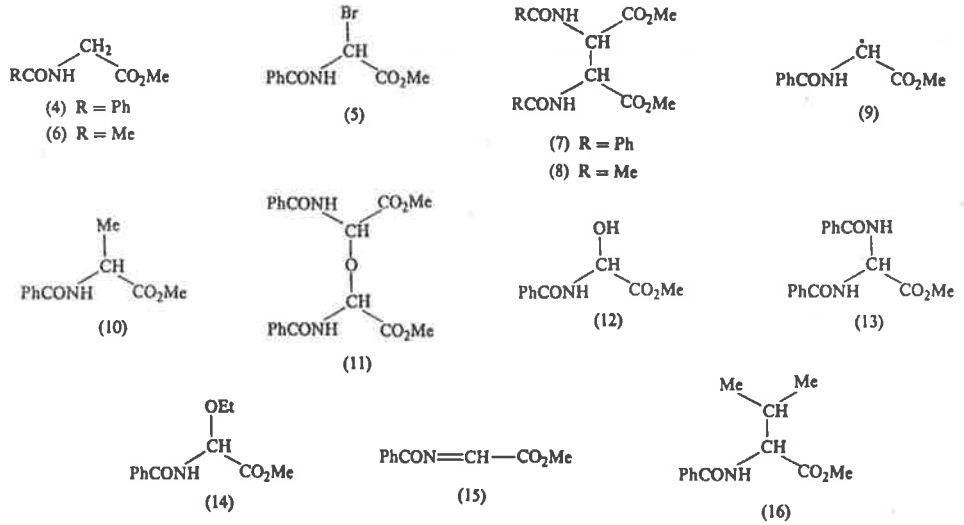
(1) (2) X = Br, Cl (3)

[1] Zoller, U., and Ben-Ishai, D., *Tetrahedron*, 1975, **31**, 863; Bernstein, Z., and Ben-Ishai, D., *Tetrahedron*, 1977, **33**, 881.
[2] Lidert, Z., and Gronowitz, S., *Synthesis*, 1980, 322.
[3] Kober, R., and Steglich, W., *Justus Liebigs Ann. Chem.*, 1983, 599.
[4] Kober, R., Papadopoulos, K., Miltz, W., Enders, D., Steglich, W., Reuter, H., and Puff, H., *Tetrahedron*, 1985, **41**, 1693.
[5] Rich, D. H., and Dhaon, M. K., *Tetrahedron Lett.*, 1983, **24**, 1671; Shiono, S., and Harada, K., *Bull. Chem. Soc. Jpn*, 1985, **58**, 1061.
[6] Castelhano, A. L., Horne, S., Billedeau, R., and Krantz, A., *Tetrahedron Lett.*, 1986, **27**, 2435.
[7] Sinclair, P. J., Zhai, D., Reibenspies, J., and Williams, R. M., *J. Am. Chem. Soc.*, 1986, **108**, 1103; Williams, R. M., Zhai, D., and Sinclair, P. J., *J. Org. Chem.*, 1986, **51**, 5021.

0004-9425/88/050701$03.00

method for the cross-linking of glycine residues in small peptides, in view of our recent study of the selective bromination of glycine derivatives.[8]

The dehydrodimerization of the glycine derivative (6) to produce a diastereoisomeric mixture of the glycine dimers (8) has been reported.[9] We have compared the dehydrodimerization of (4) with the debromodimerization of the bromide (5) prepared by treatment of (4) with *N*-bromosuccinimide.



Results and Discussion

Irradiation of a mixture of the glycine derivative (4) and di-t-butyl peroxide in benzene resulted in formation of the alanine derivative (10) in addition to diastereoisomers of the glycine dimer (7). On standing, one diastereoisomer of (7) crystallized selectively from the reaction mixture. Subsequent concentration of the mother liquor, followed by dissolution in ethyl acetate/hexane, led to crystallization of the other diastereoisomer of (7). Repeated fractional crystallization of the individual dimers (7), from benzene and from ethyl acetate/hexane, afforded pure compounds. Alternatively, the alanine derivative (10) and the glycine dimers (7) were isolated from reaction mixtures by chromatography on silica.

The alanine derivative (10) was identified by comparison with an authentic sample.[10] The identities of the glycine dimers (7) were confirmed by elemental analysis and on the basis of their spectral properties. The mass spectrum of each of the diastereoisomers of (7) showed a molecular ion at *m/z* 384. For each diastereoisomer of (7) the ^{13}C n.m.r. spectrum confirmed the presence of only eight distinct carbons, and the ^{1}H n.m.r. spectrum displayed a doublet resonance for the α,α'-hydrogens, at δ 5·34 (J 7 Hz) for the compound which crystallized directly from the reaction mixture and at δ 5·32 (J 7 Hz) for the other isomer. The diastereoisomer with a doublet resonance at δ_H 5·34 was shown to be racemic (7) by resolution into two components upon h.p.l.c. analysis by using a column with L-phenylglycine as the stationary phase.

[8] Easton, C. J., and Hay, M. P., *J. Chem. Soc., Chem. Commun.*, 1986, 55.
[9] Obata, N., and Niimura, K., *J. Chem. Soc., Chem. Commun.*, 1977, 238.
[10] Hein, G. E., and Niemann, C., *J. Am. Chem. Soc.*, 1962, **84**, 4487.

The other isomer was not resolved by h.p.l.c. analysis, consistent with assignment as the *meso*-dimer (7).

Production of the alanine derivative (10) and the dimers (7) in the reaction of the glycine derivative (4) with di-t-butyl peroxide may be rationalized as shown in equations (i)–(v). The radical (9) produced by hydrogen-atom transfer from the glycine derivative (4) to t-butoxy radical [equation (iii)] reacts either by dimerization to give (7) [equation (v)], or by coupling with methyl radical [equation (iv)] to give (10). The methyl radical is produced by β-scission of t-butoxy radical [equation (ii)].

$$Bu^tO{-}OBu^t \xrightarrow{h\nu} Bu^tO^\bullet \quad \text{(i)}$$

$$Bu^tO^\bullet \rightarrow {}^\bullet CH_3 + \text{acetone} \quad \text{(ii)}$$

$$Bu^tO^\bullet + (4) \rightarrow (9) + Bu^tOH \quad \text{(iii)}$$

$$(9) + {}^\bullet CH_3 \rightarrow (10) \quad \text{(iv)}$$

$$2(9) \rightarrow (7) \quad \text{(v)}$$

Analysis of reaction mixtures by h.p.l.c. and ^1H n.m.r. spectroscopy showed that the diastereoisomers of the dimer (7) were produced in equal proportions, and the sum concentration of the dimers (7) was approximately equal to the concentration of the alanine derivative (10) produced in the reaction. The ratio of products was not affected by changing the solvent from benzene to t-butyl alcohol, nor by variation of the light intensity incident on reaction mixtures. The ratio of production of the dimers (7) to the alanine derivative (10) varied only slightly as the concentration of the glycine derivative (4) used in the reaction was varied. It is apparent that one of the limitations to the dehydrodimerization of glycine derivatives by reaction with di-t-butyl peroxide is competing formation of the corresponding alanine derivatives.

Treatment of the glycine derivative (4) with *N*-bromosuccinimide afforded the bromide (5) in virtually quantitative yield. Irradiation of a mixture of hexabutylditin and the bromide (5) in benzene at reflux under nitrogen, under strictly anhydrous conditions, afforded the diastereoisomeric dimers (7). They were isolated by chromatography on silica in a total yield of 67% based on the quantity of the glycine derivative (4) used to prepare (5). Their production may be rationalized as follows:

$$Bu_3Sn{-}SnBu_3 \xrightarrow{h\nu} Bu_3Sn^\bullet \quad \text{(vi)}$$

$$Bu_3Sn^\bullet + (5) \rightarrow (9) + Bu_3SnBr \quad \text{(vii)}$$

$$2(9) \rightarrow (7) \quad \text{(v)}$$

When the reaction of the bromide (5) with hexabutylditin was carried out without rigorous exclusion of moisture, the dimers (7) were identified as only minor components of the product mixture. The two major components of the product mixture, present in equal proportions and comprising approximately 60% of the total products, were identified as diastereoisomers of the ether (11). The structure of the racemic diastereoisomer (11) was determined by X-ray crystallography. The asymmetric unit contains two molecules of the ether (11) and a disordered chloroform molecule.

Fig. 1 shows perspective views and atom labelling of the two independent molecules viewed in similar orientations [and both arbitrarily chosen as the (*R,R*)-enantiomer]. Comparable bond lengths and bond angles (Table 1) are similar within the two halves of each molecule and between the two independent molecules. These bond lengths and angles are similar to those found in structurally related compounds.[11]

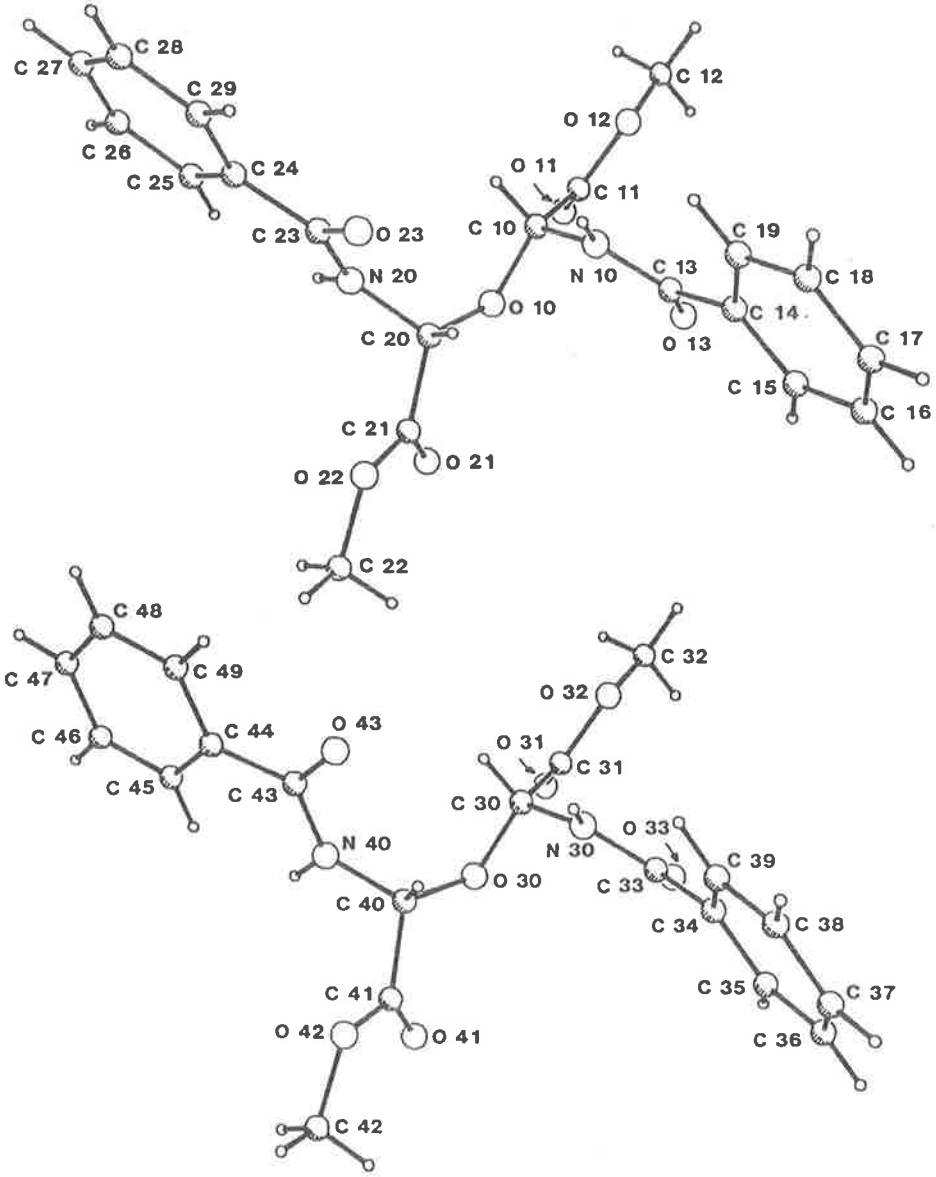


Fig. 1. Perspective views and atom labelling, used in Tables 1–3, of the two independent molecules of (11).

[11] International Union of Crystallography, 'Molecular Structures and Dimensions' Vols 1–15, and references cited therein.

Table 1. Comparison of equivalent bond lengths (Å) and angles (deg) in (11)

Feature	Atoms	Molecule A		Molecule B	
Distances[A]	O(10)–C(10)	1·414(5)	1·417(5)	1·422(4)	1·423(5)
	C(10)–C(11)	1·511(6)	1·517(6)	1·524(5)	1·523(5)
	C(11)–O(11)	1·201(4)	1·199(5)	1·200(4)	1·187(5)
	C(11)–O(12)	1·327(5)	1·331(6)	1·329(4)	1·336(5)
	O(12)–C(12)	1·447(7)	1·441(6)	1·447(5)	1·442(5)
	C(10)–N(10)	1·447(5)	1·435(5)	1·438(5)	1·440(6)
	N(10)–C(13)	1·357(5)	1·352(5)	1·364(6)	1·351(5)
	C(13)–O(13)	1·227(5)	1·227(4)	1·226(5)	1·226(4)
	C(13)–C(14)	1·492(5)	1·488(6)	1·492(6)	1·490(6)
	C(14)–C(15)	1·380(6)	1·386(5)	1·382(7)	1·383(5)
	C(14)–C(19)	1·382(5)	1·390(6)	1·394(6)	1·395(6)
	C(15)–C(16)	1·390(6)	1·385(6)	1·383(7)	1·384(7)
	C(16)–C(17)	1·364(6)	1·365(8)	1·384(7)	1·369(7)
	C(17)–C(18)	1·370(6)	1·383(8)	1·367(8)	1·380(6)
	C(18)–C(19)	1·385(6)	1·393(7)	1·378(6)	1·382(8)
Angles[B]	C(10)–O(10)–C(20)	112·7(3)		112·5(3)	
	O(10)–C(10)–C(11)	108·3(3)	106·8(3)	106·5(3)	107·0(3)
	O(10)–C(10)–N(10)	111·8(3)	112·2(3)	111·8(3)	112·3(3)
	C(11)–C(10)–N(10)	113·1(3)	110·9(3)	113·0(3)	108·7(3)
	C(10)–C(11)–O(11)	124·8(3)	125·9(4)	124·4(3)	125·7(3)
	C(10)–C(11)–O(12)	109·5(3)	108·6(3)	110·2(3)	108·3(3)
	O(11)–C(11)–O(12)	125·3(4)	125·5(4)	125·1(3)	126·0(3)
	C(11)–O(12)–C(12)	117·2(3)	115·6(3)	115·6(3)	115·6(3)
	C(10)–N(10)–C(13)	120·1(3)	123·2(3)	120·3(3)	121·8(3)
	N(10)–C(13)–O(13)	120·9(3)	121·6(4)	121·5(4)	120·8(4)
	N(10)–C(13)–C(14)	116·7(3)	116·5(3)	116·1(4)	117·1(3)
	O(13)–C(13)–C(14)	122·4(3)	121·8(3)	122·3(4)	122·1(4)
	C(13)–C(14)–C(15)	117·8(3)	123·9(3)	117·3(4)	123·6(4)
	C(13)–C(14)–C(19)	123·3(4)	116·6(3)	123·3(4)	117·1(3)
	C(15)–C(14)–C(19)	118·9(4)	119·5(4)	117·3(4)	119·2(4)
	C(14)–C(15)–C(16)	120·5(4)	120·5(4)	119·8(4)	120·3(4)
	C(15)–C(16)–C(17)	119·8(4)	119·9(4)	119·3(4)	120·0(4)
	C(16)–C(17)–C(18)	120·5(4)	120·6(5)	120·0(4)	120·5(5)
	C(17)–C(18)–C(19)	120·0(4)	119·9(5)	120·2(4)	119·8(5)
	C(14)–C(19)–C(18)	120·3(4)	119·6(4)	120·3(5)	120·1(4)

[A] The four columns of values refer to bonds between atoms labelled $1X$, $2X$, $3X$ and $4X$ respectively.
[B] The four columns of values refer to angles subtended at atoms labelled $1X$, $2X$, $3X$ and $4X$ respectively.

Table 2. Hydrogen bonding parameters for (11)

Hydrogen bond	Distance or angle	Hydrogen bond	Distance or angle
N(10)–H(10)···O(43)[A]	N–O 2·835 Å	N(30)–H(30)···O(23)[C]	N–O 2·835 Å
	H–O 1·98 Å		H–O 2·02 Å
	N–H–O 148°		N–H–O 144°
N(20)–H(20)···O(21)[B]	N–O 3·028 Å	N(40)–H(40)···O(31)[D]	N–O 2·947 Å
	H–O 2·48 Å		H–O 2·12 Å
	N–H–O 116°		N–H–O 136°

[A] O(43)[$1+x$, y, z]. [B] O(21)[$1-x$, $-y$, $-z$]. [C] O(23)[$x-1$, y, z]. [D] O(31)[$-1-x$, $1-y$, $-1-z$].

Significant torsional angle differences exist within the two halves of each molecule. For example, the two hydrogens attached to C(10) and N(10) in molecule A are almost eclipsed (i.e. *syn*-coplanar) (H–N–C–H 13°) whilst the hydrogens attached to C(20) and N(20) are approximately *anti*-coplanar (H–N–C–H 178°). Conformational differences also exist between the two molecules. For example, the mean planes through the two phenyl rings in molecule A are mutually inclined at an angle of 12·6(2)° whilst the corresponding value for molecule B is 30·5(2)°. These torsional differences are related to the molecular packing which is controlled by a complex network of hydrogen bonds. As listed in Table 2 all four independent amide hydrogens are hydrogen-bonded to carbonyl oxygens of adjacent molecules.

The elemental analysis and spectral data for racemic (11) are consistent with the structure determined by X-ray crystallography. The elemental analysis is satisfactory for the formula $C_{20}H_{20}N_2O_7 \cdot \frac{1}{2}CHCl_3$. A molecular ion is not observed in the mass spectrum, but an ion with *m/z* 208 is apparent, consistent with fragmentation of either of the ether C–O bonds. The ^{13}C n.m.r. spectrum shows the presence of eight distinct carbons, and the ^1H n.m.r. spectrum shows a doublet resonance for the α,α′-hydrogens at δ 6·22 (*J* 8 Hz).

The structure of the *meso*-diastereoisomer (11) was assigned on the basis of the elemental analysis, which is satisfactory for the molecular formula $C_{20}H_{20}N_2O_7$, and on the basis of the striking similarities between its spectral data and those recorded for racemic (11).

Other products isolated by chromatography of the reaction mixture were identified as the α-hydroxyglycine derivative (12), the α-benzamidoglycine derivative (13), and the α-ethoxyglycine derivative (14). The ratio of the products of the reaction was determined by h.p.l.c. and ^1H n.m.r. spectroscopy as follows: (±)-(7), 5%; *meso*-(7), 5%; (±)-(11), 31%; *meso*-(11), 31%; (12), 10%; (13), 18%. The ethoxyglycine derivative (14) was not detected in crude reaction mixtures.

The formation of (11)–(13) may be rationalized as follows:

$$(5) \xrightarrow{-HBr} (15) \xrightarrow{H_2O} (12) \qquad \text{(viii)}$$

$$(12) + (15) \rightarrow (11) \qquad \text{(ix)}$$

$$(12) \rightarrow PhCONH_2 \qquad \text{(x)}$$

$$PhCONH_2 + (15) \rightarrow (13) \qquad \text{(xi)}$$

Dehydrobromination of the bromide (5) affords the *N*-acyl imine (15) which reacts with adventitious water to produce the hydroxyglycine derivative (12) [equation (viii)]. Subsequent reaction of the hydroxyglycine derivative (12) with the *N*-acyl imine (15) affords the diastereoisomeric ethers (11) [equation (ix)]. Similar reactions of an analogue of the *N*-acyl imine (15) have recently been reported.[12] Formation of the benzamidoglycine derivative (13) may be attributed to reaction of benzamide, produced by cleavage of the hydroxyglycine derivative (12) [equation (x)], with the *N*-acyl imine (15) [equation (xi)]. Presumably the ethoxyglycine derivative (14) was formed from either the hydroxyglycine derivative (12) or the *N*-acyl imine (15), during

[12] Malassa, I., and Matthies, D., *Justus Liebigs Ann. Chem.*, 1986, 1133.

chromatography by reaction with ethanol present as a stabilizer in the chloroform used as eluent.

The major difficulty associated with carrying out the reaction of the bromide (5) with hexabutylditin under strictly anhydrous conditions is the hygroscopic nature of (5). It is necessary to prepare, isolate and react (5) under an atmosphere of dry nitrogen.

In conclusion, our results show that the glycine dimers (7) can be produced in good yield by debromodimerization of the bromide (5), provided moisture is rigorously excluded from the reaction. Otherwise the diastereoisomeric ethers (11) are the major products. Formation of the dimers (7) by dehydrodimerization of the glycine derivative (4) is complicated by competing formation of the alanine derivative (10). On this basis, we expect that the procedure for debromodimerization is likely to be more suitable than that for dehydrodimerization as a method for the cross-linking of glycine residues in small peptides. Another factor likely to favour use of the debromodimerization procedure is that the selectivity for reaction of glycine residues is greater in reactions with *N*-bromosuccinimide than in reactions with di-t-butyl peroxide. For example, whereas the glycine derivative (4) reacts 23 times faster than the valine derivative (16) in reactions with *N*-bromosuccinimide,[8] in reactions with di-t-butyl peroxide the glycine derivative (4) reacts only 5·3 times faster than (16).[13]

Experimental

Melting points are uncorrected. Solvents were purified and dried by standard methods. ^{1}H n.m.r. spectra were recorded on either a Varian T-60, Varian CFT-20, Varian XL-300 or Bruker CXP-300 spectrometer. ^{13}C n.m.r. spectra were recorded on either a Varian CFT-20 or Varian XL-300 spectrometer. N.m.r. spectra were recorded as dilute solutions in (D) chloroform with tetramethylsilane as an internal standard. Infrared spectra were recorded on either a Shimadzu IR-27G or Pye–Unicam SP3-300 spectrometer as Nujol mulls. Mass spectra were recorded on either a Kratos MS-9, AEI MS-902, or AEI MS-3010 spectrometer. H.p.l.c. was performed on a Shimadzu LC-4A instrument fitted with a Rheodyne 7125 injector and a Shimadzu SPD-2AS ultraviolet spectrophotometric detector. Analyses were carried out on a Dupont Zorbax cyanopropyl column (25 cm by 4·6 mm i.d.) (column *1*) or a Regis Pirkle covalent L-phenylglycine column (25 cm by 4·6 mm i.d.) (column *2*) with hexane/propan-2-ol as eluent. Chromatography was carried out on a Chromatotron 7924T (Harrison Research, Palo Alto/TC Research, Norwich) by using Merck silica gel 60 PF_{254}, eluting with a gradient of hexane/chloroform/ethyl acetate. Microanalyses were performed by the Microanalytical Laboratory, University of Otago.

Reaction of the Glycine Derivative (4) with Di-t-butyl Peroxide

In a typical experiment a mixture of the glycine derivative (4)[14] (0·5 g, 2·6 mmol) and di-t-butyl peroxide (2·5 ml, 12 mmol) in benzene (20 ml), contained in a quartz tube under nitrogen, was photolysed for 16 h in a Rayonet photochemical reactor equipped with 12 RPR 3500 lamps. Crystals which separated from the reaction mixture on standing were isolated by filtration, and recrystallized from benzene to give (±)-*dimethyl-2,3-dibenzamidobutanedioate* (7) as colourless crystals (46 mg, 9%), m.p. 189–193° (Found: C, 62·7; H, 5·1; N, 7·3. $C_{20}H_{20}N_2O_6$ requires C, 62·5; H, 5·2; N, 7·3%). ν_{max} 3420, 1740, 1640, 1540 cm^{-1}. ^{1}H n.m.r. δ 3·84, s, 2×CH_3; 5·34, d, *J* 7 Hz, 2×CH; 7·3–7·9, m, ArH; 7·98, br d, *J* 7 Hz, 2×NH. ^{13}C n.m.r. δ 53·2, 56·2, 127·4, 128·7, 132·2, 132·9, 168·2, 169·1. Found: $M^{+\bullet}$, 384·1301; $C_{20}H_{20}N_2O_6$ requires 384·1320; found: 263·0798; $C_{13}H_{13}NO_5$ requires 263·0794. H.p.l.c. analysis (column *2*) resolved the material into two components.

[13] Burgess, V. A., unpublished data.

[14] Huang, H. T., and Niemann, C., *J. Am. Chem. Soc.*, 1952, 74, 4634.

The mother liquor obtained by filtration of the reaction mixture was concentrated under reduced pressure. The residual oil crystallized from ethyl acetate/hexane to give meso-*dimethyl 2,3-dibenzamidobutanedioate* (7) as colourless crystals (18 mg, 4%), m.p. 206–207° (Found: C, 62·2; H, 5·1; N, 7·2. $C_{20}H_{20}N_2O_6$ requires C, 62·5; H, 5·2; N, 7·3%). ν_{max} 3350, 1745, 1635, 1540 cm^{-1}. ^1H n.m.r. δ 3·89, s, 2×CH_3; 5·32, d, *J* 7 Hz, 2×CH; 7·19, br d, *J* 7 Hz, 2×NH; 7·4–7·8, m, ArH. ^{13}C n.m.r. δ 53·4, 54·5, 127·4, 128·7, 132·1, 133·2, 167·4, 170·3. Found: $M^{+\bullet}$, 384·1306; $C_{20}H_{20}N_2O_6$ requires 384·1320; found: 263·0805; $C_{13}H_{13}NO_5$ requires 263·0794. H.p.l.c. analysis (column *2*) showed only one component.

In an alternative experiment chromatography of the reaction mixture on silica afforded the alanine derivative (10) (182 mg, 34%), identical with an authentic sample,[10] in addition to the racemic dimer (7) (105 mg, 20%) and the *meso*-dimer (7) (91 mg, 17%). The ratio of (10)/(±-(7)/*meso*-(7) in the crude reaction mixture was shown to be *c.* 2 : 1 : 1 by integration of ^1H n.m.r. spectra and by h.p.l.c. analysis (column *1*).

The reaction was carried out under identical conditions with t-butyl alcohol as solvent. The light intensity was varied by removing lamps from the photochemical reactor.

N-*Benzoyl-2-bromoglycine Methyl Ester (5)*[4]

A mixture of the glycine derivative (4) (2·0 g, 10 mmol) and *N*-bromosuccinimide (2·0 g, 11 mmol) in carbon tetrachloride (100 ml) was heated at reflux under nitrogen while irradiated with a 250-W mercury lamp for 0·5 h. The mixture was cooled in ice, filtered under nitrogen, and concentrated under a stream of dry nitrogen to give crude *N*-benzoyl-2-bromoglycine methyl ester (5) as pale yellow crystals which were used without further purification. ^1H n.m.r. δ 3·93, s, CH_3; 6·65, d, *J* 10 Hz, CH; 7·3–7·9, m, ArH and NH.

Reaction of the Bromoglycine Derivative (5) with Hexabutylditin

With extreme care to preclude moisture, a mixture of the crude bromoglycine derivative (5) prepared as described above from the glycine derivative (4) (2·0 g, 10 mmol) was dissolved in benzene (200 ml). Hexabutylditin (4·0 g, 7 mmol) was added, and the solution was heated at reflux under nitrogen for 2 h while irradiated with a 250-W mercury lamp. The solution was cooled to room temperature and washed with saturated aqueous sodium fluoride (4×250 ml). The organic layer was dried ($MgSO_4$) and concentrated. The residue was chromatographed on silica to give the racemic diastereoisomer (7) (0·67 g, 35%) and the *meso*-diastereoisomer (7) (0·61 g, 32%), identical in all respects to the samples obtained as described above.

When water was not rigorously excluded from the reaction, for example, when the bromide (5) was exposed even briefly to the atmosphere, a different product mixture was obtained. In a typical experiment, chromatography of the reaction mixture afforded the racemic dimer (7) (47 mg, 2%), the *meso*-dimer (7) (43 mg, 2%), and five other components.

(±)-*Dimethyl 2,4-dibenzamido-3-oxapentanedioate* (11), colourless crystals from chloroform (450 mg, 23%), m.p. 154–156° (Found: C, 53·6; H, 4·5; N, 6·1. $C_{20}H_{20}N_2O_7.\frac{1}{2}CHCl_3$ requires C, 53·5; H, 4·5; N, 6·1%). ^1H n.m.r. δ 3·77, s, 2×CH_3; 6·22, d, *J* 8 Hz, 2×CH; 7·4–7·9, m, ArH and 2×NH. ^{13}C n.m.r. δ 53·2, 76·5, 127·5, 128·7, 132·4, 132·7, 167·4, 168·0. Mass spectrum: *m/z* 208 (4%), 193 (3), 177 (10), 121 (94), 105 (100), 77 (76).

meso-*Dimethyl 2,4-dibenzamido-3-oxapentanedioate* (11), colourless crystals from chloroform (200 mg, 10%), m.p. 128–129° (Found: C, 60·0; H, 5·1; N, 7·0. $C_{20}H_{20}N_2O_7$ requires C, 60·0; H, 5·0; N, 7·0%). ^1H n.m.r. δ 3·81, s, 2×CH_3; 6·17, d, *J* 8 Hz, 2×CH; 7·4–8·0, m, ArH and 2×NH. ^{13}C n.m.r. δ 53·3, 75·4, 127·5, 128·7, 133·0, 132·8, 167·5, 168·1. Mass spectrum: *m/z* 208 (11%), 193 (12), 177 (35), 121 (54), 105 (100), 77 (46).

N-Benzoyl-2-hydroxyglycine methyl ester (12), colourless crystals (176 mg, 8%), m.p. 108–110° (lit.[15] 117–118°) (Found: C, 57·7; H, 5·2; N, 6·7. Calc. for $C_{10}H_{11}NO_4$: C, 57·4; H, 5·3; N, 6·7%). ^1H n.m.r. δ 3·88, s, 3H; 5·19, d, *J* 6 Hz, OH; 5·83, t, *J* 6 Hz, CH; 7·4–7·9, m, ArH and NH. ^{13}C n.m.r. δ 53·4, 72·7, 127·3, 128·7, 132·5, 132·7, 168·0, 169·0.

2-Benzamido-N-*benzoylglycine methyl ester* (13), colourless crystals (239 mg, 8%), m.p. 204–206° [Found: *m/z* 253·0975. $C_{15}H_{13}N_2O_2$ ($M^{+\bullet} - CO_2CH_3$) requires *m/z* 253·0977].

[15] Shemuakin, M. M., Chaman, E. S., Denisova, L. I., Ravidal, G. A., and Rodionov, V. Ya., *Bull. Chem. Soc. Fr.*, 1959, 530.

ν_{max} 3400, 1760, 1645, 1540 cm^{-1}. ^1H n.m.r. δ 3·86, s, CH_3; 5·91, t, *J* 7 Hz, CH; 7·4–8·0, m, ArH and 2×NH. ^{13}C n.m.r. δ 53·5, 58·0, 127·4, 128·7, 132·3, 132·7, 167·7, 168·6.

N-*Benzoyl-2-ethoxyglycine methyl ester* (14), colourless crystals (43 mg, 2%), m.p. 65–66° (Found: C, 61·0; H, 6·5; N, 5·9. $C_{12}H_{15}NO_4$ requires C, 60·8; H, 6·4; N, 5·9%). ^1H n.m.r. δ 1·25, t, *J* 8 Hz, CH_2CH_3; 3·78, q, *J* 8 Hz, CH_2CH_3; 3·84, s, OCH_3; 5·86, d, *J* 9 Hz, CH; 7·22, br d, *J* 9 Hz, NH; 7·4–7·9, m, ArH. ^{13}C n.m.r. δ 15·9, 53·0, 65·3, 77·4, 127·3, 128·7, 132·3, 133·2, 167·4, 168·8.

Crystallography

General.—Intensity data were collected with a Nicolet R3m four-circle diffractometer by using monochromatized Mo Kα radiation (λ 0·7107 Å). Cell parameters were determined by least-squares refinement, the setting angles of 25 accurately centred reflections ($2\theta > 20°$) being used. The intensities of three standard reflections (3 0 0, 0 6 0, 0 0 9) were monitored throughout data collection, and this test indicated no significant crystal decomposition. Intensities were corrected for Lorentz and polarization effects, but no correction was made for absorption. Reflections with intensities $I > 3\sigma(I)$ were used for structure solution and refinement.

The structure was solved by direct methods and refined by blocked cascade least-squares procedures. The asymmetric unit contains two independent molecules of the racemic ether (11), and a molecule of chloroform which is disordered over two orientations with relative site

Table 3. Atom coordinates and temperature factors ($Å^2$) for (11)

U_{eq} is one-third trace of orthogonalized U_{ij} tensor

Atom	10^4x	10^4y	10^4z	10^3U_{eq}	Atom	10^4x	10^4y	10^4z	10^3U_{eq}
		Molecule A					Molecule B		
O(10)	9229(3)	6345(2)	4674(2)	26(1)	O(30)	−613(3)	8684(2)	534(2)	23(1)
O(11)	10362(3)	7002(2)	5741(2)	36(1)	O(31)	−3066(3)	9658(2)	328(2)	31(1)
O(12)	10766(3)	8512(2)	4820(2)	35(1)	O(32)	−4008(3)	8673(2)	1505(2)	34(1)
O(13)	7656(3)	8362(2)	5036(2)	35(1)	O(33)	−2823(3)	7269(2)	383(2)	37(1)
N(10)	9000(3)	8112(2)	3904(2)	27(1)	N(30)	−1671(3)	7248(2)	1419(2)	26(1)
C(10)	9867(4)	7280(3)	4368(2)	26(1)	C(30)	−1641(4)	8369(3)	1195(2)	25(1)
C(11)	10314(4)	7573(3)	5073(2)	27(1)	C(31)	−2978(4)	8967(3)	934(2)	25(1)
C(12)	11434(5)	8851(3)	5381(3)	43(2)	C(32)	−5335(4)	9206(4)	1339(3)	44(2)
C(13)	7923(4)	8614(3)	4293(2)	28(1)	C(33)	−2189(4)	6765(3)	940(3)	31(2)
C(14)	7073(4)	9455(3)	3769(2)	28(1)	C(34)	−1927(4)	5595(3)	1130(3)	31(2)
C(15)	5742(5)	9699(4)	4082(3)	41(2)	C(35)	−1913(5)	5116(4)	502(3)	47(2)
C(16)	4889(5)	10459(4)	3618(3)	48(2)	C(36)	−1611(6)	4038(4)	633(3)	57(2)
C(17)	5367(5)	10958(4)	2844(3)	42(2)	C(37)	−1334(5)	3437(4)	1390(3)	50(2)
C(18)	6690(5)	10734(3)	2527(3)	40(2)	C(38)	−1367(5)	3906(3)	2016(3)	45(2)
C(19)	7543(5)	9976(3)	2987(3)	35(2)	C(39)	−1655(4)	4980(3)	1891(3)	36(2)
O(21)	7613(3)	4797(2)	5129(2)	37(1)	O(41)	1527(3)	8597(2)	−630(2)	34(1)
O(22)	7550(3)	4838(2)	3816(2)	38(1)	O(42)	2887(3)	7873(2)	339(2)	32(1)
O(23)	10366(3)	6355(2)	2462(2)	35(1)	O(43)	523(3)	8741(2)	2349(2)	34(1)
N(20)	10295(3)	5343(2)	3743(2)	27(1)	N(40)	1016(3)	9438(2)	1012(2)	24(1)
C(20)	9049(4)	5872(3)	4052(2)	25(1)	C(40)	705(4)	8534(3)	784(2)	24(1)
C(21)	7992(4)	5107(3)	4417(3)	29(1)	C(41)	1726(4)	8349(3)	62(2)	25(1)
C(22)	6538(5)	4107(4)	4063(3)	52(2)	C(42)	4016(4)	7740(4)	−276(3)	41(2)
C(23)	10884(4)	5627(3)	2963(2)	28(1)	C(43)	912(4)	9476(3)	1799(2)	23(1)
C(24)	12187(4)	5007(3)	2743(3)	30(2)	C(44)	1304(4)	10436(3)	1964(2)	27(1)
C(25)	12789(4)	4159(3)	3279(3)	33(2)	C(45)	1711(4)	11291(3)	1359(3)	34(2)
C(26)	14015(5)	3631(4)	3032(3)	45(2)	C(46)	2013(5)	12172(3)	1559(3)	44(2)
C(27)	14648(5)	3954(4)	2258(4)	58(2)	C(47)	1892(5)	12206(4)	2358(3)	47(2)
C(28)	14065(6)	4797(5)	1710(4)	67(3)	C(48)	1513(4)	11354(4)	2971(3)	46(2)
C(29)	12827(5)	5329(4)	1952(3)	51(2)	C(49)	1215(4)	10469(4)	2777(3)	36(2)
		Chloroform[A]					Chloroform[B]		
C(50)	4969(7)	7507(5)	3340(4)	55(3)	C(50a)	4422(20)	6743(15)	3607(12)	47(5)[C]
Cl(1)	5689(2)	7011(1)	4252(1)	41(1)	Cl(1a)	3091(7)	7673(7)	3220(6)	155(4)
Cl(2)	4964(2)	6517(1)	2842(1)	71(1)	Cl(2a)	5173(6)	6285(4)	2701(5)	84(3)
Cl(3)	3319(2)	8091(2)	3540(1)	66(1)	Cl(3a)	5552(12)	7114(8)	3984(7)	151(6)

[A] Occupancy 75%. [B] Occupancy 25%. [C] Isotropic.

occupancies of 0·75 and 0·25 (as determined by refinement). Except for the carbon of the minor chloroform contributor all non-hydrogen atoms were refined with anisotropic thermal parameters. Hydrogen atoms were included in calculated positions with isotropic thermal parameters equal to the isotropic equivalent of their carrier atoms. The function minimized was $\Sigma w(|F_o|-|F_c|)^2$, with $w = [\sigma^2(F_o)+0{\cdot}00054F_o^2]^{-1}$. All calculations were performed on Nova 4X or DG30 computers by using SHELXTL.[16] Table 3 lists the final coordinates for the non-hydrogen atoms, with estimated standard deviations in parentheses. Tabulations of structure factors, hydrogen atom coordinates and anisotropic thermal parameters have been deposited with the Australian Journal of Chemistry, 314 Albert Street, East Melbourne, Vic. 3002.

Crystal data for (±)-*(11) at 140 K.*—$C_{20}H_{20}N_2O_7{\cdot}\frac{1}{2}CHCl_3$, formula wt 460·1, triclinic, space group $P\bar{1}$, a 10·010(3), b 13·254(4), c 17·154(6) Å, α 74·83(3), β 80·73(3), γ 81·76(2)°, U 2156 Å^3, $F(000)$ 956, $D_c(Z = 4)$ 1·417 g cm^{-3}, μ 2·8 cm^{-1}, colourless crystal with dimensions 0·58 by 0·26 by 0·20 mm, ω scans, $2\theta_{max}$ 50°, N 7312, N_o 4690, 590 parameters, R 0·060, R_w 0·076.

Acknowledgments

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[16] Sheldrick, G. M., SHELXTL Users Manual, Revision 4, Nicolet XRD Corporation, Madison, WI, 1984.

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REACTIONS OF N-METHYL-γ-, δ-, AND ε-LACTAMS WITH t-BUTYL PERBENZOATE

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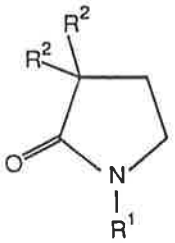
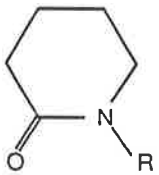
Abstract - N-Methyl-γ-, δ-, and ε-lactams reacted with t-butyl perbenzoate to give, in each case, products of oxidation at endocyclic and exocyclic carbon adjacent to amide nitrogen.

The photochemical alkylation of 2-pyrrolidinone (1a) has been reported to give products from reaction at the methylenes adjacent to the amide carbon and the amide nitrogen.[1] This is at variance with reports of the photochemical and anodic oxidation of the lactams (1a,b) and (2a,b) where the only products described were from reaction at methylene adjacent to amide nitrogen.[2-5] No products resulting from anodic or photochemical oxidation of the methyl substituent in (1b) or (2b) have been reported. In direct contrast, anodic oxidation of N-methylcaprolactam (3a) occurs regioselectively at the exocyclic carbon and no products of direct endocyclic oxidation have been reported.[4,6]



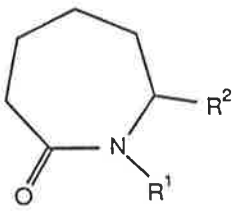
(1)

a; $R^1 = R^2 = H$
b; $R^1 = CH_3$, $R^2 = H$
c; $R^1 = R^2 = CH_3$



(2)

a; R = H
b; $R = CH_3$



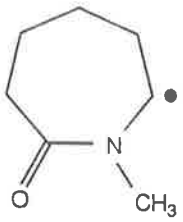
(3)

a; $R^1 = CH_3$, $R^2 = H$
b; $R^1 = CH_2OCH_3$, $R^2 = OCH_3$
c; $R^1 = CH_2OCH_3$, $R^2 = H$
d; $R^1 = CH_3$, $R^2 = OCH_3$

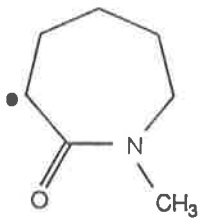
Production of the dimethoxylactam (3b) from reaction of (3a) has been attributed to subsequent endocyclic oxidation of (3c), the primary product of exocyclic oxidation, since (3c) but not (3d) was also formed in the course of the reaction.[6] The regioselectivity of anodic oxidation of N-alkylcaprolactams other than (3a) has been noted to vary from that of (3a),[7] but full details have not been reported.

Recently we reported the benzoyloxylation of β-lactams at exo- and endo-cyclic carbons adjacent to amide nitrogen.[8] The regioselectivity observed in these copper-catalysed reactions with t-butyl perbenzoate is similar to that reported for the anodic oxidation of β-lactams.[9] In view of the intriguing range of regioselectivities observed in the anodic and photochemical oxidation of the lactams (1a,b), (2a,b), and (3a), we have extended our earlier work with β-lactams to investigate reactions of the N,3,3-trimethyl-γ-lactam (1c)[10] and the N-methyl-δ- and ε-lactams (2b) and (3a)[11] with t-butyl perbenzoate. We found that pyrrolidinones without substituents at C-3 reacted to give complex intractable product mixtures.

Impetus for this work was provided by an EPR study of the reaction of N-methylcaprolactam (3a) with titanous chloride - hydrogen peroxide in a flow cell.[12] The major signal observed in the resultant spectrum indicated formation of either radical (4) or (5) [a_H (α) 22G doublet, a_H (β) 25G triplet] by hydrogen-atom transfer from (3a) to hydroxyl radical. While this experiment does not necessarily indicate the preferential formation of either (4) or (5), since other radicals may be produced and react at faster rates, it does indicate that (3a) reacts at least to some extent at endocyclic methylene adjacent to either amide nitrogen or amide carbon.

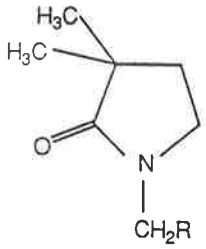


(4)



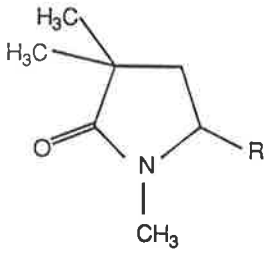
(5)

The copper-catalysed reaction of (1c) with t-butyl perbenzoate afforded, after chromatography of the reaction mixture on silica, the hydroxylactams (6a)[13,14] and (7a)[15] in yields of 16 and 17%, respectively. Analysis of crude reaction mixtures using ^{1}H nmr spectroscopy indicated that the corresponding benzoates (6b) (^{1}H nmr δ 5.61, s) and (7b) (^{1}H nmr δ 6.35, dd, J 2 and 6 Hz) were



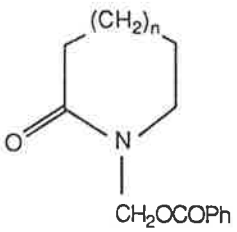
(6)

a; R = OH
b; R = OCOPh



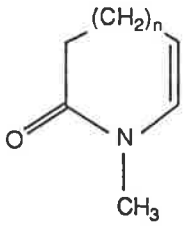
(7)

a; R = OH
b; R = OCOPh



(8)

a; n = 1
b; n = 2



(9)

a; n = 1
b; n = 2

formed in the ratio ca. 1:3, but the esters (6b) and (7b) hydrolysed to the corresponding alcohols (6a) and (7a) during chromatography. The δ-lactam (2b) reacted with t-butyl perbenzoate to give the exocyclic benzoate (8a)[16] and the olefin (9a)[17] in yields of 26 and 11%, respectively, while the ε-lactam (3a) gave (8b)[18] in 27% yield and (9b)[19] in 5% yield. Analysis of crude reaction mixtures after they were treated with triethylammonium chloride at reflux in benzene for 2 h, to facilitate elimination of any endocyclic benzoates, indicated that the δ-lactam (2b) produced (8a) and (9a) in the ratio ca. 2:3, while (8b) and (9b) were formed in the ratio ca. 3:2 by reaction of (3a). Presumably the relatively low yields of (7a), (9a), and (9b) reflect the decomposition of these compounds during isolation. Facile reactions of (9a) and related compounds on silica have been reported.[17,20] Since (8a) was isolated in 26% yield, and (8a) and (9a) were produced in the ratio ca. 2:3, it follows that the products (8a) and (9a) reflect major reaction pathways. By similar reasoning, compounds (6a), (7a), (8b), and (9b) also reflect major reaction pathways. Thus it is clear that with each of the lactams (1c), (2b), and (3a), oxidation occurs at endocyclic and exocyclic carbon adjacent to amide nitrogen. While the major reaction of the γ- and δ-lactams (1c) and (2b) is at the endocyclic

position, with the ε-lactam (3a) and with N,3,3-trimethylazetidin-2-one[8] reaction occurs predominantly at the exocyclic carbon.

ACKNOWLEDGEMENT

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REFERENCES AND NOTES

1. J. Sinnreich and D. Elad, Tetrahedron, 1968, **24**, 4509.
2. H. L. Needles and R. E. Whitfield, J. Org. Chem., 1966, **31**, 341.
3. J.-C. Gramain, R. Remuson, and Y. Troin, J. Chem. Soc., Chem. Commun., 1976, 194.
4. M. Okita, T. Wakamatsu, and Y. Ban, J. Chem. Soc., Chem. Commun., 1979, 749.
5. J. W. Pavlik and S. Tantayanon, J. Am. Chem. Soc., 1981, **103**, 6755.
6. T. Nishitani, H. Horikawa, T. Iwasaki, K. Matsumoto, I. Inoue, and M. Miyoshi, J. Org. Chem., 1982, **47**, 1706.
7. M. Okita, T. Wakamatsu, M. Mori, and Y. Ban, Heterocycles, 1980, **14**, 1089.
8. C. J. Easton and S. G. Love, Tetrahedron Lett., 1986, **27**, 2315.
9. M. Okita, M. Mori, T. Wakamatsu, and Y. Ban, Heterocycles, 1985, **23**, 247.
10. P. G. Gassman and B. L. Fox, J. Org. Chem., 1966, **31**, 982.
11. R. E. Benson and T. L. Cairns, J. Am. Chem. Soc., 1948, **70**, 2115.
12. W. T. Dixon and R. O. C. Norman, J. Chem. Soc., 1963, 3119.
13. All new compounds were fully characterized.
14. For (6a), ^{1}H nmr ($CDCl_3$) δ 1.15 (s, 3H), 1.17 (s, 3H), 1.93 (m, 2H), 3.45 (m, 2H), 4.75 (d, J 5 Hz, 2H) and 5.30 (br. s, 1H).
15. For (7a), ^{1}H nmr ($CDCl_3$) δ 1.08 (s, 3H), 1.20 (s, 3H), 1.76 (dd, J 3 and 14 Hz, 1H), 2.15 (dd, J 6 and 14 Hz, 1H), 2.78 (s, 3H), 5.00 (br. s, 1H) and 5.05 (dd, J 3 and 6 Hz, 1H).
16. For (8a), ^{1}H nmr ($CDCl_3$) δ 1.90 (m, 4H), 2.40 (m, 2H), 3.60 (m, 2H), 5.62 (s, 2H), 7.3-7.6 (m, 3H) and 7.9-8.3 (m, 2H).
17. J. C. Hubert, J. B. P. A. Wijnberg, and W. N. Speckamp, Tetrahedron, 1975, **31**, 1437.
18. For (8b), ^{1}H nmr ($CDCl_3$) δ 1.60 (m, 6H), 2.40 (m, 2H), 3.50 (m, 2H), 5.68 (s, 2H), 7.3-7.7 (m, 3H) and 7.9-8.3 (m, 2H).
19. For (9b), ^{1}H nmr ($CDCl_3$) δ 1.9-2.7 (m, 6H), 3.02 (s, 3H), 5.25 (m, 1H) and 5.88 (d, J 9 Hz, 1H).
20. J. B. P. A. Wijnberg, W. N. Speckamp, and J. J. J. de Boer, Tetrahedron Lett., 1974, 4077.

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The occurrence of polyenoic very long chain fatty acids with greater than 32 carbon atoms in molecular species of phosphatidylcholine in normal and peroxisome-deficient (Zellweger's syndrome) brain

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The $n-6$ tetra- and pentaenoic fatty acids with carbon chain lengths > 32 found in normal brain are located predominantly in a separable species of phosphatidylcholine. A similar phospholipid is found in increased amounts in the brain of peroxisome-deficient (Zellweger's syndrome) patients, but the fatty acid composition differs in that penta- and hexaenoic derivatives predominate. Our data strongly suggest that the polyenoic very long chain fatty acids are confined to the *sn*-1 position of the glycerol moiety, while the *sn*-2 position is enriched in saturated, monounsaturated and polyunsaturated fatty acids with < 24 carbon atoms. It is postulated that these unusual molecular species of phosphatidylcholine may play some, as yet undefined, role in brain physiology.

INTRODUCTION

Polyenoic fatty acids with carbon chain lengths > 22 (very long chain fatty acids, VLCFA) have been reported to be present in rat testes (Bridges & Coniglio, 1970), human brain (Poulos *et al.*, 1986*b*), mammalian spermatozoa (Poulos *et al.*, 1986*a*), and the retina of a number of different mammalian species (Aveldaño, 1987; Aveldaño & Sprecher, 1987) and to be synthesized by mouse spermatocytes and spermatids (Grogan & Huth, 1983), and human endothelial cells in culture (Rosenthal & Hill, 1984).

In bovine retina these lipids are predominantly $n-3$ series acids and occur in unusual dipolyunsaturated molecular species of phosphatidylcholine (PC) (Aveldaño & Sprecher, 1987); in contrast, the corresponding fatty acids in ram spermatozoa are found almost exclusively in a novel molecular species of sphingomyelin (Poulos *et al.*, 1987).

We have recently observed that the polyenoic VLCFA in the brain of patients with Zellweger's syndrome, a rare inherited disorder characterized by a marked deficiency of peroxisomes, are $n-6$ series fatty acids and are confined to two lipid classes, cholesterol esters and an unidentified phospholipid (Sharp *et al.*, 1987). In contrast to the cholesterol esters, the latter contained relatively high concentrations of polyenoic VLCFA with 34, 36 and 38 carbon atoms. Similar VLCFA were also detected in normal neonatal brain, indicating that these fatty acids are normal brain components.

We report here that the brain lipid which contains such a high proportion of 34–38 carbon polyenoic fatty acids is an unusual molecular species of PC.

MATERIALS AND METHODS

Post-mortem samples were obtained from patients with biochemically and clinically confirmed diagnoses of Zellweger's syndrome (Poulos *et al.*, 1985), and stored at −70 °C for periods ranging from a few months to 8 years, prior to analysis. The ages of these patients ranged from 4 days to 6 weeks. Control brain samples were obtained from patients (aged 10 days to 5 months) who had died with sudden infant death syndrome.

Lipids were extracted from small amounts (< 2 g) of brain and individual components isolated as described in an earlier report (Sharp *et al.*, 1987). For extractions from larger amounts of brain, the following procedure was adopted: 45 g of brain was extracted by the Bligh & Dyer (1959) technique except for the substitution of 0.1 M-KCl for water at the partitioning stage. The total lipid (approx. 1.6 g was extracted from both control and Zellweger brain) was applied to a 3 cm diameter column of silicic acid (40 g, 325 mesh; Sigma) and eluted successively with 1000 ml of chloroform, 600 ml of chloroform/methanol (4:1, v/v), 800 ml of chloroform/methanol (1:1, v/v) and 600 ml of methanol. The chloroform/methanol (1:1, v/v) eluate was evaporated to dryness in a rotary evaporator at 40 °C, and the residue was applied to a 2 cm diameter column of DE-52 (20 g, Whatman) and the non-acidic lipids were eluted with 450 ml of chloroform/methanol (1:1, v/v). The lipid eluted from the DE-52 column (400–450 mg) was applied to eight 20 cm × 20 cm silica gel 60 t.l.c. plates (Merck) and chromatograms were developed in chloroform/methanol/water (14:6:1, by vol.). After spraying with 0.2% dichlorofluorescein in 95% ethanol to visualize

Abbreviations used: VLCFA, fatty acids with carbon chain lengths > 22; PC, phosphatidylcholine; VLCFA-PC, phosphatidylcholine enriched in VLCFA; FAB, fast atom bombardment.

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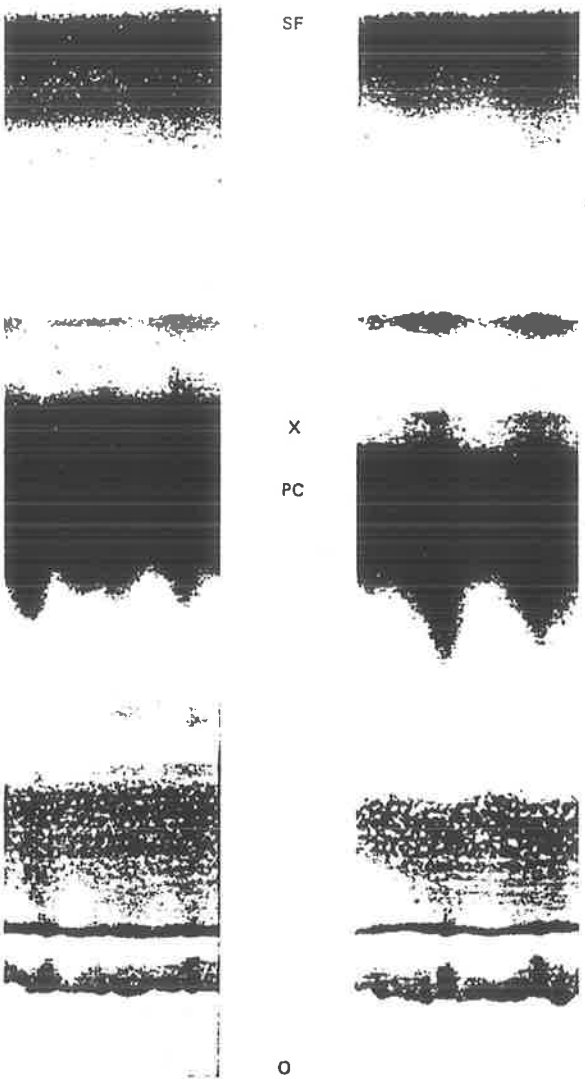

Fig. 1. T.l.c. of phospholipids from normal and Zellweger brain

Lipids were extracted from normal and Zellweger brain and were partially purified by silicic acid column chromatography as described in the text. The chromatograms shown above were obtained using aliquots of the chloroform/methanol (1:1, v/v) eluates. The various zones were detected with a phosphomolybdic acid spray reagent. Zellweger brain, left lane; normal brain, right lane. O, origin; SF, solvent front; X, phospholipid X; PC, phosphatidylcholine.

the various lipids, the zone migrating directly above the major PC zone was scraped from the plates, pooled, and eluted with 2 × 30 ml of chloroform/methanol/water (5:5:1, by vol.). The eluate was partitioned (Folch *et al.*, 1957) to remove the dye and evaporated to dryness in a rotary evaporator at 40 °C. The dried residue was dissolved in a small volume of chloroform/methanol (2:1, v/v). Further purification was achieved by t.l.c. on 20 cm × 10 cm high performance thin-layer silica gel 60 plates (HPTLC Kieselgel 60, Merck) in chloroform/methanol/water (14:6:1, by vol.). Zones were located and eluted in a similar fashion to that described for the t.l.c. on ordinary silica gel 60 plates.

Lipids were extracted from retina and purified by preparative t.l.c. as described for the brain. Aliquots of the various eluates were transesterified (Poulos *et al.*, 1986*b*) and the resulting fatty acid methyl esters were subjected to the combined g.c.-m.s. technique described earlier (Fellenberg *et al.*, 1987). This technique permits the assignment of the *n*-series for individual polyenoic fatty acids. Phosphorus analyses were carried out as described by Owens & Hughes (1970). Phospholipase A_2 hydrolysis was carried out using a commercial preparation of the bee venom enzyme (Sigma, lyophilized enzyme). Lipid samples (0.07 μmol lipid phosphorus) were shaken vigorously at room temperature (20–22 °C) for 30 min with 500 μl of diethyl ether and 150 μl of 25 mM-Tris/HCl buffer, pH 8.3, containing 5 mM-$CaCl_2$ and 0.65 units of enzyme. At the end of the incubation period, 2.5 ml of chloroform/methanol (2:1, v/v) was added and the mixture was evaporated to dryness under N_2 at 40 °C. The residue was dissolved in a small volume of chloroform/methanol (2:1, v/v), applied as a 2 cm zone to a silica gel 60 (Merck) thin-layer plate and a chromatogram was developed in chloroform/methanol/water (14:6:1, by vol.). The reaction products (1-acyl-*sn*-glycero-3-phosphocholine and unesterified fatty acid) were located with dichlorofluorescein and the lipids were eluted with chloroform/methanol/water (5:5:1, by vol.). After elution the lipids were transesterified and the resulting methyl esters were subjected to g.l.c. (Poulos *et al.*, 1986*b*). The specificity of the enzyme for the *sn*-2 position was confirmed by using 1-stearoyl 2-arachidonoyl *sn*-glycero-3-phosphocholine (Sigma) as a model substrate. Under the conditions described, the products were 1-stearoyl *sn*-glycero-3-phosphocholine and arachidonic acid, indicating that there had been little cleavage of the *sn*-1 position by the enzyme.

Tentative identification of lipids was based on a comparison of t.l.c. mobility with authentic standards using chloroform/methanol/water (14:6:1, by vol.) as the developing solvent and either normal or high performance t.l.c. plates. Lipids were detected either with iodine or with a phosphomolybdic acid spray reagent (Skipski & Barclay, 1969). For more definitive identification the techniques of n.m.r. spectrometry and fast atom bombardment (FAB) mass spectrometry were employed. Proton n.m.r. spectra were recorded on a Fourier Transform Bruker CXP-300 spectrometer operating at 300 MHz. Spectra were determined in deuteriochloroform/deuterated methanol, using tetramethylsilane as an internal standard.

Collision activation mass analysed ion kinetic energy spectroscopy (CAMIKES) of ions produced by FAB was carried out as described by Easton *et al.* (1988). For these studies mass spectra were measured on a Vacuum Generator ZAB 2 HF mass spectrometer operating in the FAB mode.

RESULTS

Polyenoic VLCFA with 32 or more carbon atoms were previously detected in significant amounts in normal and Zellweger brain in a phospholipid (designated as 'X') which migrated between PC and phosphatidylethanol-

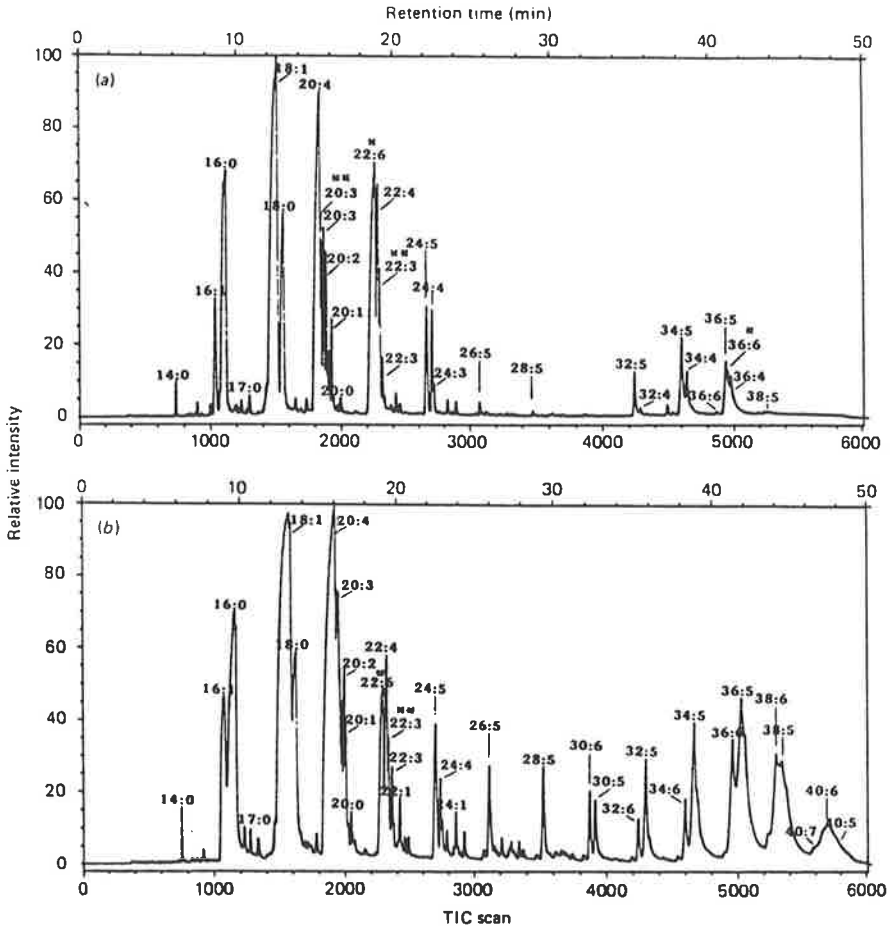

Fig. 2. G.c.-m.s. of normal and Zellweger brain phospholipid-bound fatty acids

Fatty acids (methyl esters) were released from normal (*a*) and Zellweger (*b*) brain polyenoic VLCFA-PC and were subjected to g.c.-m.s. analysis as described in the text. The figures shown above represent the total ion chromatogram. *, $n-3$ polyenoic fatty acids; **, $n-9$ polyenoic fatty acids; all other fatty acids with three or more double bonds are $n-6$ series acids.

amine zones on t.l.c. (Sharp *et al.*, 1987) (Fig. 1). (R_F lipid X, 0.61; PC, 0.52; R_F relative to PC 1.17.) This lipid was only a trace component of normal brain but was increased in Zellweger brain. Based on phosphate analysis, 0.9 and 2.8 μmol of the lipid were isolated from 45 g of control and Zellweger brain respectively. It generally migrated on normal silica gel t.l.c. plates as a single band, but high performance t.l.c. demonstrated that X isolated from normal and Zellweger brain may have comprised a number of closely migrating compounds. The complete band was used for analysis.

Although the normal and Zellweger brain lipid contained polyenoic VLCFA with 32, 34, 36 and 38 carbon fatty acids, there were subtle differences. In particular, Zellweger brain contained predominantly penta- and hexaenoic derivatives while tetra- and pentaenoic fatty acids were the major 32–38 carbon fatty acids in normal brain (Fig. 2). Zellweger brain also contained small amounts of $n-6$ polyenoic fatty acids with 40 carbon atoms. M.s. analysis confirmed that both normal and Zellweger brain fatty acids were predominantly $n-6$ series derivatives (Fellenberg *et al.*, 1987).

FAB mass spectra were obtained from the Zellweger and control brain lipid, and MIKES experiments were performed on the strong ion at m/z 184. These experiments demonstrated that the quaternary nitrogenous base choline was a constituent (Easton *et al.*, 1988) and suggested that the lipid was probably a species of PC enriched in polyenoic VLCFA (polyenoic VLCFA-PC) similar to that described in bovine retina (Aveldaño, 1987). T.l.c. of a bovine retina extract confirmed the presence of a lipid which migrated with a similar mobility to Zellweger and control polyenoic VLCFA-PC. The bovine retina lipid also showed a strong ion at m/z 184.

The p.m.r. spectrum of bovine retina PC (Fig. 3*a*) contained a number of easily identifiable peaks. These included a sharp singlet at δ 3.2 from the nine choline methyl hydrogens, a complex multiplet centred at δ 5.4 due to olefinic hydrogens, and a complex multiplet centred at δ 2.8 attributed to doubly allylic hydrogens.

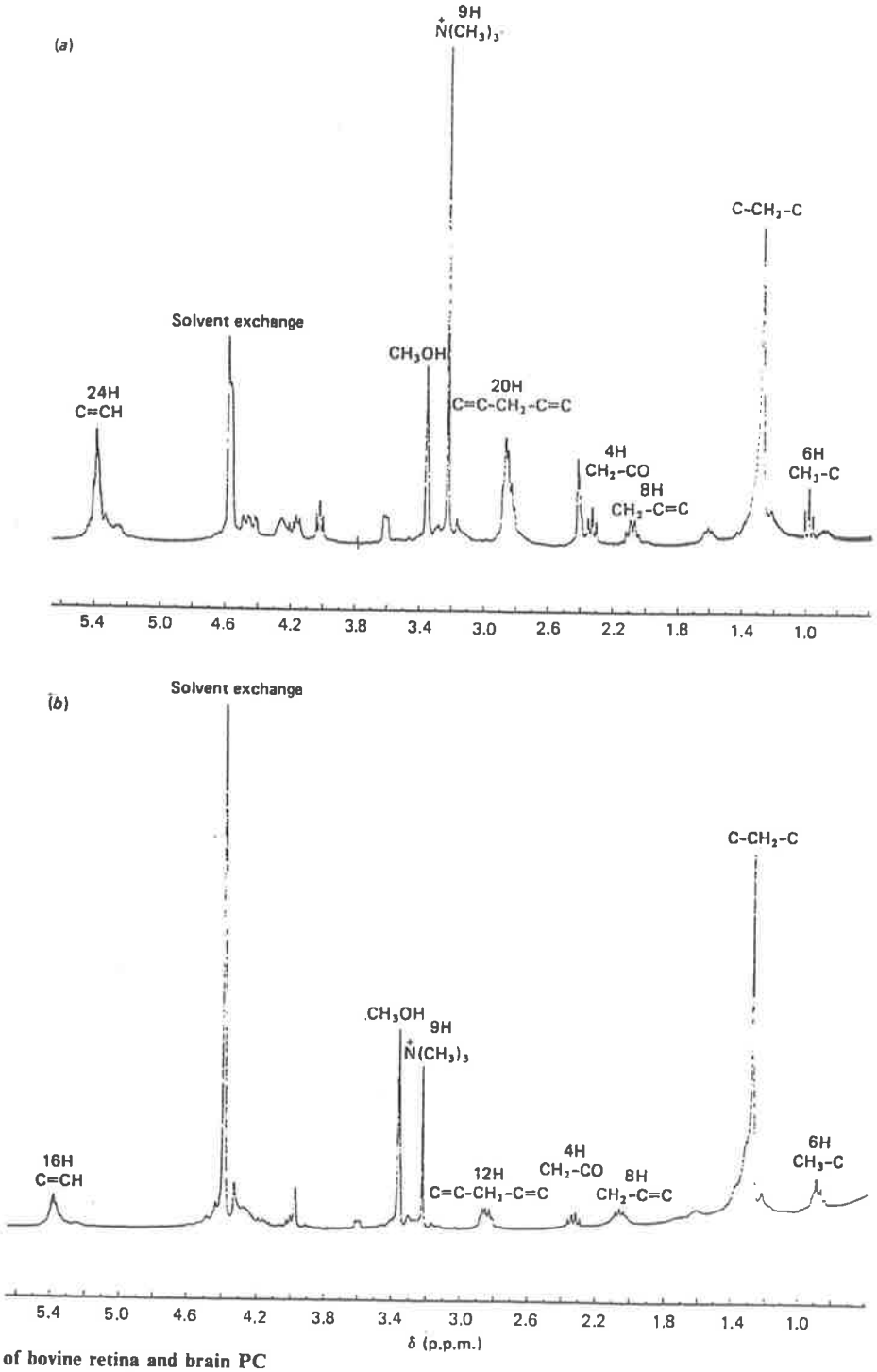

Fig. 3. P.m.r. of bovine retina and brain PC

P.m.r. spectrometry of bovine retina (*a*) and Zellweger brain (*b*) polyenoic VLCFA-PC was carried out as described in the text.

The p.m.r. spectrum of the Zellweger brain lipid (Fig. 3*b*) contained those same peaks with different relative integrated areas. There was insufficient of the normal brain lipid to obtain a p.m.r. spectrum.

The high mass region of the FAB mass spectrum of bovine retina polyenoic VLCFA-PC (Fig. 4*a*) showed three major clusters. Our analysis of the fatty acid composition of this lipid confirmed that 22:6 ($n-3$), 32:6 ($n-3$) and 34:6 ($n-3$) were major components. The major ion of each cluster neatly fits with a PC

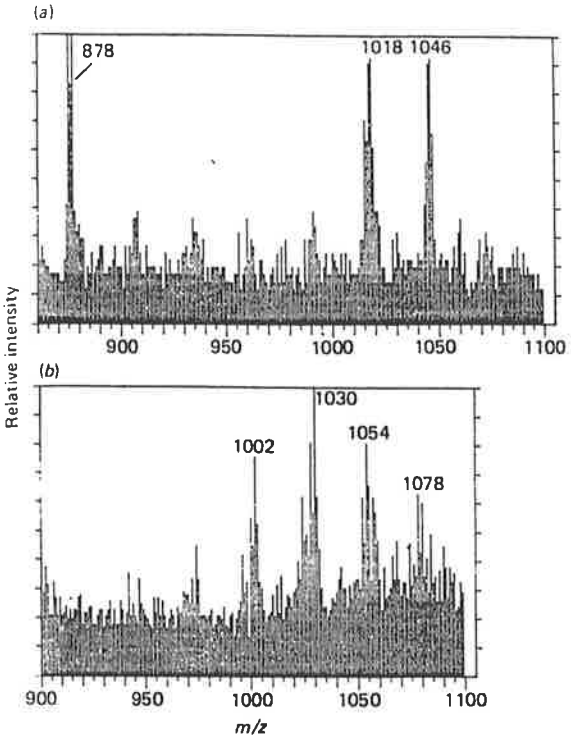

Fig. 4. FAB mass spectrometry of bovine retina and brain PC

FAB mass spectrometry of bovine retina (*a*) and Zellweger brain (*b*) polyenoic VLCFA-PC was carried out as described in the text.

containing two 22:6 (*m/z* 878), 22:6 and 32:6 (*m/z* 1018) and 22:6 and 34:6 (*m/z* 1046) fatty acids.

The corresponding high mass region of the FAB mass spectrum of Zellweger brain PC (Fig. 4*b*) contained four clusters of ions. Again it was simple to match the ions to a series of PCs, each of which contained a long chain (18–22) fatty acid and a very long chain (34–36) polyenoic fatty acid. The major ion of each cluster could be rationalized to belong to a PC containing either 18:1 and 34:5, or 18:0 and 34:6 (*m/z* 1002), 18:1 and 36:5, or 18:0 and 36:6 (*m/z* 1030), 20:3 and 36:5, or 20:2 and 36:6 (*m/z* 1054), and 22:5 and 36:5, or 22:4 and 36:6 (*m/z* 1078) fatty acids respectively. Similar ions could not be detected in the mass spectra of normal brain polyenoic VLCFA-PC, probably because the amount of lipid was insufficient to generate ions in the high mass region of the spectra.

Two products were formed by phospholipase A_2 hydrolysis of the normal and Zellweger polyenoic VLCFA-PC, unesterified fatty acid, and a lipid migrating with a slightly greater mobility than the product formed by hydrolysis of brain PC containing fatty acids with carbon chain lengths < 24, i.e. 1-acyl-*sn*-glycero-3-phosphocholine. No unreacted PC was detected on t.l.c. indicating complete hydrolysis. More than 95% of the polyenoic VLCFA with carbon chain lengths > 32 were found in the released 1-acyl-*sn*-glycero-3-phosphocholine indicating that these fatty acids were bound to the *sn*-1 position of the glycerol moiety. The major fatty acids released were 18:1, 20:4, 20:3, 20:2, 20:1, 22:4 and 22:6.

DISCUSSION

These investigations were prompted by our earlier observation that Zellweger brain contains a homologous series of polyenoic VLCFA (Poulos *et al.*, 1986*b*). Later studies demonstrated that most of these fatty acids were distributed in two lipids, cholesterol esters and an unidentified polar lipid (Sharp *et al.*, 1987). Although the latter is only a minor lipid in Zellweger brain, comprising $< 0.2\%$ of the total extracted lipid, it contains virtually all of the long chain fatty acids with carbon chain lengths > 32. Our m.s. and p.m.r. data show clearly that this lipid is a highly hydrophobic species of PC containing up to 58 carbons with as many as ten olefinic centres. Our earlier inability to detect a choline moiety using the Dragendorff reagent as a spray reagent may have been due to the lack of sensitivity of the reagent at the low lipid levels used (Sharp *et al.*, 1987). A similar molecular species is also present in bovine retina, but the polyenoic VLCFA are mostly $n-3$ rather than $n-6$ series acids (Aveldaño, 1987). The retina differs as well in that the major molecular species are dipolyunsaturated and contain docosahexaenoic acid in at least one of the positions (Fig. 4*a*).

The greatly increased t.l.c. mobility of the unusual PC species is perhaps not surprising, although the resolution of individual molecular species of phospholipids has been thought to require more specialized techniques such as argentation t.l.c. and reverse-phase h.p.l.c. (Christie, 1982). Our recent detection of a unique molecular species of ram sperm sphingomyelin containing 28–34 carbon $n-3$ polyenoic fatty acids, which migrated considerably faster on t.l.c. than conventional sphingomyelin species (Poulos *et al.*, 1987), indicates the phenomenon is not confined to glycerophospholipids but affects sphingolipids as well. It is possible that the increased mobility of phospholipids containing polyenoic VLCFA is an indication that, with increasing carbon chain length, partition assumes a proportionally greater importance in influencing chromatographic mobility due to the increased solubility of the lipid in the mobile solvent phase.

It is clear that polyenoic 34, 36 and 38 carbon fatty acids are present in both normal and in Zellweger brain with increased levels in the latter. Of particular interest is the subtle difference which exists between normal and mutant tissue. Thus tetra- and pentaenoic $n-6$ derivatives are the major 32–38 carbon fatty acids in normal brain, although we have detected smaller amounts of $n-3$ hexaenoic acids as well. In contrast, Zellweger polyenoic VLCFA-PC contains mostly pentaenoic and hexaenoic fatty acids, while the corresponding tetraenoics are barely detectable. These differences are intriguing but difficult to explain, particularly in view of the lack of information on the mechanisms for biosynthesis of the polyenoic VLCFA. However, it is likely that the carbon chains of the tetraenoic acids in normal brain are all derived by chain elongation of the shorter chain $n-6$ precursors, 20:4 $n-6$, 22:4 $n-6$, 24:4 $n-6$, all of which occur in significant amounts in normal and Zellweger brain. Similarly, the pentaenoic acids are probably formed by chain elongation of 22:5 $n-6$ and 24:5 $n-6$. The origin of the corresponding hexaenoic acids whose

synthesis requires both elongation as well as desaturation steps remains unclear. Whether the relative absence of tetraenoic VLCFA in Zellweger syndrome is due to an abnormality in the elongation of shorter chain precursors, or whether it simply reflects an increased synthesis of penta- and hexaenoic fatty acids, is clearly worthy of further investigation.

The relatively high degree of specificity of incorporation of 34–38 carbon polyenoic VLCFA into the *sn*-1 position of PC is of considerable interest because this position is thought to be mostly occupied by saturated fatty acids (Christie, 1982), and therefore indicates the existence of enzymological specificity at some stage along the biosynthetic pathway. Further supporting evidence for some degree of enzyme specificity is provided by comparing the fatty acid compositions of Zellweger brain cholesterol esters and PC (Poulos *et al.*, 1986*b*). Whereas the former lipid contains few longer (i.e. > 32 carbon) chains, and large proportions of 26–30 carbon compounds, the more polar PC is enriched in the longer chain polyenoic fatty acids. At present the exact stage at which the polyenoic VLCFA are introduced into the phospholipid molecule is not known. There have been reports that PCs enriched in polyenoic fatty acids in rat brain are formed by methylation of phosphatidylethanolamine (Blusztajn & Wurtman, 1981; Tacconi & Wurtman, 1985). However, as we have been unable to detect the longer chain polyenoic VLCFA in brain phosphatidylethanolamine (Sharp *et al.*, 1987), it would seem unlikely that this particular lipid is a precursor, although we cannot discount the possibility that there is a rapid turnover of molecular species of phosphatidylethanolamine enriched in polyenoic VLCFA.

The function of these unusual molecular species of PC remains unknown. It is probable however, that any biological role is associated with the presence of the polyenoic VLCFA. Whether the latter are released from the phospholipid and possibly converted to some as yet undefined physiologically active compounds, as has been suggested by some workers (Rosenthal & Hill, 1984), or whether the diacylglycerol or the parent phospholipid is the active molecule remains to be established.

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REFERENCES

Aveldaño, M. I. (1987) J. Biol. Chem. **262**, 1172–1179
Aveldaño, M. I. & Sprecher, H. (1987) J. Biol. Chem. **262**, 1180–1186
Bligh, B. G. & Dyer, W. J. (1959) Can. J. Biochem. Physiol. **37**, 911–916
Blusztajn, J. K. & Wurtman, R. J. (1981) Nature (London) **290**, 417–418
Bridges, R. B. & Coniglio, J. G. (1970) J. Biol. Chem. **245**, 49–56
Christie, W. W. (1982) Lipid Analysis, 2nd edn., Pergamon Press, Oxford
Easton, C., Johnson, D. W. & Poulos, A. (1988) J. Lipid Res., **29**, 109–112
Fellenberg, A. J., Johnson, D. W., Poulos, A. & Sharp, P. (1987) Biomed. Environ. Mass Spectrom. **14**, 127–129
Folch, J., Lees, M. & Sloane-Stanley, G. (1957) J. Biol. Chem. **226**, 497–509
Grogan, W. M. & Huth, E. G. (1983) Lipids **18**, 275–284
Owens, K. & Hughes, B. P. (1970) J. Lipid Res. **11**, 486–493
Poulos, A., Johnson, D. W., Beckman, K., White, I. G. & Easton, C. (1987) Biochem. J. **248**, 961–964
Poulos, A., Sharp, P., Fellenberg, A. J. & Danks, D. M. (1985) Hum. Genet. **70**, 172–177
Poulos, A., Sharp, P., Johnson, D., White, I. G. & Fellenberg, A. J. (1986*a*) Biochem. J. **240**, 891–895
Poulos, A., Sharp, P., Singh, H., Johnson, D., Fellenberg, A. J. & Pollard, A. C. (1986*b*) Biochem. J. **235**, 607–610
Rosenthal, M. & Hill, J. R. (1984) Biochim. Biophys. Acta **795**, 171–178
Sharp, P., Poulos, A., Fellenberg, A. J. & Johnson, D. W. (1987). Biochem. J. **248**, 61–67
Skipski, V. P. & Barclay, M. (1969) Methods Enzymol. **14**, 531–598
Tacconi, M. & Wurtman, R. J. (1985) Proc. Natl. Acad. Sci. U.S.A. **82**, 4828–4831

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Reaction of *N*-Benzoyl-2-bromoglycine Methyl Ester with Deprotonated Nitroalkanes: Synthesis of β-Nitro and α,β-Dehydro Amino Acid Derivatives

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Abstract

N-Benzoyl-2-bromoglycine methyl ester (1) reacted with the alkyl nitronates (2a–e) to give the corresponding β-nitro amino acid derivatives (4a–e). Elimination reactions of (4a–d) afforded the α,β-dehydro amino acid derivatives (5a–d). Treatment of the β-nitrovaline derivative (4b) with tributyltin hydride gave the valine derivative (8). Reduction of the β-nitroalanine derivative (4a) gave the β-aminoalanine derivative (9), characterized by hydrolysis to 2,3-diaminopropionic acid hydrochloride.

Introduction

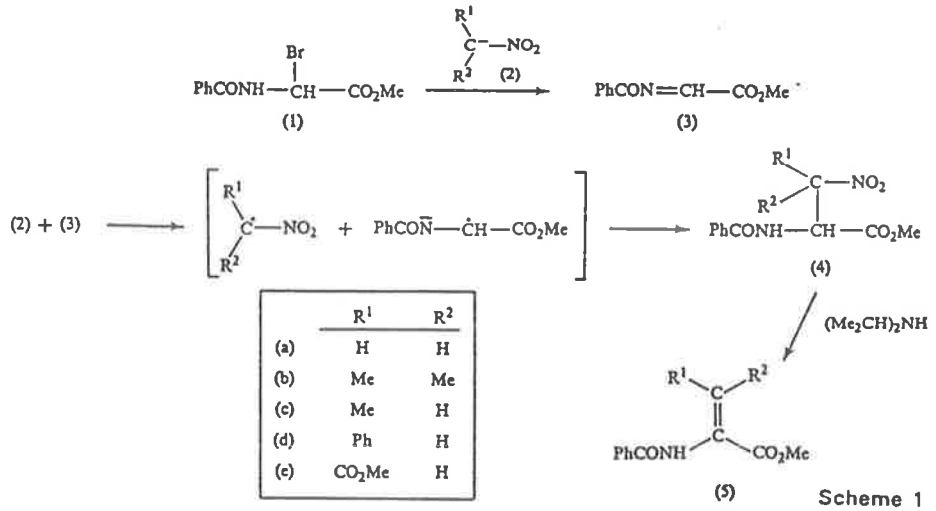
With over 500 naturally occurring α-amino acids now known,[1] there is considerable interest in methods of synthesis of this class of compounds. One approach that has received much attention in recent years involves the elaboration of α-halogenated glycine derivatives, mostly through reactions with nucleophilic species. This approach has been used, for example, in the synthesis of alkyl,[2,3] aryl,[2] γ-oxo,[2,4] dialkyl malonyl,[5–7] α,β-dehydro,[8] β,γ-dehydro[9] and γ,δ-dehydro amino acid derivatives.[3,7]

In this report we describe a complementary method for the synthesis of amino acid derivatives through reaction of *N*-benzoyl-2-bromoglycine methyl ester (1) with alkyl nitronates. The product β-nitro amino acid derivatives belong to a class of compounds that are of particular importance for a variety of reasons. There are two known examples of naturally occurring β-nitro amino acid derivatives,

[1] Wagner, I., and Musso, H., *Angew. Chem., Int. Ed. Engl.*, 1983, 22, 816.
[2] Munster, P., and Steglich, W., *Synthesis*, 1987, 223.
[3] Sinclair, P. J., Zhai, D., Reibenspies, J., and Williams, R. M., *J. Am. Chem. Soc.*, 1986, 108, 1103.
[4] Kober, R., Papadopoulos, K., Miltz, W., Enders, D., Steglich, W., Reuter, H., and Puff, H., *Tetrahedron*, 1985, 41, 1693.
[5] Rich, D. H., and Dhaon, M. K., *Tetrahedron Lett.*, 1983, 24, 1671.
[6] Shiono, S., and Harada, K., *Bull. Chem. Soc. Jpn*, 1985, 58, 1061.
[7] Easton, C. J., Scharfbillig, I. M., and Tan, E. W., *Tetrahedron Lett.*, 1988, 29, 1565.
[8] Kober, R., and Steglich, W., *Justus Liebigs Ann. Chem.*, 1983, 599.
[9] Castelhano, A. L., Horne, S., Billedeau, R., and Krantz, A., *Tetrahedron Lett.*, 1986, 27, 2435.

0004-9425/88/071063$03.00

nitropeptin[10] and 1-amino-2-nitrocyclopentanecarboxylic acid,[11] which are effective antibiotics. In addition, there exists the potential for modifying the nitro group[12] to produce β-functionalized amino acid derivatives of interest, for example, as inhibitors of pyridoxal phosphate-dependent enzymes.[13] Furthermore, elimination of the nitro group[12,14] produces α,β-dehydro amino acids, as illustrated in the present work. α,β-Dehydro amino acids are components of a large number of natural products, and they have been used to prepare chiral amino acids through asymmetric hydrogenation.[15]



	R^1	R^2
(a)	H	H
(b)	Me	Me
(c)	Me	H
(d)	Ph	H
(e)	CO_2Me	H

Scheme 1

Results and Discussion

Treatment of *N*-benzoyl-2-bromoglycine methyl ester (1) with the alkyl nitronates (2a–e) gave the corresponding β-nitro amino acid derivatives (4a–e). Conditions for the reaction of (1) with methyl nitronate (2a) were typical. A solution of butyllithium was added to a solution of nitromethane (1 equiv.) in tetrahydrofuran/hexamethylphosphoramide at −78°. The α-bromoglycine derivative (1) (0·5 equiv.) dissolved in tetrahydrofuran was then added at −78° and, after 4 h at that temperature, the reaction was quenched with acetic acid, and the product mixture was worked up to give the β-nitroalanine derivative (4a) in 62% yield. With

[10] Ohba, K., Nakayama, H., Furihata, K., Shimazu, A., Endo, T., Seto, H., and Otake, N., *J. Antibiot.*, 1987, **40**, 709.

[11] Brian, P. W., Elson, G. W., Hemming, H. G., and Radley, M., *Nature*, 1965, **207**, 998; Burrows, B. F., and Turner, W. B., *J. Chem. Soc. C*, 1966, 255.

[12] Seebach, D., Colvin, E. W., Lehr, F., and Weller, T., *Chimia*, 1979, **33**, 1.

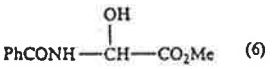
[13] Walsh, C. T., *Tetrahedron*, 1982, **38**, 871; *Ann. Rev. Biochem.*, 1984, **53**, 493; Wang, E., and Walsh, C. T., *Biochemistry*, 1978, **17**, 1313.

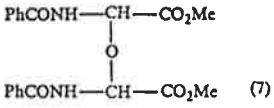
[14] Rudiger, W., and Klose, W., *Tetrahedron Lett.*, 1967, **8**, 1177; Patterson, J. W., and McMurry, J. E., *J. Chem. Soc., Chem. Commun.*, 1971, 488.

[15] Glaser, R., Geresh, S., Twaik, M., and Benoiton, N. L., *Tetrahedron*, 1978, **34**, 3617; Glaser, R., and Geresh, S., *Tetrahedron*, 1979, **35**, 2381; Onuma, K., Ito, T., and Nakamura, A., *Bull. Chem. Soc. Jpn*, 1980, **53**, 2016.

longer reaction times, or if the temperature was allowed to rise above −60° before the reaction was quenched, a lower yield of (4a) was obtained.

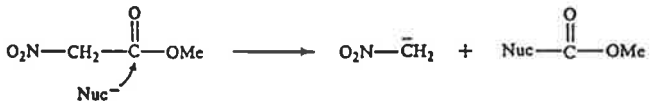
In common with previous studies involving the elaboration of α-halogenated glycine derivatives through reaction with nucleophilic species,[2,5,9] we found that 2 mol. equiv. of methyl nitronate (2a) were required to produce the β-nitro amino acid derivative (4a). When only 1 mol. equiv. of (2a) was used, the product mixture contained the α-hydroxyglycine derivative (6) and the dimeric product (7), which were identified by comparison with authentic samples.[16] These results indicate that (1) reacts with (2a), to produce the acyliminoacetate (3).[2,4] In the presence of excess (2a), species (3) reacts by addition to give (4a) (Scheme 1), otherwise (3) reacts with water during workup of the reaction mixture to give (6) and (7).[16,17]


(6)


(7)

The glycine derivative (1) reacted with 2 equiv. of the alkyl nitronate (2b) to give the β-nitrovaline derivative (4b) in 86% yield. Similarly, treatment of (1) with (2c) gave a 2 : 1 mixture of diastereoisomers of (4c), which were separated by chromatography on silica in yields of 34 and 17%. Reaction of (1) with benzyl nitronate (2d) gave the β-nitrophenylalanine derivative (4d) in 68% yield, as a 1 : 1 mixture of diastereoisomers.

When methyl nitroacetate was treated with butyllithium (1 equiv.) and, subsequently with (1) (0·5 equiv.), the only product isolated from the reaction mixture was the β-nitroalanine derivative (4a). Presumably (4a) resulted from reaction of (3) with methyl nitronate (2a), and (2a) was produced by nucleophilic substitution of methyl nitroacetate [equation (i)]. Expecting that (2a) would react with excess methyl nitroacetate to produce the nitronate ester (2e) [equation (ii)], we repeated the reaction with a mole ratio of methyl nitroacetate, butyllithium and (1) of 10 : 2 : 1. Under those conditions (4e) was produced in 66% yield as a 1 : 1 mixture of diastereoisomers.


(i)

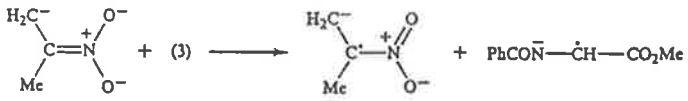

(ii)

In contrast to these reactions of (1) with (2a–e) where good yields of the *C*-alkylated aliphatic nitro compounds (4a–e) were obtained, alkylation of alkyl nitronates generally takes place overwhelmingly on oxygen and only doubly deprotonated nitroalkanes give reasonable yields of *C*-alkylated products.[12,18,19] Other exceptional cases of *C*-

[16] Burgess, V. A., Easton, C. J., Hay, M. P., and Steel, P. J., *Aust. J. Chem.*, 1988, 41, 701.
[17] Malassa, I., and Matthies, D., *Justus Liebigs Ann. Chem.*, 1986, 1133.
[18] Seebach, D., and Lehr, F., *Angew. Chem., Int. Ed. Engl.*, 1976, 15, 505.
[19] Seeback, D., Henning, R., Lehr, F., and Gonnermann, J., *Tetrahedron Lett.*, 1977, 18, 1161.

alkylation of alkyl nitronates have been attributed to an electron transfer mechanism.[20] Accordingly, it is possible that an electron transfer between (3) and (2a–e) first takes place, giving a pair of free radicals which then combine (Scheme 1).

In the present work, derivatives (4a–d) were produced when (1) was treated with 2 equiv. of the corresponding doubly deprotonated nitroalkane analogues of (2a–d). Yields were comparable to those obtained from reactions of (2a–d). The only difference observed was in the ratio of diastereoisomers of (4c). Whereas reaction of (1) with ethyl nitronate (2c) gave a 2 : 1 mixture of diastereoisomers of (4c), reaction with the dianion of nitroethane gave a 1 : 1·3 mixture of diastereoisomers of (4c). In the reaction of (1) with 2 equiv. of doubly deprotonated 2-nitropropane, the expected product of β-alkylation of 2-nitropropane[19,21] was not observed. Formation of (4b) in this reaction may be attributed to an electron transfer reaction involving either the dianion of 2-nitropropane [equation (iii)] or the alkyl nitronate (2b) (Scheme 1) generated by reaction of (1) with the dianion of 2-nitropropane to give (3).



(iii)

Treatment of the β-nitroalanine derivative (4a) with 1 equiv. of diisopropylamine in chloroform at room temperature for 6 h[14] gave the dehydroalanine derivative (5a)[22] in 60% yield. Considerably more vigorous conditions, i.e., a 40-fold excess of diisopropylamine at reflux in chloroform for 48 h, were required to bring about the conversion of (4b) into (5b). The relative ease of elimination in (4a) as compared to (4b) may be attributed to the extent of non-bonding interactions in the transition states leading to the respective products (5a) and (5b). The transition state leading to (5b) will be destabilized compared to that leading to (5a) by developing non-bonding interactions associated with the change in hybridization at the α- and β-carbons of (4a) and (4b) (Fig. 1). A similar rationale has been used to explain the selective reaction of glycine residues in free-radical reactions of amino acid derivatives.[23]

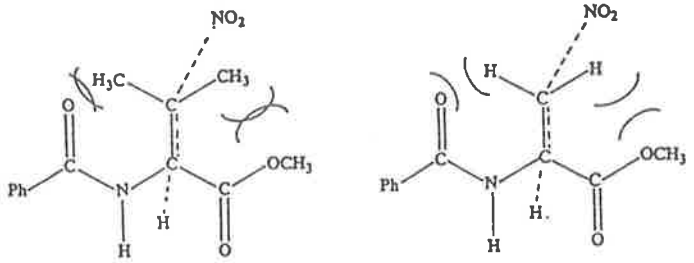
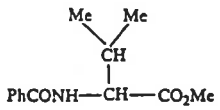


Fig. 1. Non-bonding interactions.

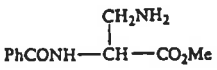
[20] Kerber, R. C., Kornblum, N., and Urry, G. W., *J. Am. Chem. Soc.*, 1964, **86**, 3904; Kornblum, N., Boyd, S. D., and Ono, N., *J. Am. Chem. Soc.*, 1974, **96**, 2580.
[21] Henning, R., Lehr, F., and Seebach, D., *Helv. Chim. Acta*, 1976, **59**, 2213.
[22] Morgenstern, A. P., Schutij, C., and Nauta, W. Th., *J. Chem. Soc., Chem. Commun.*, 1969, 321; Brown, A. G., and Smale, T. C., *J. Chem. Soc., Chem. Commun.*, 1969, 1489.
[23] Easton, C. J., and Hay, M. P., *J. Chem. Soc., Chem. Commun.*, 1986, 55.

Each of the diastereoisomers of (4c) reacted with 1 equiv. of diisopropylamine at room temperature to give the dehydro amino acid derivative (5c). Similarly, the β-nitrophenylalanine derivative (4d) underwent elimination to give the dehydrophenylalanine derivative (5d). The less stable (*E*)-isomers of (5c) and (5d) were not detected in the respective reactions.[24,25]

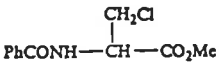
In other studies of the removal or elaboration of the nitro group in the amino acid derivatives (4a–e), the β-nitrovaline derivative (4b) reacted with tributyltin hydride[26] to give the valine derivative (8), and the β-nitroalanine derivative (4a) was reduced with hydrogen over palladium to give the β-aminoalanine derivative (9), which was characterized by hydrolysis to give 2,3-diaminopropionic acid hydrochloride. The conversion of (4a) into (9) completes a formal general synthesis of β-functionalized alanine derivatives such as the chloride (10) and serine which have been prepared from (9) via the corresponding diazonium salt.[27]



(8)



(9)



(10)

In summary, the reactions of (1) with the alkyl nitronates (2a–e) to give the corresponding β-nitro amino acid derivatives (4a–e) illustrate a new method for the synthesis of amino acid derivatives through the elaboration of α-halogenated glycine derivatives. The method is suitable for the synthesis of α,β-dehydro amino acid derivatives, as shown by the conversion of (4a–d) into (5a–d), and is potentially useful for the synthesis of β-functionalized amino acid derivatives through modification of the nitro group. In light of our studies of the selective halogenation of glycine residues in peptides,[7,23] we expect that the methodology described in this report will prove useful for the modification of glycine residues in peptides.

Experimental

Melting points are uncorrected. Solvents were purified and dried by using standard procedures. ^{1}H n.m.r. spectra were recorded on either a Varian T-60 or Bruker CXP-300 spectrometer. Unless otherwise stated, n.m.r. spectra were recorded as dilute solutions in (D)chloroform with tetramethylsilane as an internal standard. Infrared spectra were recorded on a Jasco IRA-1 spectrometer. Mass spectra were recorded on an AEI MS-3010 spectrometer. Chromatography was carried out on a Chromatotron 7924T (Harrison Research, Palo Alto/TC Research, Norwich) with Merck silica gel 60 PF_{254}, and elution with a gradient of light petroleum/ethyl acetate. Microanalyses were performed by the Microanalytical Laboratory, Research School of Chemistry, Australian National University. Nitromethane, nitroethane, 2-nitropropane and nitro(phenyl)methane were commercially available and distilled before use.

[24] Srinivasan, A., Stephenson, R. W., and Olsen, R. K., *J. Org. Chem.*, 1977, 42, 2256; Srinivasan, A., Richards, K. D., and Olsen, R. K., *Tetrahedron Lett.*, 1976, 17, 891.
[25] Chaloner, P. A., *J. Chem. Soc., Perkin Trans. 1*, 1980, 1028.
[26] Ono, N., Miyake, H., Fujii, M., and Kaji, A., *Tetrahedron Lett.*, 1983, 24, 3477; Korth, H.-G., Sustmann, R., Dupuis, J., and Giese, B., *Chem. Ber.*, 1987, 120, 1197.
[27] Okumura, K., Iwasaki, T., Okawara, T., and Matsumoto, K., *Bull. Inst. Chem. Res., Kyoto Univ.*, 1972, 50, 209.

N-Benzoyl-3-nitroalanine Methyl Ester (4a)

A solution of butyllithium (2·5 M in hexane, 0·88 ml, 2·2 mol) was added dropwise to a solution of nitromethane (0·12 ml, 135 mg, 2·2 mmol) in tetrahydrofuran (10 ml) and hexamethylphosphoramide (2 ml) maintained at −78°. A solution of *N*-benzoyl-2-bromoglycine methyl ester (1)[4] (300 mg, 1·1 mmol) in tetrahydrofuran (2 ml) was then added at −78° and, after 4 h at that temperature, acetic acid (0·35 ml) was added. The mixture was dissolved in ethyl acetate (50 ml), washed with saturated aqueous sodium bicarbonate (2×50 ml), dried ($MgSO_4$) and concentrated. The residue was chromatographed on silica to give the product (4a) as colourless *needles* from ethyl acetate/light petroleum (60–80°) (172 mg, 62%), m.p. 118–119° (Found: C, 53·1; H, 4·9; N, 11·0. $C_{11}H_{12}N_2O_5$ requires C, 52·4; H, 4·8; N, 11·1%). ν_{max} 3420, 1750, 1660, 1560 cm^{-1}. ^1H n.m.r. δ 3·88, s, 3H; 4·99, dd, *J* 4, 16 Hz, 1H; 5·10, dd, *J* 4, 16 Hz, 1H; 5·17, td, *J* 4, 8 Hz, 1H; 7·10, br d, *J* 8 Hz, 1H; 7·4–7·7, m, 5H. Mass spectrum: *m/z* 253 (51%), 205 (100), 193 (78), 173 (61), 146 (56), 105 (88), 77 (100).

When the reaction was repeated with 1·1 mmol of butyllithium, the β-nitroalanine derivative (4a) was not detected in the crude reaction mixture. *N*-Benzoyl-2-hydroxyglycine methyl ester (6) and diastereoisomers of dimethyl 2,4-dibenzamido-3-oxapentanedioate (7) were detected by t.l.c., h.p.l.c., and ^1H n.m.r. spectrometric comparison with authentic samples.[16]

When the reaction was repeated with 4·4 mmol of butyllithium, the product (4a) was isolated in 43% yield.

Reaction of methyl nitroacetate[28] with butyllithium (1 equiv.) and, subsequently with the glycine derivative (1) (0·5 equiv.), as described above for the reaction of (1) with methyl nitronate (2a), gave the β-nitroalanine derivative (4a) in 23% yield.

N-Benzoyl-3-nitrovaline Methyl Ester (4b)

Reaction of the glycine derivative (1) with isopropyl nitronate (2b), as described above for the preparation of (4a) from methyl nitronate (2a), gave the product (4b) as an *oil* (132 mg, 86%) [Found: *m/z* 281·1152. $C_{13}H_{17}N_2O_5$ ($M^{+\bullet}+1$) requires *m/z* 281·1138]. ν_{max} 3425, 1740, 1670, 1550 cm^{-1}. ^1H n.m.r. δ 1·69, s, 3H; 1·89, s, 3H; 3·78, s, 3H; 5·35, d, *J* 9 Hz, 7·13, br d, *J* 9 Hz, 1H; 7·3–7·9, m, 5H. Mass spectrum: *m/z* 281 (52%), 234 (72), 221 (100), 202 (38), 192 (45), 175 (17), 105 (86), 77 (24).

Methyl 2-Benzamido-3-nitrobutanoate (4c)

Reaction of the glycine derivative (1) with ethyl nitronate (2c), as described above for the preparation of (4a) from methyl nitronate (2a), gave the product (4c) as a 2:1 mixture of diastereoisomers (4c)(i)) and (4c(ii)), which were separated by chromatography on silica.

One of the diastereoisomers (4c(i)) was recrystallized from ethyl acetate/light petroleum as colourless *needles* (17 mg, 34%), m.p. 89–90° (Found: C, 53·6; H, 5·4; N, 10·2. $C_{12}H_{14}N_2O_5$ requires C, 54·1; H, 5·3; N, 10·5%). ν_{max} 3450, 1750, 1670, 1555 cm^{-1}. ^1H n.m.r. δ 1·70, d, *J* 6 Hz, 3H; 3·83, s, 3H; 5·34, m, 2H; 6·96, br d, *J* 11 Hz, 1H; 7·4–7·9, m, 5H. Mass spectrum: *m/z* 220 (3%), 219 (7), 207 (4), 205(3), 177 (4), 175 (3), 160 (4), 149 (4), 105 (100), 77 (68).

The other diastereoisomer (4c(ii)) was obtained as an *oil* in 17% yield [Found: *m/z* 267·0992. $C_{12}H_{15}N_2O_5$ ($M^{+\bullet}+1$) requires *m/z* 267·0981]. ν_{max} 3420, 1750, 1655, 1555 cm^{-1}. ^1H n.m.r. δ 1·82, d, *J* 6 Hz, 3H; 3·87, s, 3H; 5·06, m, 1H; 5·16, m, 1H; 7·14, br d, *J* 11 Hz, 1H; 7·4–7·9, m, 5H. Mass spectrum: *m/z* 267 (6%), 235 (3), 220 (23), 219 (12), 207 (30), 188 (4), 160 (42), 105 (100), 77 (93).

When the reaction was repeated with doubly deprotonated nitroethane, the diastereoisomers (4c(i)) and (4c(ii)) were produced in the ratio 1:1·3, and isolated in yields of 23 and 28%, respectively.

[28] Zen, S., Koyama, M., and Koto, S., *Org. Synth.* 1976, **55**, 77.

N-Benzoyl-3-nitrophenylalanine Methyl Ester (4d)

Reaction of the glycine derivative (1) with benzyl nitronate (2d), as described above for the preparation of (4a) from methyl nitronate (2a), gave the product (4d), recrystallized from chloroform/light petroleum as colourless crystals of a 1 : 1 *mixture* of diastereoisomers (111 mg, 68%), m.p. 160–165° [Found: *m/z* 329·1142. $C_{17}H_{16}N_2O_5$ ($M^{+\bullet}+1$) requires *m/z* 329·1138]. ν_{max} 3410, 1750, 1670, 1560, cm^{-1}. ^1H n.m.r. δ 3·78, s, 3H; 5·63, m, 1H; 6·26, d, *J* 10 Hz, 1H; 7·2–7·6, m, 5H; 7·79, br d, *J* 9 Hz, 1H. Mass spectrum: *m/z* 329 (0·3%), 282 (4), 281 (1), 250 (3), 223 (4), 222 (4), 192 (14), 162 (2), 121 (10), 105 (100), 77 (50).

N-Benzoyl-3-nitroaspartic Acid Dimethyl Ester (4e)

Treatment of methyl nitroacetate with butyllithium (0·2 equiv.) and, subsequently with the glycine derivative (1) (0·1 equiv.), under the conditions described above for the reaction of (1) with methyl nitronate (2a), gave a 1 : 1 mixture of diastereoisomers of the product (4e) as an *oil* (152 mg, 66%) [Found: *m/z* 311·0879. $C_{13}H_{15}N_2O_7$ ($M^{+\bullet}+1$) requires *m/z* 311·0885]. ν_{max} 3440, 1760, 1740, 1670, 1570 cm^{-1}. ^1H n.m.r. δ 3·82, s, 3H; 3·87, s, and 3·94, s, total 3H; 5·64, dd, *J* 3, 6 Hz, and 5·80, dd, *J* 4, 9 Hz, total 1H; 5·98, d, *J* 3 Hz, and 5·99, d, *J* 4 Hz, total 1H; 7·20, m, 1H; 7·4–7·9, m, 5H. Mass spectrum: *m/z* 311 (4%), 279 (2), 264 (4), 263 (2), 251 (21), 231 (4), 204 (17), 143 (3), 121 (3), 105 (100), 77 (31).

Methyl 2-Benzamidopropenoate (5a)

Diisopropylamine (0·04 ml, 29 mg, 0·285 mmol) was added to a solution of the β-nitroalanine derivative (4a) (72 mg, 0·285 mmol) in chloroform (2 ml) at room temperature, After 6 h at room temperature, the mixture was dissolved in ethyl acetate (10 ml), washed with water (2×10 ml), dried ($MgSO_4$) and concentrated. The residue was recrystallized from ethyl acetate/light petroleum to give the product (5a) as colourless crystals (35 mg, 60%), m.p. 151–156°, with spectral characteristics consistent with those previously reported.[22] ^1H n.m.r. δ 3·87, s, 3H; 6·01, d, *J* 2 Hz, 1H; 6·80, s, 1H; 7·3–8·3, m, 6H.

Methyl 2-Benzamido-3-methylbut-2-enoate (5b)

A mixture of the β-nitrovaline derivative (4b) (77 mg, 0·275 mmol), diisopropylamine (1·5 ml, 1·1 g, 11 mmol) and chloroform (2 ml) was heated at reflux for 48 h, then dissolved in ethyl acetate (10 ml), washed with water (2×10 ml), dried ($MgSO_4$) and concentrated. The residue was recrystallized from ethyl acetate/light petroleum to give the product (5b) as colourless crystals (32 mg, 50%), m.p. 134–136° (lit.[29] 137–137·5°). ^1H n.m.r. δ 1·90, s, 3H; 2·24, s, 3H; 3·73, s, 3H; 7·3–7·9, m, 6H.

Methyl (Z)-2-Benzamidobut-2-enoate (5c)

Treatment of methyl 2-benzamido-3-nitrobutanoate (4c(ii)) with diisopropylamine, as described above for the preparation of (5a), gave the product (5c) as colourless crystals from ethyl acetate/light petroleum (15 mg, 61%), m.p. 77–78° (lit.[24,30] 78–79°). ^1H n.m.r. δ 1·82, d, *J* 8 Hz, 3H; 3·76, s, 3H; 6·84, q, *J* 8 Hz, 1H; 7·3–7·9, m, 6H.

Reaction of (4c(i)) as described above gave (5c), identical in all respects to the sample obtained from reaction of (4c(ii)).

Methyl (Z)-2-Benzamido-3-phenylpropenoate (5d)

A 1 : 1 mixture of the diastereoisomers of the β-nitrophenylalanine derivative (4d) was treated with diisopropylamine as described above for the preparation of (5a). The product (5d) was recovered as colourless crystals from ethyl acetate/light petroleum in 88% yield, m.p. 135–140° (lit.[25,31] 142–143°), and had spectral characteristics consistent with those previously reported.[25,31] ^1H n.m.r. δ 3·86, s, 3H; 7·30–7·65, m, 9H; 7·70–7·90, m, 3H.

[29] Galantay, E., Szabo, A., and Fried, J., *J. Org. Chem.*, 1963, 28, 98.
[30] Kloetzel, M. C., *J. Am. Chem. Soc.*, 1948, 70, 3571.
[31] Brocklehurst, K., Price, H. S., and Williamson, K., *J. Chem. Soc., Chem. Commun.*, 1968, 884.

N-Benzoylvaline Methyl Ester (8)

A mixture of the β-nitrovaline derivative (4b) (85 mg, 0·3 mmol), tributyltin hydride (160 mg, 0·55 mmol) and azobisisobutyronitrile (*c.* 1 mg), in benzene (2 ml), was heated at reflux under nitrogen for 6 h. The mixture was cooled, concentrated and chromatographed on silica to give the product (8) as colourless needles from ethyl acetate/light petroleum (25 mg, 36%), m.p. 84–86° (lit.[32] 86°).

2,3-Diaminopropionic Acid Hydrochloride

A solution of the β-nitroalanine derivative (4a) (190 mg, 0·75 mmol) in methanol with 5% palladium on carbon (270 mg) was hydrogenated at 2 atm and room temperature for 4 h. The mixture was filtered through Celite, and concentrated to give the crude β-aminoalanine derivative (9). ^{1}H n.m.r. δ 1·8, br s, 2H; 3·17, dd, *J* 4, 10 Hz, 1H; 3·27, dd, *J* 4, 10 Hz, 1H; 3·80, s, 3H; 4·80, m, 1H; 7·20, br d, *J* 8 Hz, 1H; 7·4–7·9, m, 5H. The crude (9) was heated at reflux with dilute hydrochloric acid (25 ml) for 12 h. The mixture was cooled, washed with chloroform (2×25 ml), and concentrated to give 2,3-diaminopropionic acid hydrochloride which was recrystallized from methanol/water as colourless crystals (48 mg, 46%), m.p. 230° (dec.) (lit.[33] 225°). ^{1}H n.m.r. δ (D_2O) 3·30, d, *J* 7 Hz, 2H; 3·96, t, *J* 7 Hz, 1H.

Acknowledgment

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[32] Oh-Hashi, J., and Harada, K., *Bull. Chem. Soc. Jpn*, 1966, 39, 2287.
[33] Fischer, E., and Raske, K., *Ber. Dtsch. Chem. Ges.*, 1907, 40, 3717.

Selective Reaction of Glycine Residues in Hydrogen Atom Transfer from Amino Acid Derivatives

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Abstract: Relative rates of reaction of the *N*-benzoylamino acid methyl esters **1a–4a** with *N*-bromosuccinimide and of **1a–4a** with di-*tert*-butyl peroxide are reported. The selective reaction of glycine derivatives in these and other reactions of *N*-acylamino acid derivatives is attributed to the relative stability of intermediate radicals produced by hydrogen atom transfer. Radicals formed by hydrogen abstraction from *N*-acylglycine derivatives may adopt planar conformations which are relatively free of nonbonding interactions and in which there is maximum delocalization of the unpaired electron, whereas radicals produced by similar reactions of derivatives of other amino acids are relatively unstable because of nonbonding interactions. In accord with this hypothesis, methyl pyroglutamate (**5a**) reacts at a faster rate than *N*-benzoylglycine methyl ester (**1a**) in reactions with either *N*-bromosuccinimide or di-*tert*-butyl peroxide. Anomalous rates of reaction of *N*-benzoylproline methyl ester (**6a**) are rationalized in terms of the regioselectivity of hydrogen atom transfer. Evidence for the mechanisms of reactions of **1a–6a** is derived from product studies and by comparison of the relative rates of reactions of **1a–6a** with those of the deuteriated amino acid derivatives **1b**, **2b**, **3b,c**, **5b**, and **6b,c**.

The preferential reactivity of glycine residues observed in the photoalkylation of peptides and proteins has been attributed to the formation of α-centered radicals by selective hydrogen atom transfer from glycine derivatives.[2] Irradiation experiments with polycrystalline and single crystal samples of amino acid derivatives have also displayed selective reaction of glycine residues.[3] Two main types of radicals are produced by irradiation, as shown by EPR spectroscopy. One of these gives EPR spectra that are broad and anisotropic. These radicals are thought to be sulfur-centered, mainly because similar spectra have been observed for a number of thiols and disulfides. The other type of radical, which displays

(1) (a) University of Adelaide. (b) University of Canterbury.
(2) Elad, D.; Sperling, J. *J. Chem. Soc. C* **1969**, 1579. Elad, D.; Schwarzberg, M.; Sperling, J. *J. Chem. Soc., Chem. Commun.* **1970**, 617. Sperling, J.; Elad, D. *J. Am. Chem. Soc.* **1971**, *93*, 967. Sperling, J.; Elad, D. *J. Am. Chem. Soc.* **1971**, *93*, 3839. Schwarzberg, M.; Sperling, J.; Elad, D. *J. Am. Chem. Soc.* **1973**, *95*, 6418.

(3) Henrikson, T.; Melo, T. B.; Saxebol, G. *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic Press: New York, 1976; Vol. 2, p 213.

0002-7863/89/1511-1047$01.50/0

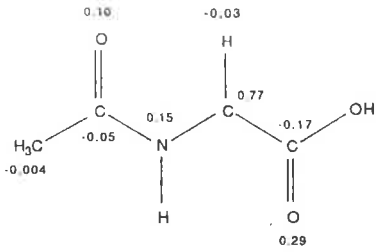
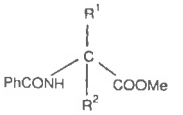


Figure 1. Distribution of the unpaired spin density in the radical formed by hydrogen atom abstraction from the α-position of *N*-acetylglycine.

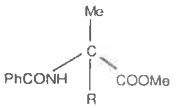
a doublet resonance in EPR spectra, is derived by hydrogen atom abstraction from the α-position of glycine derivatives. While the reactivity of amino acid residues is affected by the tertiary structure and the location of the amino acid in peptides and proteins, glycine residues are intrinsically more reactive than other amino acid derivatives.[2,3]

Radicals formed by hydrogen transfer from the α-position of *N*-acylglycine derivatives are stabilized by extensive delocalization of the unpaired electron. Molecular orbital calculations of the distribution of unpaired spin density in the radical formed by abstraction of an α-hydrogen from *N*-acetylglycine have shown it to be distributed in p orbitals perpendicular to the plane of the molecule (Figure 1).[4] While the regioselective formation of α-centered radicals by hydrogen atom transfer from *N*-acylamino acid derivatives is consistent with the degree of delocalization of the unpaired electron in the product radicals, the selective hydrogen atom abstraction from *N*-acylglycine derivatives is contrary to the expectation that tertiary radicals should form in preference to secondary ones.[5] Glycine residues afford secondary radicals by α-C–H bond homolysis, whereas analogous reactions of derivatives of other amino acids such as alanine and valine produce tertiary radicals.

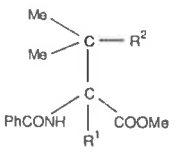
In our preliminary investigation of this anomaly[6] we studied reactions of the amino acid derivatives **1a–3a** and the deuteriated analogues **1b–3b** with *N*-bromosuccinimide (NBS). NBS was chosen as the reagent because the initial reaction of reactive substrates in brominations with NBS involves hydrogen atom abstraction by bromine atom, a reaction in which there is relatively extensive C–H bond homolysis in the transition state and which is, therefore, relatively sensitive to the stability of the product radical.[5,7] We proposed that the particular reactivity of glycine residues in free-radical reactions could be attributed to the stability of the radicals produced by atom-transfer reactions. Thus, radicals formed by hydrogen abstraction from glycine derivatives may adopt conformations which are relatively free of nonbonding interactions and in which there is maximum delocalization of the unpaired electron, whereas radicals derived by analogous reactions of derivatives of other amino acids such as alanine and valine are destabilized by nonbonding interactions. To examine this hypothesis, we have now studied reactions of *N*-benzoylsarcosine methyl ester (**4a**), methyl pyroglutamate (**5a**), *N*-benzoylproline methyl ester (**6a**), and the deuteriated analogues **5b**, **6b**, and **6c** with NBS. For comparative purposes and in order to check for major deviations from proposed mechanistic schemes, reactions of **1a–6a** and **6b,c** with di-*tert*-butyl peroxide (DTBP) have also been studied. Reactions with DTBP involve hydrogen atom transfer from substrate to *tert*-butoxy radical. There is comparatively less C–H bond homolysis in the transition state of reactions involving hydrogen atom abstraction by *tert*-butoxy radical than in those involving hydrogen transfer to bromine atom. Reactions with DTBP are, therefore, less susceptible to radical-stability effects and more susceptible to polar and steric effects.[5]



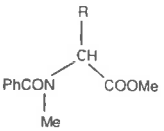
1a $R^1 = R^2 = H$
1b $R^1 = R^2 = D$
1c $R^1 = Br$, $R^2 = H$



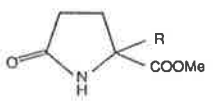
2a R = H
2b R = D



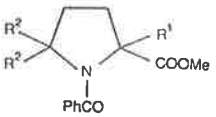
3a $R^1 = R^2 = H$
3b $R^1 = D$, $R^2 = H$
3c $R^1 = H$, $R^2 = D$
3d $R^1 = R^2 = Br$



4a R = H
4b R = Br



5a R = H
5b R = D



6a $R^1 = R^2 = H$
6b $R^1 = D$, $R^2 = H$
6c $R^1 = R^2 = D$

Methods

Relative rates of reaction of the amino acid derivatives **1a,b**, **2a,b**, **3a,b**, **4a**, **5a,b**, and **6a–c** with NBS and of **1a–6a** and **6b,c** with DTBP were determined by measuring the relative rates of their consumption from mixtures. For any two substrates X and Y, the ratio of their rates of reaction can be measured by comparing the concentration of each substrate after reaction ($t = 1$) relative to the original ($t = 0$) concentration of that substrate.[8–10]

$$k_X/k_Y = \ln ([X]_{t=1}/[X]_{t=0})/\ln ([Y]_{t=1}/[Y]_{t=0})$$

This method applies only when the reactions of X and Y are irreversible and provided neither X nor Y is produced during the reaction. In the present work, single enantiomers of the amino acid derivatives **2a,b** and **3a,b** were used, and reaction mixtures were analyzed on a Chrompack XE-60-S-VAL-S-A-PEA GLC column or a Regis Pirkle Covalent L-Phenylglycine HPLC column, each of which resolved the enantiomers of **2a,b** and **3a,b**. The fact that no epimerization of **2a,b** or **3a,b** was observed in these reactions indicates that the reactions are irreversible. Although the glycine derivative **1a** reacted with DTBP to produce the racemic alanine derivative **2a**,[11] the relative rates of consumption of the glycine derivative **1a** and the alanine derivative **2a** could be determined by using the (2*S*)-alanine derivative **2a** and measuring the enantiomeric excess of **2a** after reaction to determine the quantity of unreacted substrate. In all of the systems studied, no reaction occurred in the absence of ultraviolet irradiation. This observation is consistent with the expectation that the reactions are free-radical processes.

Where it was feasible, products of reactions were examined to gain insight into the reaction mechanisms. Reactions of **1a**, **3a**, and **4a** with NBS to give the α-bromoglycine derivative **1c**,[12] the dibromovaline derivative **3d**,[13] and the α-bromosarcosine derivative

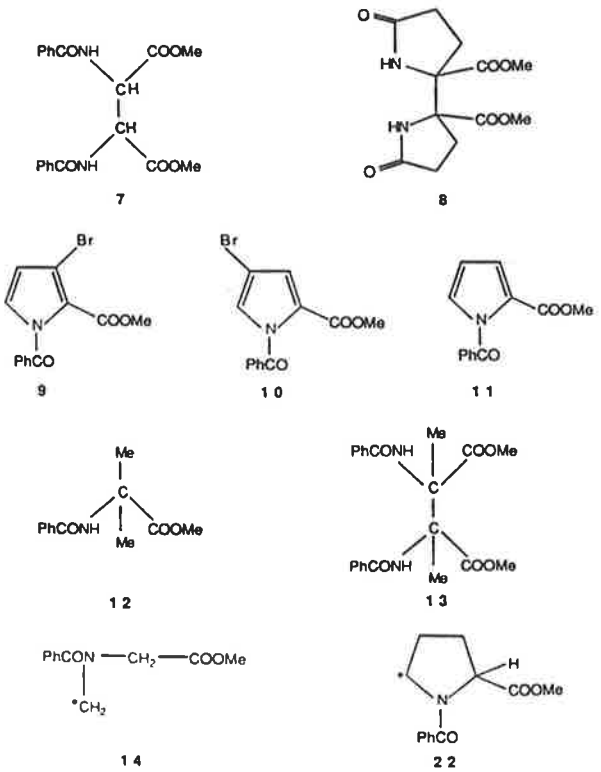
(4) Augenstein, J. A.; Ray, B. R. *J. Phys. Chem.* **1957**, *61*, 1385.
(5) Russell, G. A. *Free Radicals*; Kochi, J. K., Ed.; Wiley: New York, 1973; Vol. 1, p 275.
(6) Easton, C. J.; Hay, M. P. *J. Chem. Soc., Chem. Commun.* **1986**, 55.
(7) Poutsma, M. L. *Free Radicals*; Kochi, J. K., Ed.; Wiley: New York, 1973; Vol. 2, p 211.
(8) Hayday, K.; McKelvey, R. D. *J. Org. Chem.* **1976**, *41*, 2222.
(9) Walling, C.; Gibian, M. J. *J. Am. Chem. Soc.* **1965**, *87*, 3361.
(10) Beckwith, A. L. J.; Easton, C. J. *J. Am. Chem. Soc.* **1981**, *103*, 615.
(11) Burgess, V. A.; Easton, C. J.; Hay, M. P.; Steel, P. J. *Aust. J. Chem.* **1988**, *41*, 701.
(12) Kober, R.; Papadopoulos, K.; Miltz, W.; Enders, D.; Steglich, W.; Reuter, H.; Puff, H. *Tetrahedron* **1985**, *41*, 1693.

4b,[13] respectively, have been reported. Treatment of the glycine derivative **1a** with DTBP is known to produce diastereoisomers of the dimer **7** in addition to the alanine derivative **2a**.[11] Reaction of methyl pyroglutamate (**5a**) with DTBP has been reported to produce diastereoisomers of the dimer **8**.[14]

Reaction of the sarcosine derivative **4a** with *tert*-butoxy radical was studied directly by irradiating a mixture of **4a** and DTBP in the cavity of an EPR spectrometer. A range of solvents was examined for preparing mixtures of **1a–3a**, **5a**, or **6a** with DTBP; however, solutions suitable for study by EPR spectroscopy could not be obtained due to the low solubility of the amino acid derivatives in solutions containing DTBP.

Results

Reaction of the alanine derivative **2a** and of methyl pyroglutamate (**5a**) with NBS afforded, in each case, a red oil, which could not be resolved with numerous chromatographic systems. Treatment of the proline derivative **6a** with NBS (3 molar equiv) gave *N*-benzoyl-3-bromo-2-(methoxycarbonyl)pyrrole (**9**) and the 4-bromopyrrole **10**. When less than 3 equiv of NBS was used, the starting material **6a** and *N*-benzoyl-2-(methoxycarbonyl)-pyrrole (**11**) were detected in product mixtures. The assignment of structures of the bromopyrroles **9** and **10** rests heavily on the correlation of observed ^{13}C NMR shifts with those predicted by the use of additivity factors and the data available for a variety of pyrazoles.[15]



Treatment of the alanine derivative **2a** with DTBP afforded the α-methylalanine derivative **12** and diastereoisomers of the dimer **13**. Mixtures of products were obtained from reactions of the derivatives of valine **3a**, sarcosine **4a**, and proline **6a** with DTBP, from which discrete compounds could not be isolated.

Irradiation of a mixture of the sarcosine derivative **4a** and DTBP in the cavity of an EPR spectrometer gave rise to two signals of approximately equal intensity. One signal appeared as a triplet, consistent with formation of the radical **14** [$a(H_\alpha)$ = 17.5 G, g = 2.0030].[16] The other signal is consistent with formation of the radical **15**, appearing as a doublet [$a(H_\alpha)$ = 16.5 G, g = 2.0030].[16] The same doublet signals was observed when a mixture of the bromosarcosine derivative **4b**, hexabutylditin, and DTBP was irradiated. Under these conditions the expected product radical is **15**, produced by bromine atom transfer from **4b**.[17]

Relative rates of reaction of the amino acid derivatives **1a,b**, **2a,b**, **3a,b**, **4a**, **5a,b**, and **6a–c** with NBS, and of **1a–6a** and **6b,c** with DTBP are presented in Table I. The error limits represent the standard deviation of the sample population. The relative rates of reaction of the valine derivatives **3a,b** compared to those of the glycine derivative **1a** were determined indirectly from competitive experiments using the glycine derivative **1a** and the alanine derivative **2a**, and **2a** and the valine derivatives **3a,b**. The errors shown for the relative rates of reaction of **3a,b** are the cumulative errors. No allowance was made for incomplete deuterium incorporation in the amino acid derivatives **1b–3b**, **5b**, and **6b,c**. The most significant effect of residual hydrogen on the rate of reaction would be expected with the deuteriated methyl pyroglutamate (**5b**), where the extent of deuterium incorporation was only 62%. The general correlation between the relative rates of reaction of **1a–6a** with NBS and with DTBP indicates a similarity in reaction pathways for these two reagents, and major deviations from the pathways discussed below would appear to be unlikely.

Table I. Relative Rates of Reaction of the Amino Acid Derivatives **1a–6a**, **1b–3b**, **5b**, and **6b,c** with NBS and of **1a–6a** and **6b,c** with DTBP

	NBS	DTBP
1a	1.0[a]	1.0[a]
1b	0.32 ± 0.03	
2a	0.33 ± 0.05	0.24 ± 0.02
2b	0.18 ± 0.02	
3a	0.04 ± 0.01	0.19 ± 0.03
3b	0.01 ± 0.002	
4a	0.37 ± 0.05	0.40 ± 0.03
5a	3.1 ± 0.7	2.4 ± 0.2
5b	2.1 ± 0.3	
6a	1.4 ± 0.1	1.9 ± 0.4
6b	1.2 ± 0.1	2.2 ± 0.2
6c	0.42 ± 0.04	0.7 ± 0.2

[a] Assigned as unity for each reagent.

Discussion

The deuterium isotope effects reflected in the relative rates of reaction of the amino acid derivatives **1a–6a**, **1b–3b**, **5b**, and **6b,c** (Table I) and the fact that no epimerization of enantiomers of **2a** or **3a** was observed in reactions with either NBS or DTBP indicate that hydrogen transfer to bromine atom and *tert*-butoxy radical is the irreversible rate-determining step in reactions of the amino acid derivatives **1a–6a** with NBS and DTBP, respectively. Subsequent reactions of product radicals are unlikely to affect the relative reactivities of **1a–6a**.[5] The isotope effects reflected in the relative rates of reaction of **1a–3a** and the deuteriated analogues **1b–3b** with NBS indicate that each of the amino acid derivatives **1a–3a** reacts by α-C–H bond homolysis. Thus the relative rates of reaction of **1a–3a** with NBS indicate the ease of formation of the corresponding α-centered radicals **16–18**. The production of **1c** in the reaction of **1a** may be attributed to the reaction of the radical **16** by bromine-atom incorporation. A mechanism of formation of the dibromovaline derivative **3d** from **3a** via the α-centered radical **18** has been proposed.[13]

The formation of **2a** and **7** in the reaction of **1a** with DTBP[11] indicates that this reaction of **1a** involves hydrogen atom transfer to *tert*-butoxy radical to give the radical **16**. Similarly, the production of **12** and **13** in the reaction of **2a** with DTBP can be

(13) Easton, C. J.; Hay, M. P.; Love, S. G. *J. Chem. Soc., Perkin Trans 1* **1988**, 265.
(14) Obata, N.; Niimura, K. *J. Chem. Soc., Chem. Commun.* **1977**, 238.
(15) Cabildo, P.; Claramunt, R. M.; Elguero, J. *Org. Magn. Reson.* **1984**, *22*, 607.
(16) Other hyperfine splittings were not resolved under the conditions used to record the EPR spectra. Resolution of the hyperfine coupling with nitrogen could not be achieved because the spectra were complicated by extensive long-range hyperfine couplings.
(17) Ingold, K. U.; Lusztyk, J.; Scaiano, J. C. *J. Am. Chem. Soc.* **1984**, *106*, 343.

attributed to the reaction of **2a** with *tert*-butoxy radical to give **17**. Subsequent reaction of **17** by dimerization affords **13**, while coupling of **17** with methyl radical produced by β-scission of *tert*-butoxy radical leads to the formation of **12**. Although discrete products could not be isolated from the reaction of **3a** with DTBP, a previous study of the relative rates of reaction of **3a** and the deuteriated analogues **3b** and **3c** with DTBP has indicated that **3a** reacts only in part by hydrogen atom transfer to *tert*-butoxy radical to give the α-centered radical **18**.[13] It follows that while the relative rates of reaction of **1a** and **2a** with DTBP reflect the ease of formation of the corresponding radicals **16** and **17**, the rate of formation of **18** by reaction of **3a** with *tert*-butoxy radical is slower than the overall relative rate of reaction of **3a** with DTBP given in Table I.

On this basis there is a good correlation between the relative rates of formation of the α-centered radicals **16–18** through reaction with bromine atom and with *tert*-butoxy radical. With each species the rate of formation of the α-centered radical **16** by hydrogen atom transfer from the glycine derivative **1a** is faster than the rate of reaction of the alanine derivative **2a** to give **17**, which is in turn faster than the rate of production of the α-centered radical **18** by hydrogen transfer from the valine derivative **3a**. Even on a per hydrogen basis **1a** is more reactive than either **2a** or **3a**. When regarded in the context that the selectivity for the formation of tertiary alkyl radicals in preference to secondary radicals is typically a factor of 20 in reactions involving hydrogen transfer to bromine atom and a factor of 4 in reactions with *tert*-butoxy radical,[5,7] the relative rates of formation of **16–18** in these reactions are peculiar. To the extent that thermodynamic criteria control the pathways and rates of free-radical reactions, these results indicate that, in direct contrast to expectation, the secondary radical **16** is marginally more stable than the tertiary radical **17**, and both **16** and **17** are considerably more stable than **18**.

We attribute this peculiar stability of the radical **16** to a particularly favorable geometry. Stabilization of the captodative[18] radicals **16–18** will result from overlap of the semioccupied p orbital with the π orbitals of the amido and methoxycarbonyl substituents. There will be maximum overlap of these orbitals in planar conformations of the radicals **16–18** (Figure 2). The radical **17** will be destabilized compared to **16** by nonbonding interactions associated with planar conformations of **17**, and **18** will be even less stable owing to more severe nonbonding interactions. These destabilizing influences outweigh the normal thermodynamic preference for the production of tertiary radicals. A recent EPR study has also indicated that relatively small deviations from planarity can significantly diminish the importance of the captodative effect.[19]

The formation of the monobromide **1c** in high yield in the reaction of the glycine derivative **1a** with NBS and the lack of subsequent reaction of **1c** under these conditions[12] is consistent with our rationale for the reactivity of **1a**. The radical **19** produced by hydrogen atom abstraction from **1c** would be less stable than **14** because of nonbonding interactions (Figure 2).

From the production of the α-bromosarcosine derivative **4b** in the reaction of **4a** with NBS, it appears that this reaction involves hydrogen transfer from **4a** to bromine atom to give the α-centered radical **15**. The EPR study of the reaction of **4a** with *tert*-butoxy radical indicates that in this reaction hydrogen transfer from **4a** occurs to give either radical **14** or **15**. This variation in selectivity can be attributed to the susceptibility of reactions involving *tert*-butoxy radical to polar effects.[13] The EPR spectrum showed

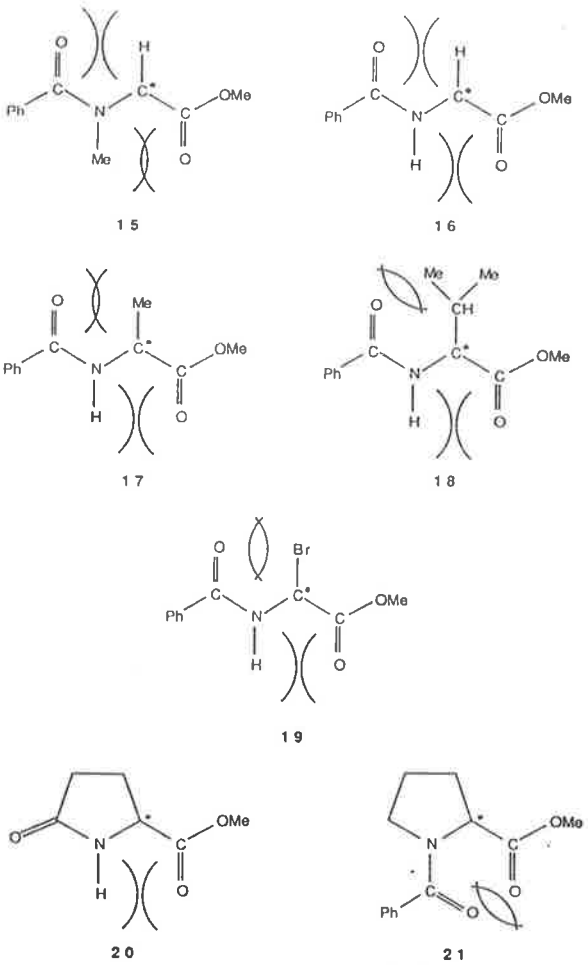


Figure 2. Nonbonding interactions associated with planar conformations of the amido- and methoxycarbonyl-substituted radicals **15–21**.

that the steady-state concentrations of **14** and **15** were approximately equal, indicating that their rates of formation were comparable.[20] The relative rate of reaction of **4a** with NBS (Table I) is a measurement, therefore, of the relative rate of production of **15**, whereas the relative rate of production of **15** by reaction of **4a** with DTBP is approximately half of the overall relative rate of reaction.

The relative rates of production of the α-centered radicals **15** and **17** in reactions with bromine atom and with *tert*-butoxy radical are very similar. This supports the hypothesis that the rate of hydrogen atom transfer from amino acid derivatives is affected by the extent of nonbonding interactions in the product radicals, since the degree of nonbonding interactions in planar conformations of **15** and **17** is also very similar (Figure 2).

The deuterium isotope effect reflected in the relative rates of reaction of methyl pyroglutamate (**5a**) and the deuteriated analogue **5b** with NBS and the production of the dimer **8** in the reaction of **5a** with DTBP indicate that with each reagent **5a** reacts by hydrogen transfer from the α-position to give the radical **20**. That the rates of reaction of **5a** with bromine atom and with *tert*-butoxy radical are faster than the corresponding rates of reaction of the glycine derivative **1a** is consistent with the rationale proposed above. The radical **20** can adopt planar conformations which are relatively free of nonbonding interactions (Figure 2). Formation of the radical **20** is favored by the relief of ring strain and by the release of steric interactions between the methoxycarbonyl substituent and the β-hydrogens in **5a**.[20,21] It is possible

(18) Radicals stabilized by the combined action of an electron-releasing amido substituent and an electron-withdrawing carboxy substituent belong to the class of captodative,[a] merostabilized,[b] or "push-pull"[c] stabilized radicals. (a) Viehe, H. G.; Merenyi, R.; Stella, L.; Janousek, Z. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 917. Viehe, H. G.; Janousek, Z.; Merenyi, R. *Acc. Chem. Res.* **1985**, *18*, 148. (b) Baldock, R. W.; Hudson, P.; Katritzky, A. R.; Soti. F. *Heterocycles* **1973**, *1*, 67. Baldock, R. W.; Hudson, P.; Katritzky, A. R.; Soti, F. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1422. (c) Balaban, A. T. *Rev. Roum. Chim.* **1971**, *16*, 725.

(19) Beckwith, A. L. J.; Brumby, S. *J. Chem. Soc., Perkin Trans. 2*, **1987**, 1801.

(20) Malatesta, V.; Ingold, K. U. *J. Am. Chem. Soc.* **1981**, *103*, 619.

that formation of the radical **20** is also favored entropically by the inflexibility of the ring in **5a**, holding the amido group in the planar orientation as required for stabilization of the product. Thus the radical **20** is more stable than the glycyl radical **16** and this is reflected in the relative rates of reaction of **1a** and **5a** with bromine atom and with *tert*-butoxy radical.

On the basis of the hypothesis presented above, reaction of *N*-benzoylproline methyl ester (**6a**) to give the α-centered radical **21** would be expected to be much slower than the rate of reaction of the glycine derivative **1a** to give **16**, because the nonbonding interactions are much more severe in **21** than in **16** (Figure 2). The anomalous relative rates of reaction of **6a** compared to **1a** with bromine atom and with *tert*-butoxy radical are due to the regioselectivity of reaction. With each species, the relative rates of reaction of **6a** and the deuteriated analogues **6b,c** show that the major reaction of **6a** occurs at the δ-position to form the radical **22** in preference to the radical **21**. The production of **9** and **10** in the reaction of **6a** with NBS provides little information on the regioselectivity of reaction, but it is not inconsistent with reaction via the radical **22**. While the rate of reaction of **6a** is faster than the rate of reaction of **1a**, the rate of formation of **21** is considerably slower than the rate of formation of **16**. In fact, steric interactions associated with planar conformations of the radical **21** are so severe that the predominant reaction of **6a** is to produce the radical **22**, instead of **21**. Analogous regioselectivity has been observed in an electrochemical reaction of *N*-(methoxycarbonyl)proline methyl ester.[22]

Conclusion

In summary, the relative rates of reaction of **1a–6a** and the deuteriated analogues **1b–3b**, **5b**, and **6b,c** with bromine atom and of **1a–6a** and **6b,c** with *tert*-butoxy radical indicate that the selective reaction of glycine residues in these and other free radical reactions of amino acid derivatives is due to the stability of radicals produced by atom-transfer reactions. Radicals produced by hydrogen transfer from *N*-acylglycine derivatives may adopt planar conformations which are relatively free of nonbonding interactions and in which there is maximum delocalization of the unpaired electron, whereas radicals produced by similar reactions of derivatives of other amino acids are relatively unstable because of nonbonding interactions. Presumably selective reactions of derivatives of pyroglutamic acid and proline have not been observed in biological systems due to the relatively rare natural occurrence of these amino acids when compared to that of glycine.[23] In view of the numerous methods that have been reported for the synthesis of amino acids through the elaboration of glycine derivatives, particularly α-halogenated glycine derivatives,[12,24] the selective bromination of glycine derivatives has considerable potential as a method for the selective modification of glycine residues in small peptides.[25]

Experimental Section

GLC analyses were carried out on either a Varian 3700, a Perkin-Elmer 990, or a Pye 104 gas chromatograph using a Chrompack XE-60-S-VAL-S-A-PEA column (50 m × 0.22 mm) or a 5% OV-17 on VarAport column (1.0 m × 3 mm). HPLC analyses were performed on either a Shimadzu (LC-4A) HPLC with a Rheodyne (7125) injector and a Shimadzu ultraviolet detector (SPD-2AS) or with a Waters Model 501 solvent delivery system and a U6K injector with a Waters Model 481 absorbance detector using a Regis Pirkle Covalent L-Phenylglycine column (25 cm × 4.6 mm), a DuPont Zorbax cyanopropyl column (25 cm × 9.4 mm), or a Waters Z-module with a μ-Porasil Radial-Pak cartridge (10 cm × 8 mm). Column eluates were monitored at 254 nm. EPR spectra were recorded on a Varian E9 EPR spectrometer. Radicals were generated directly in the spectrometer cavity by irradiating solutions with an Oriel 1000-W high-pressure mercury lamp. Mixtures of **4a** and DTBP (1:1, w/w) and of **4b**, DTBP, and hexabutylditin (1:1:1, w/w/w) were prepared and degassed by bubbling with nitrogen for 10–15 min before irradiation.

Glycine, (2*R*)-, (2*S*)-, and (2*R*,*S*)-alanine, (2*R*)- and (2*R*,*S*)-valine, sarcosine, (2*R*,*S*)-pyroglutamic acid, (2*R*,*S*)-proline, and (2*R*,*S*)-glutamic acid were purchased from Sigma Chemical Co. α-Deuteriated glycine, alanine, valine, proline, and glutamic acid were prepared by treatment of the corresponding nondeuteriated amino acids with acetic anhydride/D_2O.[26] Deuteriated alanine and valine were resolved by treatment of the respective *N*-acetylamino acid derivatives with Hog Renal Acylase I.[27] Deuteriated pyroglutamic acid was prepared by cyclization of deuteriated glutamic acid.[28] 2,5,5-Trideuterioproline was prepared by the method of Leitch.[29] The amino acid derivatives **1a,b**,[30] (2*R*)-, (2*S*)-, and (2*R*,*S*)-**2a**,[31] (2*S*)-**2b**,[31] (2*R*)- and (2*R*,*S*)-**3a**,[32] (2*S*)-**3b**,[32] **4a**,[33] (2*R*,*S*)-**5a,b**,[34] and **6a–c**[35] were all prepared from the corresponding amino acids by using standard procedures. They were characterized by ^{1}H NMR, ^{13}C NMR, and IR spectroscopy and had physical constants in agreement with those reported. Deuterium content of the amino acid derivatives was determined by mass spectrometry to be the following: **1b**, 90% D_2; **2b**, 83% D_1; **3b**, 85% D_1; **5b**, 62% D_1; **6b**, 83% D_1; and **6c**, 92% D_3.

Competitive Reactions of 1a–6a and the Deuteriated Analogues 1b–3b, 5b, and 6b,c with NBS. Mixtures of two amino acid derivatives, *tert*-butylbenzamide (internal standard), and NBS in carbon tetrachloride were irradiated with a 250-W mercury lamp at reflux under nitrogen. Aliquots were removed at intervals and analyzed by GLC and HPLC. Various mixtures of amino acid derivatives were studied, including **1a** and (2*R*,*S*)-**2a**, **1b** and (2*R*,*S*)-**2a**, **1a** and (2*S*)-**2b**, (2*R*)-**2a** and (2*S*)-**2b**, (2*R*,*S*)-**2a** and (2*R*,*S*)-**3a**, (2*R*)-**3a** and (2*S*)-**3b**, **1a** and **4a**, **1a** and (2*R*,*S*)-**5a**, **1a** and (2*R*,*S*)-**5b**, **1a** and (2*R*,*S*)-**6a**, **1a** and (2*R*,*S*)-**6b**, and **1a** and (2*R*,*S*)-**6c**. All experiments were carried out at least in triplicate and analyses were performed at least in triplicate. Results of different experiments were consistent, as were results obtained from aliquots taken from the same experiment at different times.

Competitive Reactions of 1a–6a and 6b,c with DTBP. Mixtures of two amino acid derivatives, *tert*-butylbenzamide, and DTBP in *tert*-butyl alcohol were irradiated in a Rayonet photochemical reactor equipped with 12 RPR 3500 lamps. Reaction mixtures were analyzed as described above for the reactions with NBS. Mixtures of amino acid derivatives that were studied include **1a** and (2*S*)-**2a**, (2*R*,*S*)-**2a** and (2*R*,*S*)-**3a**, **1a** and **4a**, **1a** and (2*R*,*S*)-**5a**, **1a** and (2*R*,*S*)-**6a**, **1a** and (2*R*,*S*)-**6b**, and **1a** and (2*R*,*S*)-**6c**.

Reaction of *N*-Benzoyl-(2*R*,*S*)-proline Methyl Ester 6a with NBS. A mixture of *N*-benzoyl-(2*R*,*S*)-proline methyl ester **6a** (0.5 g, 2.1 mmol) and NBS (1.14 g, 6.4 mmol) in carbon tetrachloride (80 ml) was heated at reflux while being irradiated with a 250-W mercury lamp, under nitrogen, for 1 h. The suspension was chilled in an ice/salt bath and then filtered and concentrated in vacuo. The residue was chromatographed on silica with ethyl acetate–hexane as eluent to give *N*-benzoyl-4-bromo-2-(methoxycarbonyl)pyrrole (**10**) and *N*-benzoyl-3-bromo-2-(methoxycarbonyl)pyrrole (**9**). **10** (96 mg, 14%): mp 88–90 °C; ^{1}H NMR ($CDCl_3$) δ 3.60 (s, 3 H), 7.03 (d, J = 2 Hz, 1 H), 7.22 (d, J = 2 Hz, 1 H), 7.40–7.90 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 51.9, 99.3, 122.5, 126.4, 126.6, 128.9, 130.0, 132.6, 134.0, 159.7, 167.1; MS, *m*/*z* (relative intensity) 309 (92), 307 (96), 278 (5), 276 (6), 228 (10), 205 (10), 203 (9), 176 (100). (Anal. Calcd for $C_{13}H_{10}BrNO_3$: C, 50.86; H, 3.26; N,

(21) Applequist, D. E.; Klug, J. H. *J. Org. Chem.* **1978**, *43*, 1729. Beckhaus, H.-D.; Schoch, J.; Ruchardt, Ch. *Chem. Ber.* **1976**, *109*, 1369. Hinz, J.; Ruchardt, Ch. *Liebigs Ann. Chem.* **1972**, *765*, 94.

(22) Shono, T.; Matsumura, Y.; Tsubata, K.; Sugihara, Y.; Yamane, S.; Kanazawa, T.; Aoki, T. *J. Am. Chem. Soc.* **1982**, *104*, 6697.

(23) The selectivity for reaction of glycine residues in peptides is also affected by the percentage glycine content of the peptide.[2]

(24) Zoller, U.; Ben-Ishai, D. *Tetrahedron* **1975**, *31*, 863. Bernstein, Z.; Ben-Ishai, D. *Tetrahedron* **1977**, *33*, 881. Sinclair, P. J.; Zhai, D.; Reibenspies, J.; Williams, R. M. *J. Am. Chem. Soc.* **1986**, *108*, 1103. Williams, R. M.; Zhai, D.; Sinclair, P. J. *J. Org. Chem.* **1986**, *51*, 5021. Lidert, Z.; Gronowitz, S. *Synthesis* **1980**, 322. Shiono, S.; Harada, K. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 1061. Castelhano, A. L.; Horne, S.; Billedeau, R.; Krantz, A. *Tetrahedron Lett.* **1986**, *27*, 2435. Rich, D. H.; Dhaon, M. K. *Tetrahedron Lett.* **1983**, *24*, 1671.

(25) Easton, C. J.; Scharfbillig, I. M.; Tan, E. W. *Tetrahedron Lett.* **1988**, *29*, 1565.

(26) Birnbaum, S. M.; Levintow, L.; Kingsley, R. B.; Greenstein, J. P. *J. Biol. Chem.* **1952**, *194*, 455.

(27) Baldwin, J. E.; Wan, T. S. *Tetrahedron* **1981**, *37*, 1589.

(28) Coleman, D. *J. Chem. Soc.* **1951**, 2294. Beecham, A. F. *J. Am. Chem. Soc.* **1954**, *76*, 4615.

(29) Lompa-Krzymien, L.; Boczon, W.; Leitch, L. C. *Proc. Int. Conf. Stable Isot., 2nd, 1975* **1976**, 574. Leitch, L. C., private communication.

(30) Huang, H. T.; Niemann, C. *J. Am. Chem. Soc.* **1952**, *74*, 4634.

(31) Hein, G. E.; Niemann, C. *J. Am. Chem. Soc.* **1962**, *84*, 4487.

(32) Jones, J. B.; Niemann, C.; Hein, G. E. *Biochemistry* **1965**, *4*, 1735.

(33) Cook, A. H.; Heilbron, I. M. *The Chemistry of Penicillins*; Clarke, H. T., Johnson, J. R., Robinson, R., Eds., Princeton University Press: Princeton, 1949, p 921.

(34) Papoport, H.; Castagnoli, N., Jr.; Holden, K. G. *J. Org. Chem.* **1964**, *29*, 883.

(35) Johnson, L. F.; Robertson, A. V.; Simpson, W. R. J.; Witkop, B. *Aust. J. Chem.* **1966**, *19*, 4615.

4.56. Found: C, 50.83; H, 3.05; N, 4.31.) **9** (190 mg, 29%): oil; ^{1}H NMR ($CDCl_3$) δ 3.57 (s, 3 H), 6.39 (d, J = 4 Hz, 1 H), 7.14 (d, J = 4 Hz, 1 H), 7.45–7.53 (m, 3 H), 7.73–7.77 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 51.9, 109.4, 114.9, 123.8, 126.3, 128.9, 129.8, 132.5, 133.8, 160.0, 167.1; MS, *m/z* (relative intensity) 309 (35), 307 (36), 278 (3), 276 (3), 251 (3), 249 (3), 230 (100); precise mass calcd for $C_{13}H_{10}BrNO_3$ 306.9845, found 306.9840.

Reaction of *N*-Benzoyl-(2*R*,*S*)-alanine Methyl Ester (2a) with DTBP. A mixture of *N*-benzoyl-(2*R*,*S*)-alanine methyl ester (**2a**) (0.3 g, 1.5 mmol) and DTBP (4 mL, 19 mmol) in *tert*-butyl alcohol (30 mL), contained in a quartz tube under nitrogen, was irradiated in the Rayonet photochemical reactor. After 4 days the reaction mixture was concentrated and chromatographed on silica with ethyl acetate–hexane as eluent to give dimethyl 2,3-dibenzamido-2,3-dimethylbutanedioate (**13**) and *N*-benzoyl-2,2-dimethylglycine methyl ester (**12**). **13** (60 mg, 20%): mp 170–177 °C; ^{1}H NMR (DMSO-d_6) δ 2.00 (s, 6 H), 3.80 (s, 6 H), 6.80 (br, 2 H), 7.53–7.93 (m, 10 H); MS, *m/z* (relative intensity) 413 (0.4), 381 (2), 353 (7), 231 (22), 207 (38), 175 (8), 105 (100), 77 (50). (Anal. Calcd for $C_{22}H_{24}N_2O_6$: C, 64.1; H, 5.9; N, 6.8. Found: C, 63.9; H, 5.9; N, 6.6.) **12** (32 mg, 10%) was identical in all respects with an authentic sample obtained by derivatization of the corresponding amino acid.[36]

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Registry No. 1a, 1205-08-9; **1b**, 102770-12-7; (2*S*)-**2a**, 38767-73-6; (2*R*)-**2a**, 7260-27-7; (2*RS*)-**2a**, 38767-73-6; (2*S*)-**2b**, 118013-54-0; (2*R*)-**3a**, 1492-13-3; (2*RS*)-**3a**, 14599-03-2; (2*S*)-**3b**, 116297-93-9; **4a**, 71533-21-6; (2*RS*)-**5a**, 54571-66-3; (2*RS*)-**5b**, 117918-31-7; (2*RS*)-**6a**, 114051-14-8; (2*RS*)-**6b**, 117918-32-8; (2*RS*)-**6c**, 117918-33-9; **9**, 117918-26-0; **10**, 117918-27-1; **11**, 117918-28-2; **12**, 65563-98-6; (±)-**13** (diastereomer-1), 117918-29-3; (±)-**13** (diastereomer-2), 117918-34-0; **14**, 117918-30-6; **15**, 116453-15-7.

(36) Hoefle, G.; Hoehne, G.; Respondek, J.; Schwarz, H. *Org. Magn. Reson.* **1977**, *12*, 477.

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Bromination of *N*-Phthaloylamino Acid Derivatives

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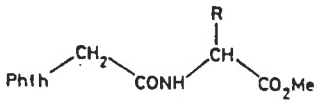
Hydrogen transfer to the bromine atom from the α-position of *N*-phthaloylamino acid derivatives is disfavoured to such an extent that bromination of *N*-phthaloyl derivatives of valine and phenylalanine occurs regiospecifically at the β-position, while halogenation of *N*-phthaloyl derivatives of glycylglycine and glycylglycylvaline occurs selectively at the *C*-terminal and non-terminal glycine residues, respectively.

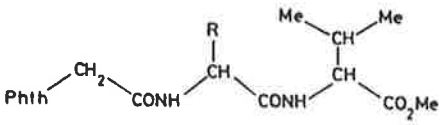
Numerous methods have been reported for the synthesis of amino acid derivatives through the elaboration of α-halogenated glycine derivatives.[1—3] A number of these procedures have been applied to the modification of halogenated glycine residues in dipeptides,[2,3] but their wider application is constrained by a lack of methods for the synthesis of peptides with an α-halogenated glycine residue. Selective bromination of glycine derivatives in reactions of dipeptide and other amino acid derivatives with *N*-bromosuccinimide has been reported.[2,4] The synthetic utility of this procedure is limited by competing reactions, however, especially if more than one glycine residue is present in the peptide. In this report we describe procedures for the selective bromination of particular glycine residues in *N*-phthaloyl-substituted di- and tri-peptides. In addition, we report a procedure for the direct β-halogenation of valine and phenylalanine derivatives. β-Halogenated amino acids are of interest as inhibitors of pyridoxal phosphate-dependent enzymes.[5]

Treatment of the peptide derivatives (**1a**) and (**2a**) with *N*-bromosuccinimide (1.0 equiv.) in dichloromethane at reflux under nitrogen for 1 h, with reaction initiated by irradiation with a 250 W mercury lamp, afforded the corresponding bromides (**1b**) and (**2b**). The bromides (**1b**) and (**2b**) were detected by ^{1}H n.m.r. spectroscopic analysis of the corresponding crude reaction mixtures after concentration under reduced pressure. The spectra of (**1b**) and (**2b**) each showed a doublet attributable to the α-proton of the brominated glycine residue, at δ 6.50 (*J* 10 Hz) for (**1b**) and at δ 6.65 (*J* 10 Hz) for (**2b**).

Since the bromides (**1b**) and (**2b**) were not sufficiently stable for isolation and purification, they were characterised by conversion to the corresponding methoxyglycine derivatives (**1c**)† [74% yield based on (**1a**), m.p. 187—188 °C, ^{1}H n.m.r. δ ($CDCl_3$) 3.48 (s, 3H), 3.83 (s, 3H), 4.52 (s, 2H), 5.62 (d, *J* 10 Hz, 1H), 7.18 (d, *J* 10 Hz, 1H), and 7.67—8.13 (m, 4H)] and (**2c**) [73% yield based on (**2a**) for a 1 : 1 mixture of diastereoisomers, m.p. 190—200 °C, ^{1}H n.m.r. δ ($CDCl_3$) 0.92 (d, *J* 7 Hz, 6H), 2.17 (m, 1H), 3.46 (s, 3H), 3.75 (s, 3H), 4.44 (s, 2H), 4.46 (m, 1H), 5.51 (d, *J* 8 Hz, 0.5H), 5.57 (d, *J* 9 Hz, 0.5H), 6.98 (m, 2H), and 7.72—7.90 (m, 4H)], and the deuteriated peptide derivatives (**1d**) (73% yield, 91% $^2H_1$) and (**2d**) (69%



(1)



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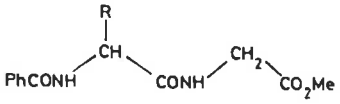

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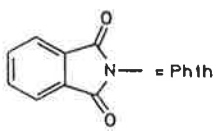

a; R = H
b; R = Br
c; R = OMe
d; R = D

† With the exception of the brominated peptide derivatives (**1b**), (**2b**), and (**3b**), all new compounds gave satisfactory ^{1}H n.m.r., i.r., high resolution mass spectral, and microanalytical data. Derivatives of (**1b**), (**2b**), and (**3b**) were fully characterised.

(4) (5)

a; R = H
b; R = Br

yield, 97% $^2H_1$), through reactions with methanol and tri-n-butyltin deuteride,[2] respectively. Mass spectrometric analysis of (**1d**) and (**2d**) indicated that the deuterium incorporation was regiospecific in each case.

In direct contrast to the selective reaction of the *C*-terminal glycine residue in (**1a**), reaction of *N*-benzoylglycylglycine methyl ester (**3a**) with *N*-bromosuccinimide occurred regioselectively at the *N*-terminal glycine residue to give (**3b**) [^{1}H n.m.r. δ 6.94 (*J* 10 Hz)]. The bromide (**3b**) was characterised by conversion to the methoxyglycine derivative (**3c**) [62% yield based on (**3a**), m.p. 56—57 °C, ^{1}H n.m.r. δ ($CDCl_3$) 3.48 (s, 3H), 3.75 (s, 3H), 4.09 (d, *J* 6 Hz, 2H), 5.83 (d, *J* 8 Hz, 1H), and 7.20—8.17 (m, 7H)] and the deuteriated dipeptide derivative (**3d**) [67% yield, 63% $^2H_1$]. Deuterium incorporation in (**3d**) was regiospecific, as shown by mass spectrometric analysis.

The regioselective bromination of the peptide derivatives (**1a**)—(**3a**) indicates that the α-position of an *N*-phthaloyl-substituted amino acid is less reactive than that of an *N*-acylamino acid derivative towards reaction with *N*-bromosuccinimide. This may be attributed to the relative stability and ease of formation of the corresponding α-centred radicals. Whereas acylamino-substituted radicals are stabilized by resonance, there is less delocalization of unpaired spin density by a phthalamido substituent.

The extent of the effect of an *N*-phthaloyl substituent to disfavour reaction at the α-position of an amino acid derivative is illustrated by the reactions of the valine and phenylalanine derivatives (**4a**) and (**5a**) with *N*-bromosuccinimide to give the corresponding β-brominated amino acid derivatives (**4b**) [87% yield, m.p. 129—131 °C, ^{1}H n.m.r. δ ($CDCl_3$) 2.00 (s, 3H), 2.16 (s, 3H), 3.71 (s, 3H), 5.18 (s, 1H), and 7.40—8.05 (m, 4H)] and (**5b**) [83% yield of a 1 : 1 mixture of diastereoisomers: one diastereoisomer had m.p. 122—123 °C, ^{1}H n.m.r. δ ($CDCl_3$) 3.80 (s, 3H), 5.55 (d, *J* 10 Hz, 1H), 5.92 (d, *J* 10 Hz, 1H) and 7.00—7.65 (m, 9H); the other diastereoisomer had m.p. 135—136 °C, ^{1}H n.m.r. δ ($CDCl_3$) 3.50 (s, 3H), 5.42 (d, *J* 10 Hz, 1H), 5.95 (d, *J* 10 Hz, 1H), and 7.00—7.85 (m, 9H)]. The regioselectivity observed in these reactions is contrary to that reported for reactions of the corresponding *N*-acylamino acid derivatives with *N*-bromosuccinimide, where reaction occurs at the α-position.[6]

In summary, the reactions of (**1a**)—(**5a**) with *N*-bromosuccinimide illustrate the effect of the *N*-phthaloyl substituent on the regioselectivity of bromination of peptide and amino acid derivatives. They exemplify procedures for the synthesis of β-brominated amino acid derivatives and for the enhancement of regiocontrol in the halogenation of peptide derivatives. Through appropriate choice of protecting groups and by exploiting the inherent selectivity for reaction of glycine residues, it is possible to control the regioselectivity of bromination of derivatives of small peptides.

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References

1 For examples see: Z. Bernstein and D. Ben-Ishai, *Tetrahedron*, 1977, **33**, 881; R. Kober, K. Papadopoulos, W. Miltz, D. Enders, W. Steglich, H. Reuter, and H. Puff, *ibid.*, 1985, **41**, 1693; P. J. Sinclair, D. Zhai, J. Reibenspies, and R. M. Williams, *J. Am. Chem. Soc.*, 1986, **108**, 1103; R. M. Williams, D. Zhai, and P. J. Sinclair, *J. Org. Chem.*, 1986, **51**, 5021; Z. Lidert and S. Gronowitz, *Synthesis*, 1980, 322; R. Kober and W. Steglich, *Liebigs Ann. Chem.*, 1983, 599; D. H. Rich and M. K. Dhaon, *Tetrahedron Lett.*, 1983, **24**, 1671; R. M. Williams, P. J. Sinclair, D. Zhai, and D. Chen, *J. Am. Chem. Soc.*, 1988, **110**, 1547; T. Bretschneider, W. Miltz, P. Munster, and W. Steglich, *Tetrahedron*, 1988, **44**, 5403; R. M. Williams and D. Zhai, *ibid.*, 1988, **44**, 5425; A. L. Castelhano, S. Horne, G. J. Taylor, R. Billedeau, and A. Krantz, *ibid.*, 1988, **44**, 5451; V. A. Burgess and C. J. Easton, *Aust. J. Chem.*, 1988, **41**, 1063.

2 C. J. Easton, I. M. Scharfbilling, and E. W. Tan, *Tetrahedron Lett.*, 1988, **29**, 1565.

3 S. Shiono and K. Harada, *Bull. Chem. Soc. Jpn.*, 1985, **58**, 1061; A. L. Castelhano, S. Horne, R. Billedeau, and A. Krantz, *Tetrahedron Lett.*, 1986, **27**, 2435.

4 C. J. Easton and M. P. Hay, *J. Chem. Soc., Chem. Commun.*, 1986, 55.

5 For reviews see: C. Walsh, *Tetrahedron*, 1982, **38**, 871; C. T. Walsh, *Annu. Rev. Biochem.*, 1984, **53**, 493.

6 C. J. Easton, M. P. Hay, and S. G. Love, *J. Chem. Soc., Perkin Trans. 1*, 1988, 265.

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Crystal and molecular structure of N-*p*-fluorobenzoyl-L-valine

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Crystal structure / Valine derivative / Hydrogen bonding

Abstract. The title compound, ($C_{12}H_{14}NO_3F$), N-*p*-fluorobenzoyl-L-valine, crystallizes in the orthorhombic space group $P2_12_12_1$ with unit cell parameters $a = 10.743(1)$, $b = 11.534(1)$, $c = 20.013(5)$ Å, $Z = 8$; $D_x = 1.281$ Mg m^{-3}. The structure was solved by direct-methods and refined by a full-matrix least-squares procedure on 2757 reflections to final $R = 0.045$. The two molecules comprising the crystallographic asymmetric unit differ from each other as a result of a rotation about the C(2)–C(3) bond. All potential hydrogen bonding sites on each molecule participate in a complicated, three-dimensional hydrogen bonding network which is described in detail.

Introduction

The ability of various cyclodextrins (cyclic oligosaccharides) to include other smaller molecules within their truncated conical cavities is well documented (Saenger, 1980). As a result of being incorporated within a cyclodextrin 'host' molecule, 'guest' species may exhibit modified properties such as stability and solubility. Consequently, much attention has been devoted to understanding the chemistry of these systems. In these laboratories the nature of the association between aromatic fluorine containing molecules and various cyclodextrins is being examined using a range of

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techniques including ^{19}F n.m.r. spectroscopy (Lincoln et al., 1988). Amongst the guest species being investigated is the title compound, N-*p*-fluorobenzoyl-L-valine, which includes in certain cyclodextrins. This paper details the single-crystal structure analysis of the title compound, hereafter L-PFBV, which was determined in order to investigate i) the conformation of the valine residue, and ii) the hydrogen bonding characteristics of the molecule in solid state.

Experimental

The compound L-PFBV was prepared using an established literature procedure (Spratt, Meng and Dorn, 1985). Crystals suitable for X-ray analysis were grown by the slow evaporation of an ethyl acetate/hexane solution of the compound; m.pt. 136.5–138°C. In order to compare the hydrogen bonding characteristics of L-PFBV with those of N-*p*-fluorobenzoyl-D,L-valine (DL-PFBV), attempts were made to obtain suitable crystals of the racemate. Although well-formed crystals of DL-PFBV were obtained, these were unsuitable for X-ray analysis as preliminary photographic work showed that the crystals were weakly scattering yielding a diffraction pattern limited to a Bragg angle of 20° for Cu radiation.

Intensity data for L-PFBV were measured at room-temperature using an Enraf-Nonius CAD4F diffractometer on a well-formed crystal with faces (and distances from centroid) ± (110) 0.39, ±(1$\bar{1}$0) 0.39, ±(011) 0.26, ±(01$\bar{1}$) 0.16, and ±(001) 0.25 mm. Metric crystal data are given in the Abstract. The compound crystallizes in the non-centrosymmetric space group $P2_12_12_1$ with two unique molecules comprising the asymmetric unit. The ω:2θ scan technique was used to measure the intensities of 4387 reflections up to a maximum Bragg angle of 25° with graphite-monochromatized Mo$K\alpha$ radiation, $\lambda = 0.71069$ Å. Cell parameters were refined by a least-squares procedure on the setting angles of 25 reflections ($7 \leq \theta \leq 12°$). No detectable decomposition of the crystal occurred during the data collection. Corrections were applied for Lorentz and polarization effects and for absorption (Sheldrick, 1976); maximum and minimum transmission factors were 0.9826 and 0.9678 respectively. Of the reflections measured 3444 were unique (R_{amal} 0.011) and of these 2757 satisfied the $I \geq 2.5\,\sigma(I)$ criterion of observability.

The structure was solved by direct-methods (Sheldrick, 1986) and refined by a full-matrix least-squares procedure in which the function $\Sigma w\Delta^2$ was minimized where $\Delta = ||F_o| - |F_c||$ and w was the weight applied to each reflection (Sheldrick 1976). Non-hydrogen atoms were refined anisotropically and except for methyl-bound hydrogen atoms which were included in the model at their tetrahedral estimates, all hydrogen atom positions were located from difference maps and refined with individual isotropic thermal

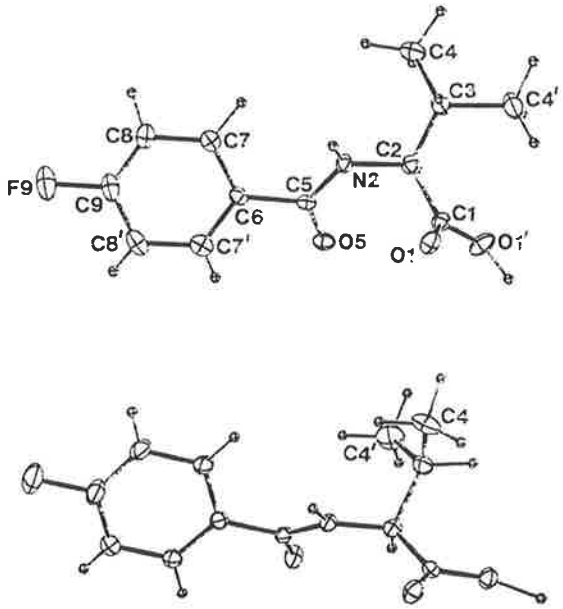

Fig. 1. An ORTEP (Johnson, 1976) diagram of the two molecules comprising the asymmetric unit for L-PFBV. The numbering scheme is also shown which is common to both molecules (upper view is molecule *a*).

parameters. A weighting scheme of the form $k/[\sigma^2(F) + gF^2]$ was introduced and the refinement continued until convergence; $R = 0.038$, ($R = 0.056$ for all data); $R_w = 0.045$ for $k = 1.08$ and $g = 0.002$. An empirical extinction correction was applied to the data such that the value of nz in SHELX76 (Sheldrick, 1976) was 0.0054(4). The maximum and minimum residual electron density peaks in the final difference maps were $+0.26$ and $-0.20\ e\text{Å}^{-3}$ respectively. The analysis of variance showed no special features.

The scattering factors used for all atoms were those incorporated in the SHELX76 program (Sheldrick, 1976). Calculations were performed on the University of Adelaide's SUN 4/280 computer system. Atomic coordinates for non-hydrogen atoms are given in Table 1, the numbering scheme used is shown in Figure 1 which was drawn at 15% probability ellipsoids with ORTEP (Johnson, 1976). Selected bond distances and angles are listed in Table 2. Listings of thermal parameters, hydrogen atom parameters and tables of F_{obs} have been deposited. [1]

[1] Additional material to this paper can be ordered from the Fachinformationszentrum Energie-Physik-Mathematik, D-7514 Eggenstein-Leopoldshafen 2, FRG. Please quote references no. CSD 53502, the name of the authors and the title of the paper.

Table 1. Fractional atomic coordinates ($\times 10^4$) and B_{eq} values (A^2) $B_{eq} = 8\pi^2(U_{11} + U_{22} + U_{33})/3$.

Atom	x	y	z	B_{eq}
F(9a)	−8084(2)	−6070(3)	−2700(1)	8.87
O(1a)	−1348(2)	−8247(2)	−1157(1)	4.76
O(1′a)	282(2)	−8119(2)	−1829(1)	5.90
O(5a)	−2443(2)	−7494(2)	−2769(1)	4.55
N(2a)	−2382(2)	−6352(2)	−1866(1)	3.63
C(1a)	−743(3)	−7716(3)	−1560(1)	3.82
C(2a)	−1041(3)	−6486(3)	−1780(1)	3.71
C(3a)	−594(3)	−5596(3)	−1269(2)	4.63
C(4a)	767(4)	−5768(4)	−1070(2)	6.95
C(4′a)	−813(5)	−4389(3)	−1522(3)	8.13
C(5a)	−2973(3)	−6863(2)	−2363(1)	3.50
C(6a)	−4346(3)	−6634(3)	−2423(1)	3.87
C(7a)	−4907(4)	−5635(4)	−2210(2)	6.53
C(7′a)	−5085(5)	−7429(5)	−2728(3)	8.71
C(8a)	−6170(4)	−5435(4)	−2303(3)	7.26
C(8′a)	−6349(5)	−7241(6)	−2802(4)	10.24
C(9a)	−6846(3)	−6250(4)	−2605(2)	6.16
F(9b)	−1361(3)	5259(2)	970(2)	9.98
O(1b)	828(2)	−286(2)	−1457(1)	4.90
O(1′b)	1257(2)	−2067(2)	−1121(1)	4.82
O(5b)	1378(2)	496(2)	695(1)	5.03
N(2b)	72(2)	333(2)	−180(1)	3.63
C(1b)	848(3)	−1011(3)	−1030(1)	3.69
C(2b)	404(3)	−860(3)	−316(1)	3.76
C(3b)	−650(4)	−1703(3)	−132(2)	5.69
C(4b)	−1810(4)	−1480(5)	−523(3)	8.40
C(4′b)	−861(6)	−1664(5)	620(2)	9.48
C(5b)	539(3)	901(3)	345(1)	3.63
C(6b)	12(3)	2066(3)	491(1)	3.79
C(7b)	−1253(3)	2288(3)	450(2)	4.66
C(7′b)	798(3)	2933(3)	715(2)	4.37
C(8b)	−1717(4)	3359(4)	621(2)	6.00
C(8′b)	340(4)	4006(3)	878(2)	5.79
C(9b)	−898(4)	4196(3)	819(2)	6.16

Results and discussion

The asymmetric unit of N-*p*-fluorobenzoylvaline, L-PFBV, is comprised of two formula units of the compound, designated molecules *a* and *b*, which are illustrated in Figure 1. Interatomic distances describing chemically equivalent connectivities are equal within experimental error in the two molecules; Table 2. Similarly, comparable bond angles are equivalent with only minor differences existing between a few pairs of angles; the maximum difference of 3.6° is found for the N(2)−C(2)−C(3) angle [108.5(2) and 112.1(3)° for molecules *a* and *b* respectively]. In a survey of crystal

Table 2. Selected bond distances (Å) and angles (°).

Atoms	Molecule *a*	Molecule *b*	Atoms	Molecule *a*	Molecule *b*
C(1)–O(1)	1.204(3)	1.196(3)	C(2)–C(1)–O(1)	123.7(3)	126.0(3)
C(1)–O(1′)	1.311(4)	1.308(3)	C(2)–C(1)–O(1′)	112.9(3)	110.1(3)
O(1′)–H(1′)	1.00(6)	0.84(4)	O(1)–C(1)–O(1′)	123.3(3)	123.9(3)
C(1)–C(2)	1.519(4)	1.517(4)	N(2)–C(2)–C(1)	109.9(3)	111.3(2)
C(2)–N(2)	1.460(4)	1.447(4)	N(2)–C(2)–C(3)	108.5(2)	112.1(3)
C(2)–C(3)	1.527(4)	1.536(5)	C(1)–C(2)–C(3)	111.6(2)	112.6(3)
C(2)–H(2)	0.96(3)	0.87(3)	C(2)–N(2)–C(5)	121.1(3)	121.4(3)
N(2)–C(5)	1.319(3)	1.337(4)	C(2)–C(3)–C(4)	112.8(3)	112.4(3)
N(2)–H(n2)	0.87(3)	0.86(3)	C(2)–C(3)–C(4′)	110.4(3)	109.1(3)
C(3)–C(4)	1.528(5)	1.495(6)	C(4)–C(3)–C(4′)	111.0(4)	112.8(4)
C(3)–C(4′)	1.500(5)	1.522(6)	O(5)–C(5)–C(6)	120.3(2)	120.7(2)
C(3)–H(3)	0.97(3)	1.14(4)	N(2)–C(5)–O(5)	122.7(3)	122.4(3)
C(5)–O(5)	1.231(3)	1.233(3)	N(2)–C(5)–C(6)	117.0(2)	117.0(3)
C(5)–C(6)	1.503(4)	1.488(4)	C(5)–C(6)–C(7)	123.7(3)	121.9(3)
C(6)–C(7)	1.369(5)	1.385(4)	C(5)–C(6)–C(7′)	119.4(3)	118.9(3)
C(6)–C(7′)	1.358(6)	1.383(4)	C(7)–C(6)–C(7′)	116.8(4)	119.0(3)
C(7)–C(8)	1.388(6)	1.375(5)	C(6)–C(7)–C(8)	121.9(4)	120.4(3)
C(7′)–C(8′)	1.382(8)	1.371(5)	C(6)–C(7′)–C(8′)	121.1(5)	120.7(3)
C(8)–C(9)	1.333(6)	1.365(6)	C(7)–C(8)–C(9)	118.4(4)	118.3(3)
C(8′)–C(9)	1.321(7)	1.353(6)	C(7′)–C(8′)–C(9)	120.1(5)	118.6(4)
F(9)–C(9)	1.359(5)	1.357(4)	C(8)–C(9)–C(8′)	121.6(4)	123.0(4)
			C(8)–C(9)–F(9)	119.3(4)	117.9(4)
			C(8′)–C(9)–F(9)	119.1(4)	119.1(4)

environments for a series of amino acid residues, Gould et al. (1985) reported the average values for selected interatomic parameters for the valine molecule. The values averaged from a total of twenty-six crystal structure determinations [C(1)–C(2) 1.524(19); C(2)–N(2) 1.469(25); C(2)–C(3) 1.537(19); C(3)–C(4) 1.523(15) Å and N(2)–C(2)–C(1) 1.08.4(14); N(2)–C(2)–C(3) 111.3(18); C(1)–C(2)–C(3) 111.4(21); C(2)–C(3)–C(4) 111.0(10)°] agree well with those reported here for L-PFBV; see Table 2.

The two molecules differ from each other by virtue of a rotation about the C(2)–C(3) bond. The valine residue may adopt three different conformations which depend on the relative disposition of the methyl substituents bound to the C(3) atom; the three ideal geometries are shown as Newman projections in Figure 2. These geometries are best defined by the torsion angles, N(2)–C(2)–C(3)–C(4) and N(2)–C(2)–C(3)–C(4′) [χ' and χ'' respectively]. Values for χ' and χ'' of 180 and 60° corresponds to the conformation where one methyl group is *trans* to N(2) and the other is *trans* to H as shown in Figure 2A. Two other conformations are possible where one of the methyl groups is gauche to both N(2) and H and i) the other methyl group is *trans* to N(2) i.e. χ' is about $-60°$ (or about 300°)

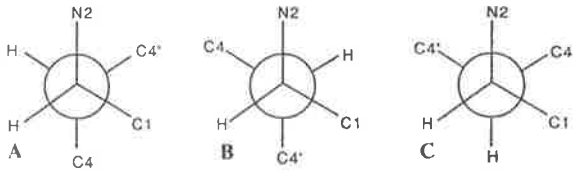

Fig. 2. Newman projections for L-PFBV viewed down the C(2)–C(3) bond.

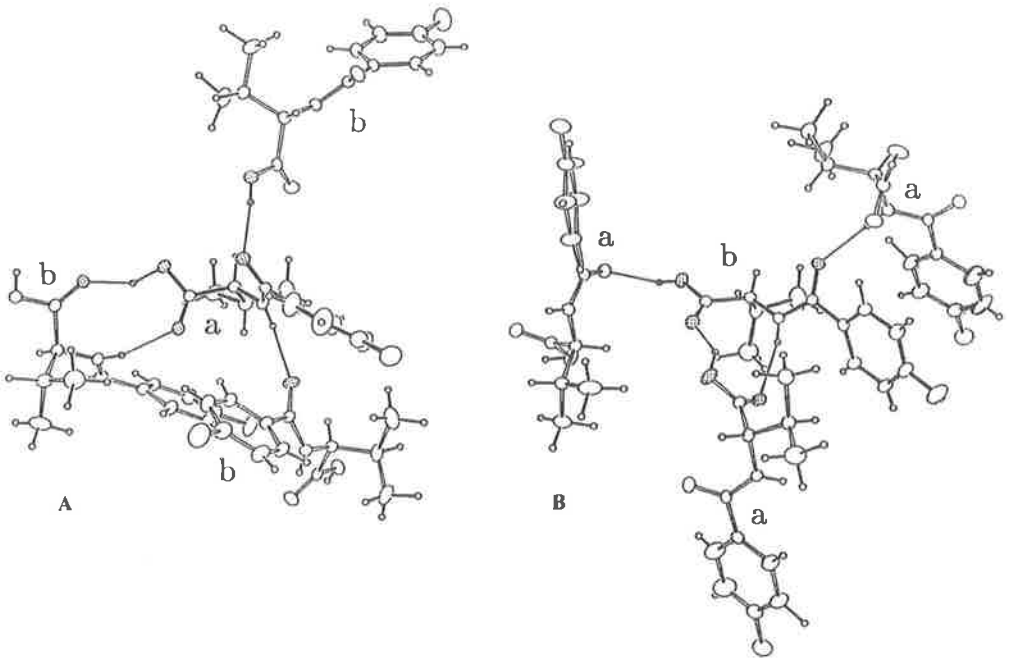

Fig. 3. Hydrogen bonding characteristics in L-PFBV. (A) view of a molecule of *a* interacting with three molecules of *b*; (B) view of a molecule of *b* interacting with three molecules of *a*.

and χ'' is approximately 180° as shown in Figure 2B or ii) the second methyl substituent is *trans* to C(1) i.e. χ' is about 60° and χ'' is approximately −60° as shown in Figure 2C. On this basis molecule *a* in PFBV is assigned conformation B with values of χ' and χ'' of 297.0 and 172.3° respectively and molecule *b* is assigned conformation C [61.2 and 295.3° respectively]. In this context it is noteworthy that there are two valine molecules in the asymmetric unit of L-valine corresponding to conformations A and B (Torii and Iitaka, 1970). Analyses of χ' for the valine side-chain in small molecule compounds (Gould et al., 1985) as well as in protein structures (Pullman and Pullman, 1974; Janin et al., 1978; Bhat et al., 1979) show that the *trans*

Table 3. Intermolecular hydrogen bonding, A—H...B, contacts.

A—H	B	Symmetry operation	H...B (Å)	A—H...B ( )
N—H(n2a)	O(5b)	$-0.5+x, 0.5-y, -z$	2.01(3)	174(2)
O—H(1'a)	O(1b)	$x, y-1, z$	1.70(3)	145(2)
N—H(n2b)	O(1a)	$x, y+1, z$	2.15(3)	165(2)
O—H(1'b)	O(5a)	$-x, 0.5+y, -0.5-z$	1.84(3)	159(2)

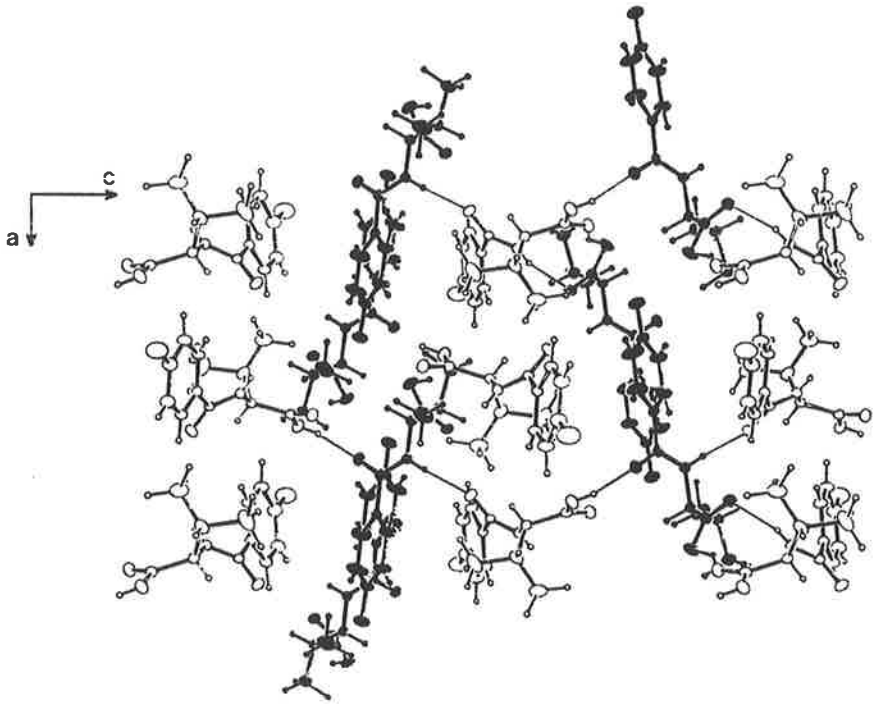

Fig. 4. The unit cell contents for L-PFBV viewed down [010] with molecules *a* shaded.

conformation is the most common with smaller contributions of the two gauche forms. Thus for example, Gould et al. (1985) report the ratio of the three conformations, A:B:C, in twenty-six small molecule structure determinations as being 42:27:31%.

Each molecule in the asymmetric unit has four sites which participates in intermolecular hydrogen bonding (the fluoride atoms are not involved in any significant intermolecular contacts). Two of these, N—H(n2) and O—H(1'), function as hydrogen atom donors and the two other sites, O(1) and O(5), act as hydrogen bond acceptors. Utilization of these potential hydrogen bonding sites in the crystal lattice of L-PFBV results in a

complicated three-dimensional hydrogen bonding network. As illustrated in Figure 3, each individual molecule *a* interacts with three different *b* molecules and similarly, molecule *b* interacts with three different *a* molecules. Despite this similarity the mode of association between the individual molecules and their nearest neighbours is quite different. The N – *H*(n2a) atom forms a hydrogen bond with the O(5b) atom of one molecule *b* and O(5a) interacts with O – *H*(1'b) of a second molecule *b*. The carboxyl group of molecule *a* is associated with a third molecule *b* via two contacts i.e. O – *H*(1'a) interacts with O(1b) and O(1a) interacts with N – *H*(n2b) which results in the formation of a nine-membered ring. The N – *H*(n2b) and O(5b) atoms function as hydrogen bond donor and acceptor respectively to O(1a) of one molecule and N – *H*(n2a) of a second molecule *a*. The O(1b) and N – *H*(n2b) atoms bridge the carboxyl group of the third molecule *a* as described above. The hydrogen bonding characteristics of both molecules are summarized in Table 3. The unit cell content for L-PFBV are projected down the [010] direction in Figure 4. From this diagram it can be seen that the structure is comprised of alternate layers of molecules *a* and *b* respectively held together by the hydrogen bonding scheme detailed above.

Acknowledgement. The Australian Research Grants Scheme is thanked for support.

References

Bhat, T. N., Sasisekharan, V., Vijayan, M.: An analysis of side-chain conformation in proteins. Int. J. Pept. Protein Res. **13** (1979) 170 – 184.

Gould, R. O., Gray, A. M., Taylor, P., Walkinshaw, M. D.: Crystal environments and geometries of leucine, isoleucine, valine, and phenylalanine provide estimates of minimum nonbonded contact and preferred van der Waals interaction distances. J. Am. Chem. Soc. **107** (1985) 5921 – 5927.

Janin, J., Wodak, S., Levitt, M., Maigret, B.: Conformation of amino acid side chains in proteins. J. Mol. Biol. **125** (1978) 357 – 386.

Johnson, C. K.: ORTEP II. Report ORNL-5138, Oak Ridge National Laboratory, Tennessee (1976).

Lincoln, S. F., Hounslow, A. M., Coates, J. H., Villani, R. P., Schiller, R. L.: The inclusion of diflunisal by γ-cyclodextrin and permethylated β-cyclodextrin. A UV-visible and ^{19}F nuclear magnetic resonance spectroscopic study. J. Inclusion Phenom. **6** (1988) 183 – 191.

Pullman, B., Pullman, A.: Molecular orbital calculations on the conformation of amino acid residues of proteins. Adv. Protein Chem. **28** (1974) 347 – 526.

Saenger, W.: Cyclodextrin inclusion compounds in research and industry. Angew. Chem. Int. Ed. Engl. **19** (1980) 344 – 362.

Sheldrick, G. M.: SHELX76. Program for crystal structure determination. University of Cambridge, England (1976).

Sheldrick, G. M.: SHELXS86. Program for crystal structure solution. University of Göttingen, Fed. Rep. of Germany (1986).

Spratt, M. P., Meng, Y., Dorn, H. C.: Characterization of amino acids and steroids by fluorine-19 nuclear magnetic resonance spectrometry of *p*-fluorobenzoyl derivatives. Anal. Chem. **57** (1985) 76 – 81.

Torii, K., Iitaka, Y.: The crystal structure of L-valine. Acta Crystallogr. **B26** (1970) 1317.

Anal. Calcd for $C_8H_6F_3N_3O$: C, 44.24; H, 2.79; N, 19.35. Found: C, 44.24; H, 2.80; N, 19.32.

Procedure B. A mixture of 5.37 g (0.030 mol) of **6** and 24.6 g (0.152 mol) of diethoxymethyl acetate was held at reflux for 64 h and concentrated. The residue was dissolved in ether, and the ether solution was extracted twice with 50 mL of 10% NaOH. The combined NaOH extracts were acidified with 50 mL of concentrated HCl. The oily precipitate was seeded with a crystal of **7** and began to solidify. The precipitate was filtered to give 3.2 g (49%) of **7** as white plates. The ether layer was dried and concentrated to 2.6 g of an oil, which was flash distilled at 1 Torr (90 °C) to give 2.3 g (35%) of **8**.

Homolytic Allyl Transfer Reactions of 1- and 3-Alkyl-Substituted Allyltributylstannanes

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Introduction

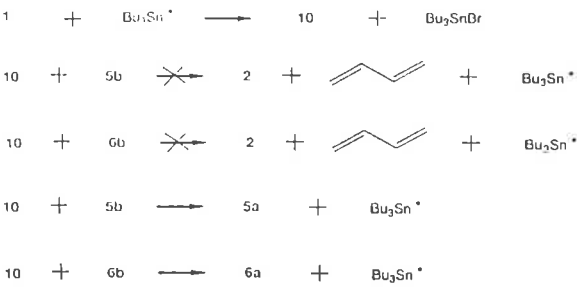
There have been several reports that homolytic allyl transfer reactions of 1- and 3-substituted allylstannanes are complicated by competing reactions.[1-4] 1,3-Rearrangement of the allylstannane, under the normal reaction conditions for homolytic allyl group transfer, can affect the integrity of the stannane and of the allylation product.[3] Alternatively, reduction of the substrate through hydrogen abstraction from the stannane can occur in preference to the allylation reaction.[1,2] This is particularly the case in reactions of 3-alkyl-substituted allylstannanes, where the steric effect of the alkyl substituent slows the rate of addition of radicals to the stannane, thus facilitating the competing reduction process. Only Pereyre and co-workers[5] have reported homolytic allyl transfer reactions of tributyl(3-methylallyl)stannane (**5b**).

In this report we describe allyl transfer reactions of *N*-benzoyl-2-bromoglycine methyl ester (**1**) with 1-, 2-, and 3-alkyl-substituted allyltributylstannanes. These reactions illustrate that allylation with 1- and 3-alkyl-substituted allylstannanes can occur without competing reduction of the substrate. The present work is based on our preliminary study[6] of the allylation of glycine derivatives through reaction of the corresponding brominated amino acid derivatives, such as **1**, with allyltributylstannane (**3b**). Independently, Baldwin et al.[7] reported analogous allyl transfer reactions of **1** with allyltriphenylstannane and 2-functionalized allyltributylstannanes. Neither our preliminary report[6] nor the account of the work of Baldwin et al.[7] dealt with reactions of 1- or 3-substituted allylstannanes.

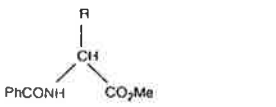
Results and Discussion

As described in our preliminary report,[6] the bromide **1** obtained through reaction of the glycine derivative **2** with

Scheme I



N-bromosuccinimide was treated with allyltributylstannane (**3b**) (2 equiv) and azobisisobutyronitrile (ca. 0.05 equiv) in benzene at reflux under nitrogen. After chromatography of the reaction mixture on silica and recrystallization of the product from ethyl acetate–petroleum ether, the allylglycine derivative **3a** was obtained in 63% yield based on the quantity of the glycine derivative **2** used to prepare the bromide **1**. The reaction of **1** with **3b** worked equally well using carbon tetrachloride instead of benzene as the solvent, or if the reaction was carried out at room temperature instead of at reflux. Thus it was possible to prepare the bromide **1** in carbon tetrachloride and react it with the stannane **3b** in situ.



1 R = Br
2 R = H
3a R = CH_2—CH=CH_2
4a R = CH_2—CMe=CH_2
5a R = CHMe—CH=CH_2
6a R = CH_2—CH=CHMe
7a R = CMe_2—CH=CH_2
8a R = CH_2—CH=CMe_2
9a R = CH—CH=CH—CH_2—CH_2

3b R = CH_2—CH=CH_2
4b R = CH_2—CMe=CH_2
5b R = CH_2—CH=CHMe
6b R = CHMe—CH=CH_2
7b R = CH_2—CH=CMe_2
8b R = CMe_2—CH=CH_2
9b R = CH—CH=CH—CH_2—CH_2

Treatment of the bromide **1** with tributyl(2-methylallyl)stannane (**4b**), in benzene at reflux, gave the 4-methyl-substituted allylglycine derivative **4a** in 56% yield based on **2**. When the bromide **1** was treated with a mixture (ca. 1:1) of the (*cis*- and *trans*-3-methylallyl)stannane **5b**, the corresponding 3-methyl-substituted allylglycine derivative **5a** was obtained in 57% yield as a 1:1 mixture of diastereomers. None of the glycine derivative **2** was detected in the reaction mixture, nor was there any evidence of formation of the 5-methyl-substituted allylglycine derivative **6a**, as determined by HPLC and ^1H NMR spectroscopic analyses of the reaction mixture. Since tributyl(1-methylallyl)stannane (**6b**) is essentially impossible to obtain in pure form due to its facile isomerization to the (3-methylallyl)stannane **5b**,[8] the reaction of **6b** with **1** was investigated by utilizing a 10-fold excess of a mixture (ca. 6:4) of the (3-methylallyl)- and (1-methylallyl)stannanes **5b** and **6b**. The reaction afforded the 3-methyl-substituted allylglycine derivative **5a** and the trans isomer of the 5-methyl-substituted analogue **6a** in yields of 5 and 19%, respectively, but none of the glycine derivative **2** was

(1) Keck, G. E.; Yates, J. B. *J. Organomet. Chem.* 1983, *248*, C21.
(2) Keck, G. E.; Enholm, E. J.; Yates, J. B.; Wiley, M. R. *Tetrahedron* 1985, *41*, 4079.
(3) Baldwin, J. E.; Adlington, R. M.; Birch, D. J.; Crawford, J. A.; Sweeney, J. B. *J. Chem. Soc., Chem. Commun.* 1986, 1339.
(4) For a review of the use of allylstannanes in homolytic allyl transfer reactions, see: Giese, B. In *Radicals in Organic Synthesis: Formation of Carbon–Carbon Bonds*; Baldwin, J. E., Ed.; Pergamon: New York, 1986; Vol. 5. in the Organic Chemistry Series.
(5) Grignon, J.; Pereyre, M. *J. Organomet. Chem.* 1973, *61*, C33. Servens, C.; Pereyre, M. *J. Organomet. Chem.* 1971, *26*, C4.
(6) Easton, C. J.; Scharfbillig, I. M.; Tan, E. W. *Tetrahedron Lett.* 1988, *29*, 1565.
(7) Baldwin, J. E.; Adlington, R. M.; Lowe, C.; O'Neil, I. A.; Sanders, G. L.; Schofield, C. J.; Sweeney, J. B. *J. Chem. Soc., Chem. Commun.* 1988, 1030.
(8) Matarasso-Tchiroukine, E.; Cardiot, P. *J. Organomet. Chem.* 1976, *121*, 169.

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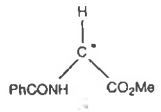
detected. Compound **6a** was assigned the trans configuration on the basis of ^{1}H NMR decoupling experiments, which indicated a vicinal coupling between the olefinic protons of 15 Hz.[9]

The reactions of the bromide **1** with the stannanes **5b** and **6b** may be rationalized as shown in Scheme I. The fact that none of the glycine derivative **2** was detected in the reaction of **1** with either **5b** or the mixture of **5b** and **6b** indicates that the expected process[1,2] of hydrogen atom transfer from the stannanes **5b** and **6b** to the glycinyl radical **10** does not occur. Production of the 3-methyl-



10

substituted allylglycine derivative **5a** through reaction of the bromide **1** with tributyl(3-methylallyl)stannane (**5b**) indicates that the intermediate glycinyl radical **10** reacts by addition to the stannane **5b**, rather than by hydrogen abstraction. Similarly, the formation of **5a** and **6a** in the reaction of the mixture of the stannanes **5b** and **6b** with **1** can be attributed to addition of **10** to **5b** and **6b**, respectively. The predominance of **6a** in the latter case, despite the use of a mixture of stannanes **5b** and **6b** in which **5b** was the major component, is consistent with the expectation that addition of **10** to the (1-methylallyl)-stannane **6b** should be faster than the addition of **10** to **5b**.[1,10] Hence the use of the 10-fold excess of the mixture of stannanes **5b** and **6b** in the reaction with **1** enables the selective reaction of the more reactive isomer **6b**.[1]

Particularly in light of the greater reactivity of **6b** compared to **5b**, the production of **5a** without concomitant formation of **6a** in the reaction of **1** with **5b** indicates that in this reaction 1,3-rearrangement of the stannane **5b** to give **6b** does not compete with allyl group transfer. Presumably this reflects the greater stability of **5b** compared to **6b**. The formation of **6a** from **1**, by utilizing a mixture of **5b** and **6b**, indicates that homolytic allyl group transfer from **6b** to the glycinyl radical **10** at least competes with 1,3-rearrangement of the stannane **6b**.

Reaction of the bromide **1** with tributyl(3,3-dimethylallyl)stannane (**7b**), in benzene at reflux, gave the 3,3-dimethyl-substituted allylglycine derivative **7a**, albeit in a modest yield of 15%. The production of **7a** is consistent with reaction via addition of the glycinyl radical **10** to the stannane **7b**. Reaction was not observed at lower temperatures, presumably due to the relatively low reactivity of **7b** toward allyl group transfer. By comparison with **5b**, the methyl substituent of tributyl(3,3-dimethylallyl)stannane (**7b**) would be expected to slow the rate of addition of the glycinyl radical **10** to the stannane **7b**, and increase the probability of allylic hydrogen atom transfer from the stannane **7b**.[10] There was no evidence of formation of the glycine derivative **2**, however, indicating that allylic hydrogen abstraction from **7b** by the glycinyl radical **10** did not occur. Nor was there any evidence of formation of the 5,5-dimethyl-substituted allylglycine derivative **8a**, indicating that 1,3-rearrangement of the stannane **7b** to give **8b** did not occur under the reaction conditions. Presumably this reflects the greater stability of **7b** compared to **8b**. The relatively low yield of **7a** can be attributed to decomposition of the bromide **1** during the reaction, as a result of the low reactivity of the stannane **7b**. Other products of the reaction were isolated only as mixtures which were not amenable to separation or characterization.

When the bromide **1** was treated with tributyl(cyclopent-2-enyl)stannane (**9b**), workup of the reaction mixture afforded the cyclopentenylglycine derivative **9a** in 37% yield, as a mixture (ca. 3:1) of diastereomers, and the glycine derivative **2** (19%). The major product **9a** results from allyl group transfer. The recovered glycine derivative **2** did not result from incomplete conversion of **2** in the preparation of the bromide **1**, as determined by analysis of the bromide **1**. It must have been derived, therefore, by reduction of the bromide **1** through hydrogen atom transfer from the stannane **9b** to **10**, competing with the allylation process. The reaction is not complicated by products resulting from 1,3-rearrangement of the stannane **9b**, since **9b** and its rearrangement product are degenerate.

From the results of these reactions of the bromoglycine derivative **1** with the stannanes **3b–7b** and **9b**, it is clear that allylstannanes and their 1-, 2- and 3-alkyl-substituted derivatives react by homolytic allyl group transfer, at least under some circumstances. While the general synthetic utility of 1- and 3-alkyl-substituted allylstannanes in allyl transfer reactions may be limited,[1-4] allylation reactions with 1- and 3-alkyl-substituted allylstannanes can occur without competing reduction of the substrate. The utility of 1- and 3-alkyl-substituted allylstannanes in allyl transfer reactions is illustrated by the synthesis of the cyclopentenylglycine derivative **9a**. Cyclopentenylglycine is a naturally occurring nonproteinogenic amino acid that has been isolated from the seeds of *Hydnocarpus anthelmintica* and the leaves of *Caloncoba echinata*.[11] Racemic cyclopentenylglycine has been shown to be a potent growth inhibitor of *Escherichia coli*[12] as well as a biogenic precursor of unusual cyclopentenyl fatty acids.[13]

In their reports of allyl transfer reactions of tributyl(3-methylallyl)stannane (**5b**), Pereyre and co-workers[5] noted that the allylation reaction is susceptible to polar effects, being faster with substrates which react via intermediate radicals substituted with electron-withdrawing groups. On this basis it seems likely that the allylation reaction is favored with electrophilic radicals such as **10** and those used by Pereyre and co-workers,[5] while nonpolar alkyl radicals such as those used by Keck et al.[1,2] react by hydrogen atom transfer.

Allyl transfer reactions of 1-alkyl-substituted allylstannanes are likely to be limited to reactive substrates such as **1**. Baldwin et al.[3] reported that as the reactivity of the alkyl halide decreased, the amount of product of allyl transfer reaction from rearranged stannane increased. With 3-alkyl-substituted allylstannanes, rearrangement of the stannanes is less likely to be a problem, due to the greater stability of the 3-substituted stannanes compared to the products of their rearrangement. With stannanes such as **9b**, which give degenerate products by rearrangement, the integrity of the allylation products will be unaffected by rearrangement of the stannanes.

Experimental Section

Melting points were determined on a hot-stage apparatus and are uncorrected. Solvents were purified and dried by using standard procedures.[14] ^{1}H NMR spectra were recorded on either

(9) Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*, 4th ed.; Wiley: New York, 1981.

(10) For a review of factors affecting the rate of addition of radicals to olefins, see: Ingold, K. U. In *Free Radicals*; Kochi, J. K., Ed.; Wiley: New York, 1973; Vol. 1, p 37.

(11) Cramer, V.; Rehfeldt, A. G.; Spener, F. *Biochemistry* **1980**, *19*, 3074.

(12) Dennis, R. L.; Plent, W. J.; Skinner, C. G.; Sutherland, G. L.; Shire, W. *J. Am. Chem. Soc.* **1955**, *77*, 2362.

(13) Cramer, V.; Spener, F. *Eur. J. Biochem.* **1977**, *74*, 495.

a Varian T-60 or Bruker CXP-300 spectrometer, as dilute solutions in deuteriochloroform with tetramethylsilane as an internal standard, unless otherwise indicated. Mass spectra were recorded on an AEI MS-3010 spectrometer, using an ionizing voltage of 70 eV, unless otherwise indicated. Chromatography was carried out on a Chromatotron 7924T (Harrison Research, Palo Alto/TC Research, Norwich) with Merck silica gel 60 PF_{254}, and elution with a gradient of petroleum ether/dichloromethane/ethyl acetate. Petroleum ether refers to the fraction with bp 60–80 °C. Microanalyses were performed by the Canadian Microanalytical Service Ltd., Vancouver.

Allyltributylstannane (**3b**) was purchased from Aldrich Chemical Company, Inc. Tributyl(2-methylallyl)stannane (**4b**),[15] tributyl(3-methylallyl)stannane (**5b**),[8] a mixture (ca. 6:4) of **5b** and tributyl(1-methylallyl)stannane (**6b**),[8] tributyl(3,3-dimethylallyl)stannane (**7b**),[16] tributyl(cyclopent-2-enyl)stannane (**9b**),[17] and *N*-benzoylglycine methyl ester (**2**)[18] were prepared and purified by using standard literature procedures. They were characterized by ^1H NMR and IR spectroscopy and had physical constants in agreement with those previously reported.

***N*-Benzoyl-2-bromoglycine Methyl Ester (1).** A mixture of the glycine derivative **2** (0.46 g, 2.4 mmol) and *N*-bromosuccinimide (0.43 g, 2.4 mmol) in carbon tetrachloride (10 mL) was heated at reflux under nitrogen while irradiated with a 250-W mercury lamp for 0.5 h. The mixture was cooled in ice, filtered under nitrogen, and concentrated under a stream of dry nitrogen to give crude **1** as pale yellow crystals, which were used without further purification: ^1H NMR δ 3.93 (s, 3 H), 6.65 (d, J = 10 Hz, 1 H), 7.30–7.90 (m, 6 H).

Methyl 2-Benzamidopent-4-enoate (3a). A mixture of crude **1** [prepared from the glycine derivative **2** (0.46 g, 2.4 mmol)], allyltributylstannane (**3b**) (1.6 g, 4.8 mmol), and azobisisobutyronitrile (ca. 20 mg) in benzene (20 mL) was heated at reflux under nitrogen for 5 h. The cooled solution was concentrated under reduced pressure, and the residue was chromatographed on silica to give **3a** (0.35 g, 63% yield based on **2**): mp 78–79 °C; ^1H NMR (CCl_4) δ 2.66 (m, 2 H), 3.76 (s, 3 H), 4.88 (dt, J = 7 and 6 Hz, 1 H), 5.15 (m, 2 H), 5.75 (m, 1 H), 6.94 (d, J = 7 Hz, 1 H), 7.40–7.80 (m, 5 H); mass spectrum, m/e (relative intensity) 233 (M^+, 6), 192 (14), 174 (8), 105 (100), 77 (32); mass spectrum, m/e 233.106 (M^+, calcd 233.105). Anal. Calcd for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48; N, 6.00. Found: C, 66.78; H, 6.43; N, 6.05.

Methyl 2-Benzamido-4-methylpent-4-enoate (4a). Treatment of **1** with tributyl(2-methylallyl)stannane (**4b**), as described above for the reaction of **1** with **3b**, gave **4a** in 56% yield based on **2**: mp 59–61 °C; ^1H NMR (CCl_4) δ 1.80 (s, 3 H), 2.60 (d, J = 8 Hz, 2 H), 3.70 (s, 3 H), 4.60–4.90 (m, 3 H), 6.99 (d, J = 8 Hz, 1 H), 7.30–7.90 (m, 5 H); mass spectrum, m/e (relative intensity) 247 (M^+, 75), 192 (51), 188 (89), 142 (62), 127 (71), 105 (100), 77 (73); mass spectrum, m/e 247.122 (M^+, calcd 247.121). Anal. Calcd for $C_{14}H_{17}NO_3$: C, 68.01; H, 6.93; N, 5.66. Found: C, 67.99; H, 6.88; N, 5.66.

Methyl 2-Benzamido-3-methylpent-4-enoate (5a). Treatment of **1** with tributyl(3-methylallyl)stannane (**5b**), as described above for the reaction of **1** with **3b**, gave **5a** in 57% yield based on **2**, as a 1:1 mixture of diastereomers: oil; ^1H NMR δ 1.150 and 1.152 (d and d, J = 7 Hz and J = 7 Hz, total 3 H), 2.78 and 2.90 (m and m, total 1 H), 3.767 and 3.780 (s and s, total 3 H), 4.82 and 4.85 (dd and dd, J = 5 and 8 Hz and J = 5 and 8 Hz, total 1 H), 5.13 (m, 2 H), 5.78 (m, 1 H), 6.52 and 6.65 (d and d, J = 8 Hz and J = 8 Hz, total 1 H), 7.35–7.90 (m, 5 H); mass spectrum, m/e (relative intensity) 247 (M^+, 21), 192 (45), 188 (25), 122 (52), 105 (100), 77 (40); exact mass calcd for $C_{14}H_{17}NO_3$ (M^+) 247.121, found 247.121.

Methyl (*E*)-2-Benzamido-5-methylpent-4-enoate (6a). Treatment of **1** with a 10-fold excess of a mixture (ca. 6:4) of tributyl(3-methylallyl)stannane (**5b**) and tributyl(1-methylallyl)stannane (**6b**), as described above for the reaction of **1** with **3b**, gave **6a** in 19% yield based on **2**: oil; ^1H NMR δ 1.67 (d, J = 7 Hz, 3 H), 2.59 (m, 2 H), 3.78 (s, 3 H), 4.85 (dt, J = 8 and 5 Hz, 1 H), 5.34 (dt, J = 15 and 7 Hz, 1 H), 5.59 (dq, J = 15 and 7 Hz, 1 H), 6.69 (d, J = 8 Hz, 1 H), 7.40–7.80 (m, 5 H); mass spectrum, m/e (relative intensity) 247 (M^+, 9), 192 (31), 188 (18), 122 (31), 105 (100), 77 (42); exact mass calcd for $C_{14}H_{17}NO_3$ (M^+) 247.121, found 247.121.

The reaction also gave **5a** in 5% yield.

Methyl 2-Benzamido-3,3-dimethylpent-4-enoate (7a). Treatment of **1** with (3,3-dimethylallyl)tributylstannane (**7b**), as described above for the reaction of **1** with **3b**, gave **7a** in 15% yield based on **2**: oil: ^1H NMR δ 1.16 (s, 6 H), 3.73 (s, 3 H), 4.70 (d, J = 9 Hz, 1 H), 5.20 (m, 2 H), 5.90 (m, 1 H), 6.70 (d, J = 9 Hz, 1 H), 7.40–8.00 (m, 5 H); mass spectrum, m/e (relative intensity) 202 ($M^+ - CO_2Me$, 5), 193 (10), 192 (9), 122 (22), 105 (100), 77 (97); mass spectrum (VG ZAB 2F mass spectrometer, operating in the positive ion fast-atom bombardment mode, with argon as the source gas and a primary beam energy of 8 kV), m/e 261 (M^+); exact mass calcd for $C_{13}H_{16}NO$ ($M^+ - CO_2Me$) 202.123, found 202.124.

***N*-Benzoyl-2-(cyclopent-2-enyl)glycine Methyl Ester (9a).** Treatment of **1** with tributyl(cyclopent-2-enyl)stannane (**9b**), as described above for the reaction of **1** with **3b**, gave **9a** in 37% yield based on **2**, as a mixture (ca. 3:1) of diastereomers: mp 91–93 °C; ^1H NMR δ 1.70–2.50 (m, 4 H), 3.34 (m, 0.25 × 1 H), 3.41 (m, 0.75 × 1 H), 3.77 (s, 0.75 × 3 H), 3.78 (s, 0.25 × 3 H), 4.90 (dd, J = 4 and 8 Hz, 0.75 × 1 H), 4.92 (m, 0.25 × 1 H), 5.59 (m, 0.75 × 1 H), 5.68 (m, 0.25 × 1 H), 5.86 (m, 0.25 × 1 H), 6.02 (m, 0.75 × 1 H), 6.46 (d, J = 8 Hz, 0.75 × 1 H), 6.59 (d, J = 8 Hz, 0.25 × 1 H), 7.40–7.80 (m, 5 H); mass spectrum, m/e (relative intensity) 200 ($M^+ - CO_2Me$, 6), 193 (45), 122 (23), 105 (100), 77 (97). Anal. Calcd for $C_{15}H_{17}NO_3$: C, 69.48; H, 6.61; N, 5.40. Found: C, 69.31; H, 6.53; N, 5.38.

The reaction also gave **2** in 19% yield.

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Registry No. 1, 101649-82-5; 2, 1205-08-9; 3a, 117290-14-9; 3b, 24850-33-7; 4a, 123642-88-6; 4b, 67883-62-9; 5a (diastereomer 1), 123642-89-7; 5a (diastereomer 2), 123642-90-0; (*E*)-5b, 35998-93-7; (*Z*)-5b, 35998-94-8; 6a, 123642-91-1; 6b, 76505-19-6; 7a, 123642-92-2; 7b, 53911-92-5; 9a (diastereomer 1), 123642-93-3; 9a (diastereomer 2), 123642-94-4; 9b, 58655-77-9; 10, 123642-95-5.

(14) Perrin, D. D.; Armarego, W. L. F.; Perrin, D. R. *Purification of Laboratory Chemicals*, 2nd ed.; Pergamon: Oxford, 1980.
(15) Seyferth, D.; Weiner, M. A. *J. Org. Chem.* 1961, *26*, 4797.
(16) Naruta, Y. *J. Am. Chem. Soc.* 1980, *102*, 3774.
(17) Schroer, U.; Neumann, W. P. *J. Organomet. Chem.* 1976, *105*, 183.
(18) Huang, H. T.; Niemann, C. *J. Am. Chem. Soc.* 1952, *74*, 4634.

0022-3263/90/1955-0386$02.50/0

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Synthesis of Novel Cross-Linked Amino Acid Derivatives

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Abstract

Reactions of the derivatives of cysteine (6), serine (7) and lysine (8a), with the bromide (1), afforded the corresponding cross-linked amino acid derivatives (11), (12) and (13). Of the linked species (11)–(13), the cysteine derivative (11) was found to be the most stable under a variety of conditions. A mixture of the cross-linked serine derivative (12) and the cysteine derivative (6) in chloroform containing triethylamine reacted to give the cross-linked cysteine derivative (11) and the serine derivative (7). Similarly, a mixture of (13) and (6) in chloroform reacted to give (11).

Introduction

Cross-linked amino acid derivatives are important in many areas of peptide and protein chemistry. For example, the introduction of cross-links between peptide strands of wool protein increases the strength of the fibre and reduces the tendency of wool to shrink on washing.[1] Cross-links serve to restrict the conformational freedom of peptides, and conformationally restricted peptides have been used in studies of the three-dimensional structure of peptides.[2] As a consequence of these and other uses of cross-linked amino acid derivatives, there is considerable interest in the development of methods for their synthesis.[1,3]

In a recent report[4] we described the reaction of *N*-benzoyl-2-bromoglycine methyl ester (1) with hexabutylditin, to give the dimeric glycine derivative (2), as a method for the cross-linking of glycine residues in small peptides. When the reaction was carried out without rigorous exclusion of moisture the diastereomers of (2) were formed in only modest yield, the major products being the diastereomers of the ether (3). A similar reaction of (4) with water to give (5) had been reported previously.[5]

[1] Hinton, E. H., *Text. Res. J.*, 1974, **44**, 233.
[2] Ovchinnikov, Y. A., and Ivanov, V. T., *Tetrahedron*, 1975, **31**, 2177; Berman, J. M., and Goodman, M., *Int. J. Pept. Protein Res.*, 1984, **23**, 610; Richman, S. J., Goodman, M., Nguyen, T. M.-D., and Schiller, P. W., *Int. J. Pept. Protein Res.*, 1985, **25**, 648; Nemethy, G., McQuie, J. R., Pottle, M. S., and Scheraga, H. A., *Macromolecules*, 1981, **14**, 975.
[3] Manesis, N. J., and Goodman, M., *J. Org. Chem.*, 1987, **52**, 5331.
[4] Burgess, V. A., Easton, C. J., Hay, M. P., and Steel, P. J., *Aust. J. Chem.*, 1988, **41**, 701.
[5] Malassa, I., and Matthies, D., *Justus Liebigs Ann. Chem.*, 1986, 1133.

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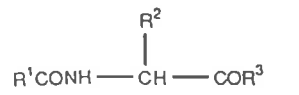
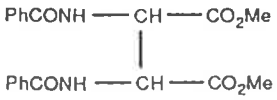
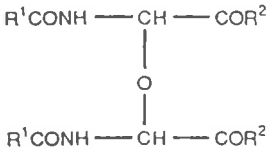
The observation that (3) and (5) are stable, crystalline species prompted us to investigate methods for the synthesis of related cross-linked amino acid derivatives. With this aim, reactions of the bromide (1) have been studied, with the amino acid derivatives (6), (7) and (8a), each having a nucleophilic residue incorporated in the side chain. Although reactions to produce hydroxy-, alkoxy-, amino-, amido- and alkylthio-substituted glycine derivatives have been



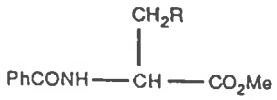
	R^1	R^2	R^3
(1)	Ph	Br	OMe
(4)	Me_3C	Cl	CMe_3



(2)



	R^1	R^2
(3)	Ph	OMe
(5)	CMe_3	CMe_3

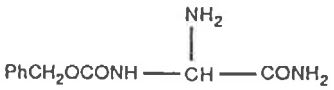


(6) R = SH

(7) R = OH

(8a) R = $(CH_2)_3NH_3^+Cl^-$

(8b) R = $(CH_2)_3NH_2$



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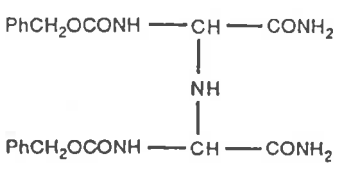
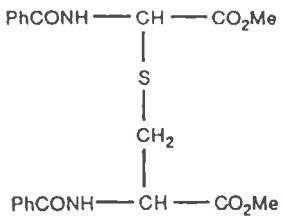


(10)



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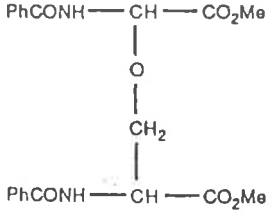
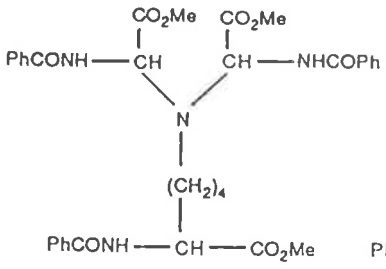
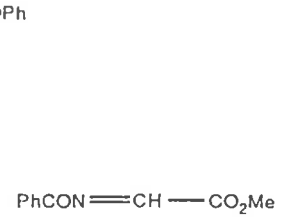


(12)



(13)



(14)

reported,[6–9] to the best of our knowledge there has been only one other report of the formation of a cross-linked amino acid derivative analogous to (3) and (5). In a preliminary communication,[9] on the synthesis of α-aminoglycine derivatives, it was reported, during the course of our work, that the amine dimer (10) was formed on attempted recrystallization of the aminoglycine derivative (9) from boiling ethanol.

Results and Discussion

When nitrogen was passed through a mixture of the bromide (1) and the cysteine derivative (6) (1 equiv.) in dichloromethane at room temperature, reaction occurred to give an 85% yield of a 1 : 1 mixture of the diastereomers of the cross-linked cysteine derivative (11). The diastereomers of (11) were separated by careful chromatography, and each was fully characterized. When the reaction was conducted without purging with nitrogen, the conversion into (11) was incomplete, and (1) and (6) remained in the reaction mixture. Presumably the bromide (1) exists in equilibrium with the *N*-acyl imine (14) and hydrogen bromide

$$(1) \rightleftharpoons (14) + HBr \qquad \text{(i)}$$

and the nitrogen serves to remove hydrogen bromide from the reaction mixture and displace the equilibrium in favour of (14). The *N*-acyl imine (14) then reacts with the cysteine derivative (6) to give the cross-linked cysteine derivative (11):

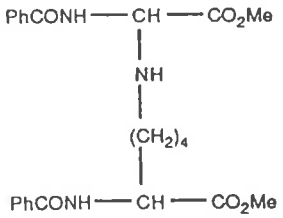
$$(14) + (6) \rightarrow (11) \qquad \text{(ii)}$$

When a mixture of the bromide (1) and the serine derivative (7) was purged with nitrogen, as described above for the preparation of (11), the reaction gave a 1 : 1 mixture of the diastereomers of the cross-linked serine derivative (12) in 43% yield. Unlike the diastereomers of (11) which recrystallized as a 1 : 1 mixture, the diastereomers of (12) were easily separated by fractional crystallization. Each of the diastereomers of (12) was fully characterized.

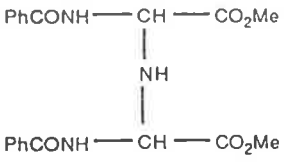
Dropwise addition of triethylamine to a mixture of the bromide (1) (0·5 equiv.) and the lysine derivative (8a) (0·25 equiv.), in chloroform at 0–5°, afforded a mixture of compounds. H.p.l.c. and ^{1}H n.m.r. spectroscopic analysis showed that the mixture consisted of approximately equal quantities of four components, although two of the components were only partly separated even by analytical h.p.l.c. By using preparative h.p.l.c. the mixture was resolved into two pure compounds and a fraction consisting of the other two components. Characterization of the three fractions showed that the four compounds produced in the reaction of (1) with (8a) were the diastereomers of

[6] Bernstein, Z., and Ben-Ishai, D., *Tetrahedron*, 1977, **33**, 881; Ben-Ishai, D., Altman, J., and Peled, N., *Tetrahedron*, 1977, **33**, 2715; Lidert, Z., and Gronowitz, S., *Synthesis*, 1980, 322; Kober, R., and Steglich, W., *Justus Liebigs Ann. Chem.*, 1983, 599.
[7] Zoller, U., and Ben-Ishai, D., *Tetrahedron*, 1975, **31**, 863.
[8] Easton, C. J., Scharfbillig, I. M., and Tan, E. W., *Tetrahedron Lett.*, 1988, **29**, 1565.
[9] Katritzky, A. R., Urogdi, L., and Mayence, A., *J. Chem. Soc., Chem. Commun.*, 1989, 337.

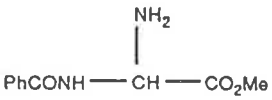
the cross-linked lysine derivative (13). There was a general similarity between the spectral characteristics of the three fractions. The mass spectrum (fast atom bombardment) of each fraction showed peaks at *m/z* 647 (M+1), 587 (M−CO_2Me) and 526 (M−PhCONH). Consistent with the structural assignments, the ^1H and ^{13}C n.m.r. spectra of each of the pure diastereomers of (13) showed that two identical glycine moieties and one lysine derivative were incorporated in each product. The fraction consisting of a mixture of two diastereomers of (13) showed the presence of two distinct types of glycine moiety, presumably one for each diastereomer.



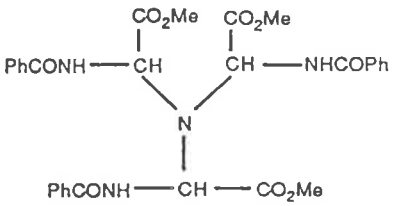
(15)



(16)



(17)



(18)

When the bromide (1) was allowed to react with 1 equiv. of the lysine derivative (8a), the products were the diastereomers of (13) which were formed in 40% yield. The secondary amine (15) was not detected in reaction mixtures. This curious preference for the production of (13) instead of (15) prompted us to examine the reaction of the bromide (1) with ammonia. Treatment of (1) with ammonia, in dichloromethane at room temperature, afforded a 1:1 mixture of the diastereomers of the secondary amine (16). The diastereomers of (16) were separated by chromatography on silica. For each diastereomer of (16) the mass spectrum (fast atom bombardment) showed peaks at *m/z* 400 (M+1) and 279 (M−PhCONH), the ^{13}C n.m.r. spectrum confirmed the presence of only eight distinct carbons, and the ^1H n.m.r. spectrum showed the presence of only one type of glycine moiety. The diastereomer of (16) with m.p. 158·5–159·5° was shown to be racemic by resolution into two components upon h.p.l.c. analysis on a column with L-phenylglycine as the stationary phase. The compound with m.p. 175–177° was not resolved by h.p.l.c. analysis, consistent with assignment as the *meso*-diastereomer (16).

Although it seems likely that the reaction of (1) with ammonia to give (16) involves the initial formation of (17), and subsequent reaction of (16) with (1) to give (18) might have been expected on the basis of the reaction of (1) with the lysine derivative (8a), neither (17) nor (18) was detected in the reaction of (1) with ammonia. Clearly there is a strong preference for the formation of the secondary amine (16), as indicated by the fact that the total yield of the diastereomers of (16) was 70%, after separation by chromatography.

The above reactions of (1) with the derivatives of cysteine (6), serine (7) and lysine (8a), to produce (11), (12) and (13), respectively, exemplify procedures for the formation of novel cross-linked amino acid derivatives. Since the stability of linked species will affect their performance characteristics, experiments were carried out to determine the stability of (11)–(13) under a variety of conditions. Some of the experiments were carried out with diastereomeric mixtures of the cross-linked amino acid derivatives (11)–(13), while others were conducted with only one diastereomer. In the latter experiments the cross-linked cysteine derivative (11a) with m.p. 176–179°, the serine derivative (12a) with m.p. 139–140°, and the lysine derivative (13b) with m.p. 61–62° were used.

The cross-linked derivatives of both cysteine (11a) and serine (12a) were stable in chloroform, or in chloroform containing 10 mM hydrogen chloride. In the presence of triethylamine, however, both (11a) and (12a) isomerized slowly without decomposition to give a 1 : 1 mixture of diastereomers. The cross-linked lysine derivative (13) was stable in chloroform, but decomposed in chloroform containing 10 mM hydrogen chloride.

The effect of nucleophiles on the integrity of the linked species (11)–(13) was also examined. There was no reaction when the cross-linked cysteine derivative (11) was mixed with the serine derivative (7) in chloroform, or in chloroform containing hydrogen chloride or triethylamine. Similarly, the integrity of the cross-linked cysteine derivative (11) was maintained in chloroform in the presence of a mixture of the lysine derivative (8a) and triethylamine. The cross-linked lysine derivative (13b) reacted with the cysteine derivative (6) in chloroform to give (11). A mixture of the cross-linked serine derivative (12) and the cysteine derivative (6) was stable in chloroform or in chloroform containing hydrogen chloride, but in chloroform containing triethylamine the mixture reacted to give the cross-linked cysteine derivative (11) and the serine derivative (7). When the bromide (1) was treated with a 1 : 1 mixture of the cysteine derivative (6) and the serine derivative (7), the cross-linked cysteine derivative (11) was produced but none of the linked serine derivative (12) was formed. This indicates that the production of (11) is faster than the production of (12), since subsequent reaction of (12) to give (11) would not be expected to occur under these reaction conditions.

The reactions of the cross-linked derivatives of serine (12) and lysine (13b) with the cysteine derivative (6), to give (11), may be rationalized as follows:

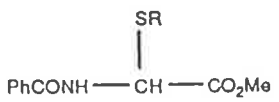
$$(12) \rightarrow (14) + (7) \qquad \text{(iii)}$$

$$(13b) \rightarrow 2(14) + (8b) \qquad \text{(iv)}$$

$$(14) + (6) \rightarrow (11) \qquad \text{(v)}$$

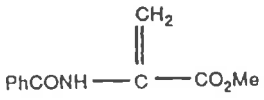
The linked species (12) and (13b) dissociate to give (7) and (8b), respectively, together with the *N*-acyl imine (14) [equations (iii) and (iv)]. Subsequent reaction of (14) with (6) affords the cross-linked cysteine derivative (11) [equation (v)]. Presumably the isomerization of (12a) in the presence of triethylamine, to give a mixture of diastereomers, also involves formation of the *N*-acyl imine (14).

Treatment of the cross-linked cysteine derivative (11a) with ethanethiol and triethylamine in chloroform resulted in the production of a mixture of diastereomers of (11), but none of the thioether (19) was formed. Similar treatment of (19) with the cysteine derivative (6) and triethylamine gave only starting materials. The cross-linked cysteine derivative (11) was treated with butyllithium and methyl iodide to give the dehydroalanine derivative (20) and the thioether (21), in 42 and 78% yields, respectively. When (11) was treated with sodium benzenethiolate and methyl iodide the dehydroalanine derivative (20) was produced in 8% yield and the lanthionine derivative (22) was formed in 14% yield. Other products of the reaction could not be isolated or identified.

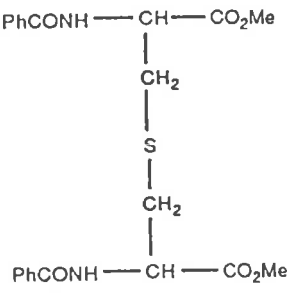


(19) R = Et

(21) R = Me



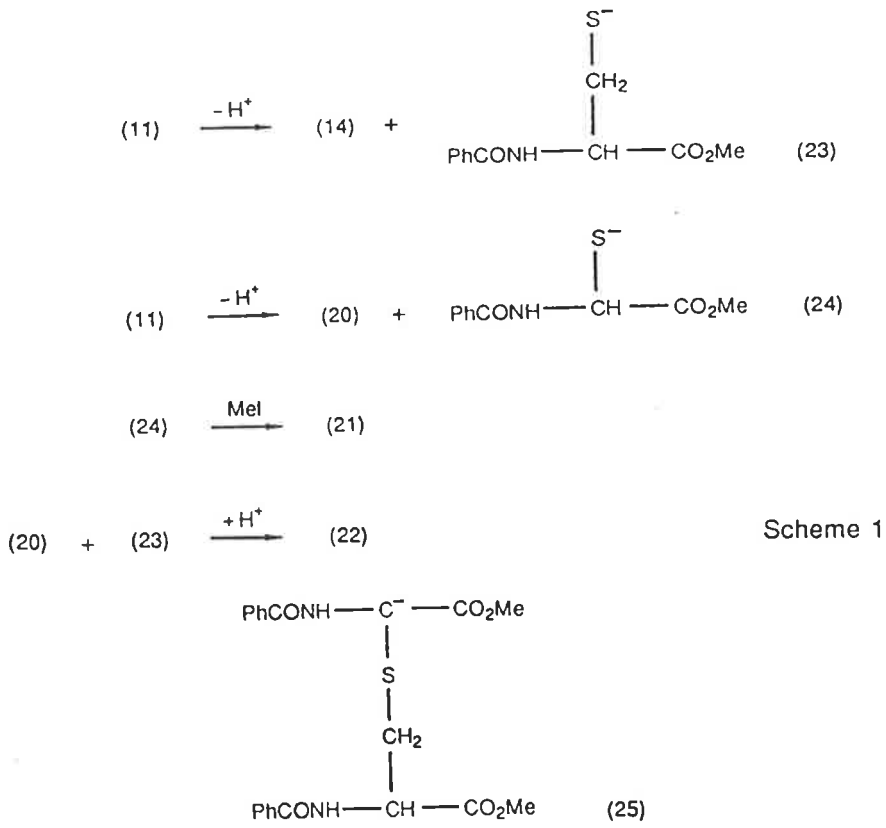
(20)



(22)

The production of (20)–(22) in these reactions may be rationalized as shown in Scheme 1. From these reactions it is evident that the cross-linked cysteine derivative (11) reacts with bases to produce the *N*-acyl imine (14) and the anions (23) and (24). It seems unlikely, however, that (11) dissociates in the presence of triethylamine to give (14) and (23), or (24), otherwise interconversion between (11) and ethanethiol and (19) and (6) would have been expected. On this basis it seems likely that the isomerization of (11a) in the presence of triethylamine, to give a mixture of diastereomers, occurs by deprotonation of (11a) to give (25).

In summary, reactions of the bromide (1) with the derivatives of cysteine (6), serine (7) and lysine (8a) produce the corresponding cross-linked amino acid derivatives (11), (12) and (13). Of the linked species (11)–(13), the cysteine derivative (11) is the most stable, under a variety of conditions, and the most easily formed. Under basic conditions, however, even the cysteine derivative (11) is susceptible to reaction. The reactions described above illustrate methods for the synthesis of novel cross-linked amino acid derivatives, and we expect that these methods will be suitable for the cross-linking of small peptides,



Scheme 1

when used in conjunction with procedures that have been reported for the selective bromination of glycine derivatives.[8,10]

Experimental

Melting points are uncorrected. Solvents were purified and dried by standard procedures. Light petroleum refers to the fraction with b.p. 66–68°. ^{1}H n.m.r. spectra were recorded on a Varian T-60, Jeol JNM-PMX 60 or Bruker CXP-300 spectrometer. ^{13}C n.m.r. spectra were recorded on either a Bruker WP-80 or a Bruker CXP-300 spectrometer. N.m.r. spectra were recorded as dilute solutions in (D)chloroform, with tetramethylsilane as internal standard. Infrared spectra were recorded on either a Jasco A-102 or a Hitachi 270-30 spectrometer. Electron impact mass spectra were recorded on an AEI MS-3010 spectrometer. Fast atom bombardment mass spectra were recorded on a Vacuum Generators ZAB 2HF spectrometer. H.p.l.c. was carried out by using a Waters 501 solvent delivery system and a U6K injector with a Waters 481 absorbance detector. Analyses were performed by using either a Waters Z-module with a μ-Porasil Radial-Pak cartridge (10 cm by 8 mm) (column 1), with light petroleum/ethyl acetate as eluent, or a Regis Pirkle covalent L-phenylglycine column (25 cm by 4·6 mm) (column 2), with light petroleum/propan-2-ol as eluent. Chromatography was carried out on a Chromatotron 7924T (Harrison Research, Palo Alto/TC Research, Norwich) by using Merck silica gel 60 PF_{254}, eluting with a gradient of light petroleum/ethyl acetate, except where stated otherwise. Microanalyses were performed by the Canadian Microanalytical Service Ltd, New Westminster, Canada.

[10] Easton, C. J., and Hay, M. P., *J. Chem. Soc., Chem. Commun.*, 1986, 55; Burgess, V. A., Easton, C. J., and Hay, M. P., *J. Am. Chem. Soc.*, 1989, **111**, 1047.

The bromide (1)[11] and the derivatives of cysteine (6),[12] serine (7)[13] and lysine (8a)[14] were obtained as previously described.

Dimethyl 2,5-Dibenzamido-3-thiahexanedioate (11)

A stream of nitrogen was passed through a solution of *N*-benzoylcysteine methyl ester (6) (280 mg, 1·2 mmol) and *N*-benzoyl-2-bromoglycine methyl ester (1) (320 mg, 1·2 mmol) in dichloromethane (35 ml), at room temperature for 4 h. The mixture was concentrated, and chromatographed on silica to give a 1:1 mixture of diastereomers of the product (11) (440 mg, 85%) (Found: C, 58·5; H, 5·0; N, 6·5. $C_{21}H_{22}N_2O_6S$ requires C, 58·6; H, 5·2; N, 6·5%). The isomers were separated by further chromatography, eluting with a gradient of dichloromethane/ethyl acetate.

One diastereomer (11a) recrystallized as colourless *needles* from acetone/light petroleum, m.p. 176–179° (Found: C, 58·3; H, 5·2; N, 6·4. $C_{21}H_{22}N_2O_6S$ requires C, 58·6; H, 5·2; N, 6·5%). ν_{max} 3250, 1740, 1635, 1520 cm^{-1}. ^1H n.m.r. δ 3·36, d, J 5 Hz, 2H; 3·77, s, 3H; 3·82, s, 3H; 5·23, dt, J 5, 8 Hz, 1H; 5·75, d, J 8 Hz, 1H; 7·1–8·0, m, 12H. Mass spectrum (electron impact): *m/z* 430 (0·1%), 325 (1), 238 (17), 192 (18), 105 (62), 77 (100).

The other diastereomer (11b) recrystallized from ethyl acetate/light petroleum as colourless *needles*, m.p. 151–155° (Found: C, 58·7; H, 4·9; N, 6·4. $C_{21}H_{22}N_2O_6S$ requires C, 58·6; H, 5·2; N, 6·5%). ν_{max} 3260, 1730, 1630, 1510 cm^{-1}. ^1H n.m.r. δ 3·29, dd, J 6, 15 Hz, 1H; 3·37, dd, J 4, 15 Hz, 1H; 3·84, s, 3H; 3·88, s, 3H; 5·13, m, 1H; 5·61, d, J 7 Hz, 1H; 7·1–7·9, m, 12H. Mass spectrum (fast atom bombardment): *m/z* 431 (19%), 105 (100), 77 (38).

Dimethyl 2,5-Dibenzamido-3-oxahexanedioate (12)

A stream of nitrogen was passed through a mixture of *N*-benzoylserine methyl ester (7) (328 mg, 1·5 mmol) and *N*-benzoyl-2-bromoglycine methyl ester (1) (400 mg, 1·5 mmol) in dichloromethane (10 ml), at room temperature for 6 h. The mixture was concentrated, and chromatographed on silica to give a 1:1 mixture of diastereomers of the product (12) (260 mg, 43%) (Found: C, 60·1; H, 5·3; N, 6·6. $C_{21}H_{22}N_2O_7$ requires C, 60·9; H, 5·4; N, 6·8%). The isomers were separated by fractional recrystallization.

One diastereomer (12a) recrystallized from benzene/ether as colourless *needles*, m.p. 139–140° (Found: C, 61·0; H, 5·3; N, 6·8. $C_{21}H_{22}N_2O_7$ requires C, 60·9; H, 5·4; N, 6·8%). ν_{max} 3250, 1750, 1670, 1630, 1540, 1520 cm^{-1}. ^1H n.m.r. δ 3·75, s, 3H; 3·82, s, 3H; 4·13, dd, J 3, 10·5 Hz, 1H; 4·42, dd, J 3, 10·5 Hz, 1H; 4·99, m, 1H; 5·71, d, J 8 Hz, 1H; 7·1–8·0, m, 12H. Mass spectrum (electron impact): *m/z* 384 (1·5%), 205 (5), 173 (8), 146 (8), 105 (100), 77 (69).

Concentration of the mother liquor followed by recrystallization from ethyl acetate/light petroleum gave the other diastereomer (12b) as colourless *needles*, m.p. 141–142° (Found: C, 60·7; H, 5·3; N, 6·7. $C_{21}H_{22}N_2O_7$ requires C, 60·9; H, 5·4; N, 6·8%). ν_{max} 3250, 1755, 1740, 1645, 1635, 1545, 1525 cm^{-1}. ^1H n.m.r. δ 3·75, s, 3H; 3·83, s, 3H; 4·19, dd, J 3, 10 Hz, 1H; 4·35, dd, J 4, 10 Hz, 1H; 4·98, m, 1H; 5·79, d, J 8 Hz, 1H; 7·1–8·0, m, 12H. Mass spectrum (electron impact): *m/z* 279 (28%), 149 (90), 105 (100), 77 (44).

(8S)-Dimethyl 2,8-Dibenzamido-3-[benzamido(methoxycarbonyl)methyl]-3-azanonanedioate (13)

Triethylamine (1·0 g, 10 mmol) was added dropwise over 0·5 h to a solution of the hydrochloride (8a) of (2*S*)-N^{α}-benzoyllysine methyl ester (0·75 g, 2·5 mmol) and *N*-benzoyl-2-bromoglycine methyl ester (1) (1·4 g, 5·1 mmol) in chloroform (30 ml), maintained at 0–5°. After 1 h the reaction mixture was washed with dilute aqueous hydrochloric acid (3×20 ml) and water (20 ml); then it was dried ($MgSO_4$), concentrated, and chromatographed on silica to give a mixture of approximately equal quantities of the four diastereomers of the product

[11] Kober, R., Papadopoulos, K., Miltz, W., Enders, D., Steglich, W., Reuter, H., and Puff, H., *Tetrahedron*, 1985, **41**, 1693.
[12] Martin, T. A., *J. Med. Chem.*, 1969, **12**, 950.
[13] Inui, T., Tanaka, S., and Takino, M., *Bull. Chem. Soc. Jpn*, 1970, **43**, 1582.
[14] Izumiya, N., Okazaki, H., Matsumoto, I., and Takiguchi, H., *J. Biochem.*, 1959, **10**, 1347.

(13) (1·0 g, 62%), as determined by h.p.l.c. analysis (column 1). The mixture was separated into three components by h.p.l.c. (column 1).

The first component consisted of a mixture of two diastereomers of the product (13a), and was obtained as a colourless *oil* which solidified on standing. ν_{max} 3432, 1740, 1658, 1602, 1580, 1516 cm^{-1}. ^1H n.m.r. δ 1·4–2·1, m, 6H; 2·94, t, J 7 Hz, 2H; 3·64, s, 3H; 3·66, s, 3H; 3·75, s, 3H; 4·78, dt, J 5, 8 Hz, 1H; 5·67, d, J 8 Hz, 1H; 5·71, d, J 8 Hz, 1H; 7·02, br d, J 8 Hz, 2H; 7·2–8·0, m, 16H. Mass spectrum (fast atom bombardment): *m/z* 647 (10%), 587 (32), 526 (20), 456 (100), 454 (40).

The second component was a single diastereomer of the product (13b), and was obtained as a colourless *solid*, m.p. 61–62° [Found: *m/z* 587·2526. $C_{32}H_{35}N_4O_7$ ($M^{+\bullet} - CO_2Me$) requires *m/z* 587·2505]. ν_{max} 3434, 1743, 1658, 1602, 1580, 1517 cm^{-1}. ^1H n.m.r. δ 1·2–2·1, m, 6H; 2·76, t, J 7 Hz, 2H; 3·73, s, 3H; 3·78, s, 6H; 4·75, dt, J 5, 8 Hz, 1H; 5·74, d, J 8 Hz, 2H; 6·86, br d, J 8 Hz, 1H; 7·3–8·3, m, 17H. ^{13}C n.m.r. δ 22·44, 27·25, 32·03, 45·62, 52·41, 52·81, 64·58, 127·02, 127·34, 128·44, 131·66, 132·00, 132·84, 133·75, 167·00, 167·66, 170·04, 172·88. Mass spectrum (fast atom bombardment): *m/z* 647 (40%), 587 (45), 526 (35), 456 (100), 454 (45).

The third component was a single diastereomer of the product (13c), and was obtained as a colourless *oil* which solidified on standing. ν_{max} 3432, 1739, 1655, 1602, 1581, 1517 cm^{-1}. ^1H n.m.r. δ 1·3–2·1, m, 6H; 2·77, t, J 7 Hz, 2H; 3·75, s, 3H; 3·80, s, 6H; 4·78, dt, J 5, 8 Hz, 1H; 5·76, d, J 8 Hz, 2H; 6·83, br d, J 8 Hz, 1H; 7·3–8·3, m, 17H. ^{13}C n.m.r. δ 22·46, 27·32, 32·22, 45·97, 52·48, 53·00, 64·71, 127·15, 127·46, 128·57, 131·68, 132·10, 133·03, 133·89, 167·06, 167·77, 170·30, 173·08. Mass spectrum (fast atom bombardment): *m/z* 647 (100%), 587 (44), 526 (59), 546 (65), 544 (53). The products (13a,c) were not sufficiently stable for microanalysis, and electron impact mass spectrometry failed to give molecular or fragment ions suitable for accurate mass determination.

Dimethyl 2,4-Dibenzamido-3-azapentanedioate (16)

A stream of ammonia was passed through a solution of *N*-benzoyl-2-bromoglycine methyl ester (1) (0·4 g, 1·5 mmol) in dichloromethane (10 ml), at room temperature for 0·1 h. The reaction mixture was washed with dilute aqueous hydrochloric acid (3x20 ml) and water (2x20 ml); then it was dried ($MgSO_4$), concentrated, and chromatographed on silica to give diastereomers of the product (16).

The (±)-diastereomer (16a) recrystallized from ethyl acetate/light petroleum as colourless *needles* (0·11 g, 37%), m.p. 158·5–159·5° (Found: C, 59·6; H, 4·7; N, 10·3. $C_{20}H_{21}N_3O_6$ requires C, 60·1; H, 5·3; N, 10·5%). ν_{max} 3434, 1750, 1661, 1602, 1581, 1515 cm^{-1}. ^1H n.m.r. δ 2·5, br, 1H; 3·80, s, 6H; 5·39, dd, J 7, 7 Hz, 2H; 7·3–7·6, m, 6H; 7·68, br d, J 7 Hz, 2H; 7·8–7·9, m, 4H. ^{13}C n.m.r. δ 53·20, 62·13, 127·28, 128·67, 132·20, 132·90, 167·71, 169·72. Mass spectrum (fast atom bombardment): *m/z* 400 (10%), 279 (80), 105 (100). H.p.l.c. analysis (column 2) resolved the material into two components.

The *meso*-diastereomer (16b) recrystallized from acetone/light petroleum as colourless *needles* (0·10 g, 33%), m.p. 175–177° (Found: C, 59·6; H, 5·3; N, 10·3. $C_{20}H_{21}N_3O_6$ requires C, 60·1; H, 5·3; N, 10·5%). ν_{max} 3436, 1747, 1662, 1602, 1581, 1513 cm^{-1}. ^1H n.m.r. δ 2·4, br, 1H; 3·71, s, 6H; 5·68, dd, J 6, 8 Hz, 2H; 7·3–7·6, m, 8H; 7·8–7·9, m, 4H. ^{13}C n.m.r. δ 53·19, 61·56, 127·27, 128·71, 132·26, 133·04, 167·52, 170·10. Mass spectrum (fast atom bombardment): *m/z* 400 (27%), 279 (100). H.p.l.c. analysis (column 2) showed only one component.

Treatment of Dimethyl 2,5-Dibenzamido-3-thiahexanedioate (11a) with Triethylamine

Treatment of dimethyl 2,5-dibenzamido-3-thiahexanedioate (11a) (50 mg, 0·12 mmol) with triethylamine (0·2 g, 2 mmol), in chloroform (2 ml) at room temperature for 48 h, gave a 1:1 mixture of diastereomers of (11), as shown by comparison with the sample obtained as described above.

Treatment of Dimethyl 2,5-Dibenzamido-3-oxahexanedioate (12a) with Triethylamine

Treatment of dimethyl 2,5-dibenzamido-3-oxahexanedioate (12a) (20 mg, 48 mmol) with triethylamine (0·3 g, 3 mmol), in refluxing chloroform (3 ml) for 15 h, gave a 1:1 mixture of diastereomers of (12), as shown by comparison with the sample obtained as described above.

Treatment of (8S)-Dimethyl 2,8-Dibenzamido-3-[benzamido(methoxycarbonyl)methyl]-3-azanonanedioate (13) with Hydrogen Chloride in Chloroform

Treatment of (8*S*)-dimethyl 2,8-dibenzamido-3-[benzamido(methoxycarbonyl)methyl]-3-azanonanedioate (13) (35 mg, 0·05 mmol) with 10 mM hydrogen chloride in chloroform (10 ml), at room temperature for 48 h, resulted in the complete decomposition of (13), as shown by h.p.l.c. and ^{1}H n.m.r. spectroscopic analysis.

Reaction of Dimethyl 2,5-Dibenzamido-3-oxahexanedioate (12) with N-Benzoylcysteine Methyl Ester (6)

A mixture of dimethyl 2,5-dibenzamido-3-oxahexanedioate (12) (50 mg, 0·12 mmol), *N*-benzoylcysteine methyl ester (6) (29 mg, 0·12 mmol) and triethylamine (0·5 g, 5 mmol), in chloroform (5 ml), was heated at reflux for 15 h to give dimethyl 2,5-dibenzamido-3-thiahexanedioate (11) and *N*-benzoylserine methyl ester (7), as shown by comparison with samples obtained as described above. Analysis of the product mixture by h.p.l.c. and ^{1}H n.m.r. spectroscopy showed neither (12) nor (6) remained.

Reaction of (8S)-Dimethyl 2,8-Dibenzamido-3-[benzamido(methoxycarbonyl)methyl]-3-azanonanedioate (13b) with N-Benzoylcysteine Methyl Ester (6)

A mixture of (8*S*)-dimethyl 2,8-dibenzamido-3-[benzamido(methoxycarbonyl)methyl]-3-azanonanedioate (13b) (6 mg, 9 μmol) and *N*-benzoylcysteine methyl ester (6) (20 mg, 84 μmol) in chloroform (1 ml) was heated at reflux for 48 h to give dimethyl 2,5-dibenzamido-3-thiahexanedioate (11), as shown by comparison with the sample obtained as described above. Analysis of the product mixture by h.p.l.c. and ^{1}H n.m.r. spectroscopy showed that no (13b) remained, although (6) was detected.

Reaction of N-Benzoyl-2-bromoglycine Methyl Ester (1) with a Mixture of N-Benzoylcysteine Methyl Ester (6) and N-Benzoylserine Methyl Ester (7)

A stream of nitrogen was passed through a mixture of *N*-benzoyl-2-bromoglycine methyl ester (1) (50 mg, 0·18 mmol), *N*-benzoylcysteine methyl ester (6) (44 mg, 0·18 mmol) and *N*-benzoylserine methyl ester (7) (41 mg, 0·18 mmol) in dichloromethane (10 ml), at room temperature for 0·3 h. The reaction mixture was washed with dilute sodium bicarbonate solution (3×20 ml) and water (2×20 ml), then it was dried ($MgSO_4$) and concentrated. H.p.l.c. and ^{1}H n.m.r. spectroscopic analysis of the crude reaction mixture showed the presence of dimethyl 2,5-dibenzamido-3-thiahexanedioate (11) and (7), but neither dimethyl 2,5-dibenzamido-3-oxahexanedioate (12) nor (6) was detected. Chromatography of the product mixture on silica gave (11) (60 mg, 78%) as a 1:1 mixture of diastereomers, identical in all respects with the sample obtained as described above.

N-Benzoyl-2-ethylthioglycine Methyl Ester (19)

A solution of *N*-benzoyl-2-bromoglycine methyl ester (1) (2·0 g, 7·4 mmol) in chloroform (10 ml) was added dropwise over 0·2 h to a solution of ethanethiol (2·7 ml, 37 mmol) and triethylamine (2·5 g, 25 mmol) in chloroform (25 ml), maintained at 0–5°. The mixture was washed with dilute aqueous hydrochloric acid (3×30 ml) and water (50 ml), then it was dried ($MgSO_4$) and concentrated. Chromatography of the residue on silica gave the product (19), as colourless *needles* from ethyl acetate/light petroleum (1·6 g, 85%), m.p. 81–82° (Found: C, 57·0; H, 6·0; N, 5·5. $C_{12}H_{15}NO_3S$ requires C, 56·9; H, 6·0; N, 5·5%). ν_{max} 3348, 1736, 1650, 1580, 1522 cm^{-1}. ^{1}H n.m.r. δ 1·32, t, *J* 6 Hz, 3H; 2·78, q, *J* 6 Hz, 2H; 3·83, s, 3H; 5·75, d, *J* 8 Hz, 1H; 6·9, br, 1H; 7·1–7·9, m, 5H. ^{13}C n.m.r. δ 14·57, 25·55, 53·13, 53·77, 127·14, 128·69, 132·13, 133·23, 166·22, 169·85. Mass spectrum (electron impact): *m/z* 193 (6%), 192 (2), 148 (4), 105 (100), 77 (40).

Treatment of Dimethyl 2,5-Dibenzamido-3-thiahexanedioate (11a) with Ethanethiol

A mixture of dimethyl 2,5-dibenzamido-3-thiahexanedioate (11a) (25 mg, 0·06 mmol), ethanethiol (40 mg, 0·6 mmol) and triethylamine (0·2 g, 2 mmol), in chloroform (2 ml), was stirred at room temperature for 48 h to give a 1:1 mixture of diastereomers of (11), as shown

by comparison with samples obtained as described above. *N*-Benzoyl-2-ethylthioglycine methyl ester (19) was not detected in the crude product mixture.

Treatment of N-*Benzoyl-2-ethylthioglycine Methyl Ester (19) with* N-*Benzoylcysteine Methyl Ester (6)*

A mixture of *N*-benzoyl-2-ethylthioglycine methyl ester (19) (90 mg, 0·36 mmol), *N*-benzoylcysteine methyl ester (6) (86 mg, 0·36 mmol) and triethylamine (1·0 g, 10 mmol) in chloroform (10 ml), stirred at room temperature for 24 h, afforded only starting materials. Some decomposition of (6) was evident, however, from t.l.c., h.p.l.c. and ^{1}H n.m.r. spectroscopic analysis of the reaction mixture.

Reaction of Dimethyl 2,5-Dibenzamido-3-thiahexanedioate (11) with Butyllithium and Methyl Iodide

Butyllithium (2·5 M in hexane, 0·18 ml, 0·46 mmol) was added dropwise to a solution of dimethyl 2,5-dibenzamido-3-thiahexanedioate (11) (0·2 g, 0·46 mmol) and methyl iodide (0·29 ml, 4·6 mmol) in tetrahydrofuran (30 ml), cooled to 0–5°. Dichloromethane (20 ml) was added and the mixture was washed with dilute hydrochloric acid (3×20 ml) and water (20 ml); then it was dried ($MgSO_4$) and concentrated. The residue was chromatographed on silica to give *N*-benzoyl-2-methylthioglycine methyl ester (21), as colourless crystals from ethyl acetate/light petroleum (86 mg, 78%), m.p. 79–80° (lit.[7] 75–76°). ^{1}H n.m.r. δ 2·26, s, 3H; 3·87, s, 3H; 5·75, d, *J* 8 Hz, 1H; 7·05, br, 1H; 7·45–7·85, m, 5H.

Further chromatography afforded methyl 2-benzamidopropenoate (20) as an oil (40 mg, 42%), with spectral properties consistent with those previously reported.[15] ^{1}H n.m.r. δ 3·90, s, 3H; 6·00, d, *J* 1 Hz, 1H; 6·81, s, 1H; 7·3–8·0, m, 5H; 8·5, br, 1H.

Reaction of Dimethyl 2,5-Dibenzamido-3-thiahexanedioate (11) with Sodium Benzenethiolate and Methyl Iodide

A solution of dimethyl 2,5-dibenzamido-3-thiahexanedioate (11) (0·2 g, 0·47 mmol) in dry tetrahydrofuran (10 ml) was added dropwise at room temperature to a solution of sodium benzenethiolate [prepared *in situ* from sodium (11 mg, 0·47 mmol) and benzenethiol (0·048 ml, 0·47 mmol)] in dry tetrahydrofuran (10 ml). Methyl iodide (0·029 ml, 0·47 mmol) was then added. After 0·1 h, dilute aqueous hydrochloric acid (5 ml) and carbon tetrachloride (10 ml) were added, the layers were separated, and the aqueous layer was extracted with carbon tetrachloride (3×10 ml). The combined organic fractions were dried ($MgSO_4$), concentrated, and chromatographed on silica to give methyl 2-benzamidopropenoate (20) (8 mg, 8%), identical in all respects with the sample obtained as described above.

Further chromatography afforded dimethyl 2,6-dibenzamido-4-thiaheptanedioate (22) as colourless crystals (29 mg, 14%), m.p. 153–155°. ν_{max} 3438, 3022, 2954, 1744, 1663, 1581, 1512, 1440 cm^{-1}. ^{1}H n.m.r. δ 3·12, dd, *J* 5, 14 Hz, 2H; 3·24, dd, *J* 5, 14 Hz, 2H; 3·80, s, 6H; 5·02, td, *J* 5, 7 Hz, 2H; 7·07, br d, *J* 7 Hz, 2H; 7·4–7·8, m, 10H. Mass spectrum (fast atom bombardment): *m/z* 445 (6%), 369 (2), 277 (7), 185 (100), 105 (12), 77 (6). The product (22) was not sufficiently stable for microanalysis, and electron impact mass spectrometry failed to give a molecular or fragment ions suitable for accurate mass determination.

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[15] Morgenstern, A. P., Schutij, C., and Nauta, W. T., *J. Chem. Soc., Chem. Commun.*, 1969, 321; Brown, A. G., and Smale, T. C., *J. Chem.. Soc., Chem. Commun.*, 1969, 1489.

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Acyloxylation at the 4-Position of Azetidin-2-ones

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The copper-catalysed reaction of azetidin-2-ones with t-butyl perbenzoate or peracetate affords the corresponding 4-benzoyloxy- and acetoxy-substituted β-lactams, respectively. *N*-Unsubstituted 4-acyloxyazetidinones can be synthesized by dearylation of the *N*-(4-methoxyphenyl)-substituted products with ceric ammonium nitrate. Acyloxylation of β-lactams that are monosubstituted at C-3 affords predominantly *trans*-products.

4-Acetoxy- and benzoyloxy-substituted azetidin-2-ones have been used extensively in organic synthesis, particularly as precursors of β-lactam antibiotics.[1–6] Consequently, there is considerable interest in the development of methods for the synthesis of compounds of this type. Here[7] we describe a method for the synthesis of 4-acyloxy-substituted azetidinones through the copper-catalysed reaction of β-lactams with t-butyl peresters.

The only alternative procedure that has been reported for the direct acyloxylation of β-lactams at the 4-position involves the electrochemical oxidation of azetidin-2-ones in acetic acid–acetonitrile.[8] This latter method is an extension of previous work on the introduction of alkoxy substituents through electrolysis of β-lactams.[9,10] It has also been reported that oxidation of *N*-hydroxyazetidines with lead tetra-acetate gives the corresponding 1,4-diacetoxyazetidinones, but this process is thought to occur by 1,4-addition of the oxidizing agent to intermediate nitrones, not by direct substitution of β-lactams.[11]

Results and Discussion

The β-lactams (**3a**—**g**) used in this work were obtained as shown in the Scheme. The substituted propionamides (**2a**—**f**), prepared from the corresponding propionic acid derivatives (**1a**—**f**), cyclized on treatment with sodium hydride to give the corresponding β-lactams (**3a**—**f**).[12] The β-lactam (**3g**) was prepared from *N*-phthaloylserine (**1g**) *via* the amide (**2g**).[13]

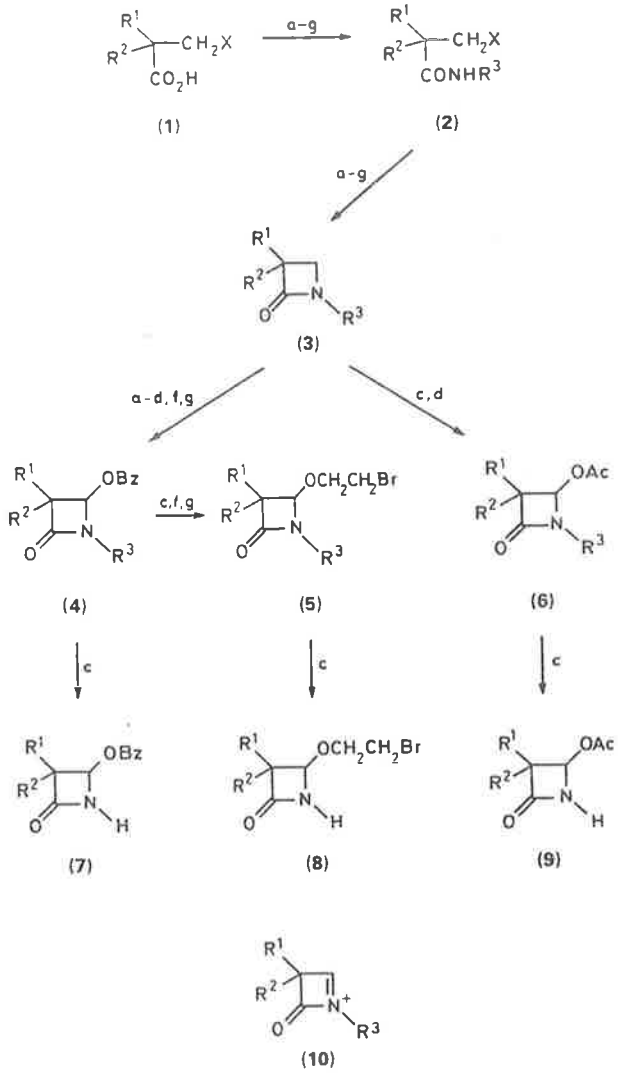
Reaction of 1-t-butylazetidin-2-one (**3a**) with t-butyl perbenzoate (*ca.* 4 equiv.) in the presence of cupric octanoate (0.025 equiv.) afforded, after chromatography of the product mixture on silica, the 4-benzoyloxy-substituted β-lactam (**4a**) in 59% yield, together with 39% unchanged (**3a**). Similar treatment of the 1-phenyl-substituted azetidinone (**3b**) gave the benzoate (**4b**) in 54% yield, with 27% unchanged (**3b**), while reaction of the 1-(*p*-methoxyphenyl)-substituted β-lactam (**3c**) afforded (**4c**) in 46% yield, together with 50% unchanged (**3c**). When larger molar excesses of t-butyl perbenzoate were used in reactions with the lactams (**3a**—**c**), lower yields of the corresponding benzoates (**4a**—**c**) were obtained. This is consistent with the observation that there was some decomposition of the benzoates (**4a**—**c**) under the reaction conditions used in their production.

The major features of the mechanism of reactions involving t-butyl perbenzoate have been elucidated.[14] Formation of (**4a**—**c**) may be attributed to hydrogen-atom transfer from the corresponding β-lactams (**3a**—**c**) to t-butoxy radical, followed by benzoate incorporation at the site of hydrogen abstraction. Clearly the methylene at the 4-position in each of the lactams (**3a**—**c**) is more reactive than the corresponding 3-methylene towards hydrogen transfer, presumably because of the activating effect of adjacent amide nitrogen.[15] For each of the lactams (**4a**—**c**), the ^{1}H n.m.r. spectrum shows unambiguously that the benzoyloxy substituent has been incorporated at the 4-position. The geminal coupling constants of 14, 16, and 16 Hz in the spectra of (**4a**—**c**), respectively, are consistent with methylene protons adjacent to the amide carbonyl group, whereas geminal coupling constants of approximately 5.5 Hz would be expected for methylene protons at the 4-position.[16]

The effect of substituents at C-3 on the reactivity of β-lactams towards reaction with t-butyl perbenzoate was investigated by studying reactions of the β-lactams (**3d**) and (**3e**). Reaction of the 3,3-dimethyl-substituted β-lactam (**3d**) afforded the corresponding benzoate (**4d**) in 44% yield. In contrast, the 3,3-diphenyl-substituted analogue (**3e**) was found to be inert under the reaction conditions, even when a twelve-fold molar excess of the perester was used. This result indicates that while the methyl substituents of the β-lactam (**3d**) do not markedly affect its reactivity, the phenyl substituents of (**3e**) reduce the reactivity of that species towards hydrogen transfer from the 4-position. As a consequence, t-butoxy radical produced by cleavage of the perbenzoate reacts by β-scission rather than by hydrogen-atom abstraction from (**3e**).

Treatment of the 3-methyl-substituted β-lactam (**3f**) with t-butyl perbenzoate afforded a mixture (*ca.* 1:3) of the *cis*- and *trans*-isomers of the corresponding benzoate (**4f**). Reaction of the 3-phthalimido-substituted lactam (**3g**) gave the *trans*-benzoate (**4g**). The stereochemistry of the benzoates (**4f**) and (**4g**) was assigned on the basis of ^{1}H n.m.r. spectral data.[16] The major isomer of (**4f**) exhibited a coupling constant of 0.9 Hz for interaction between the vicinal lactam protons, while the minor component showed a vicinal coupling constant of 4.3 Hz, consistent with the assignment of these compounds as the *trans*- and *cis*-isomers, respectively. The benzoate (**4g**) gave rise to a vicinal coupling constant of 1.3 Hz. The preferential formation of the *trans*-isomers of (**4f**) and (**4g**) may be attributed to steric interactions between the benzoyloxy group and the substituents at C-3. There is a greater selectivity for production of *trans*-(**4g**) because of the greater steric bulk of the phthalimido substituent in (**4g**) compared to the methyl substituent in (**4f**).

These reactions of the β-lactams (**3a**—**g**) with t-butyl perbenzoate illustrate a new method for the direct incorporation of a benzoyloxy substituent at the 4-position of azetidin-2-ones. The reaction products are suitable for elaboration by nucleophilic substitution of the acyloxy group. Accordingly, treatment



Scheme.

a $R^1 = R^2 = H$, $R^3 = Bu^t$, X = I
b $R^1 = R^2 = H$, $R^3 = Ph$, X = Cl
c $R^1 = R^2 = H$, $R^3 = 4$-MeOPh, X = Br
d $R^1 = R^2 = Me$, $R^3 = Bu^t$, X = Cl
e $R^1 = R^2 = Ph$, $R^3 = Bu^t$, X = Cl
f $R^1 = Me$, $R^2 = H$, $R^3 = 4$-MeOPh, X = Cl
g $R^1 = Phth$, $R^2 = H$, $R^3 = 4$-MeOPh, X = OH

of the benzoyloxy-substituted azetidin-2-one (**4c**) with 2-bromoethanol in the presence of zinc acetate[4] afforded the 4-(2-bromoethoxy)-substituted β-lactam (**5c**) in 57% yield. When the reaction was worked up before it had gone to completion, the acetoxy-substituted lactam (**6c**) was also isolated from the reaction mixture, indicating that the conversion of (**3c**) into (**5c**) proceeds *via* the acetate (**6c**) at least to some extent.

When the 3-methyl- and 3-phthalimido-substituted benzoyloxyazetidinones (**4f**) and (**4g**) were treated with 2-bromoethanol and zinc acetate, the corresponding bromoethoxy-substituted lactams (**5f**) and (**5g**) were produced, (**5f**) as a mixture (*ca.* 1:10) of the *cis*- and *trans*-isomers, (**5g**) as the *trans*-isomer. These results are in accord with previous studies[2,5] of nucleophilic substitution reactions of the acyloxy group in 3-substituted 4-acyloxyazetidinones, where the *trans*-product was found to predominate irrespective of the geometry of the reactant lactam. The preferential formation of the *trans*-isomers of (**5f**) and (**5g**) indicates that their production occurs *via* intermediates of type (**10f**) and (**10g**), respectively.[6]

Since substitution reactions of the 4-acyloxy group in β-lactams have been carried out more frequently with acetoxy-substituted azetidinones, in preference to their benzoyloxy-substituted analogues, we studied the incorporation of the acetoxy group in β-lactams through reaction of the azetidinones (**3c**) and (**3d**) with t-butyl peracetate. The reactions afforded the corresponding 4-acetoxy-substituted azetidinones (**6c**) and (**6d**), respectively. It should be noted, however, that t-butyl peracetate is more difficult to handle than the corresponding perbenzoate, owing to its greater thermal and shock sensitivity. Consequently, the reagent of choice for the functionalization of β-lactams is the perbenzoate. In light of the conversion of the benzoate (**4c**) into the acetate (**6c**) on treatment with zinc acetate as described above, the need for direct introduction of an acetoxy substituent is likely to be limited.

Finally, we examined reactions of the *p*-methoxyphenyl-substituted azetidinones (**4c**), (**5c**), and (**6c**) with ceric ammonium nitrate.[17] In each case cleavage of the *p*-methoxyphenyl substituent occurred, to give the corresponding *N*-unsubstituted lactams (**7c**), (**8c**), and (**9c**).

In summary, the methodology described above provides a new route for the synthesis of 4-acyloxy-substituted azetidinones that are suitable for elaboration by nucleophilic substitution of the acyloxy group, and the method can be applied to the synthesis of *N*-unsubstituted 4-acyloxyazetidinones.

Experimental

M.p.s are uncorrected. Solvents were purified and dried by standard methods. ^{1}H N.m.r. spectra were recorded on either a Varian T-60, a Varian XL-300, or a Bruker CXP-300 spectrometer. ^{13}C N.m.r. spectra were recorded on a Varian XL-300 spectrometer. Unless otherwise indicated n.m.r. spectra were recorded as dilute solutions in deuteriochloroform with tetramethylsilane as an internal standard. I.r. spectra were recorded on either a Shimadzu IR-27G or a Pye-Unicam SP3-300 spectrometer, as neat liquids or as Nujol mulls of solids. Mass spectra were recorded on either a Kratos MS-9, an AEI MS-902, or an AEI MS-3010 spectrometer. Chromatography was carried out on a Chromatotron 7924T (Harrison Research, Palo Alto/TC Research, Norwich) by using Merck silica gel 60 PF_{254}, and elution with a gradient of light petroleum–dichloromethane–ethyl acetate. Light petroleum refers to the fraction with b.p. 60—80 °C. Microanalyses were performed by the Microanalytical Laboratory, University of Otago, New Zealand, or by the Canadian Microanalytical Service Ltd., New Westminster, Canada.

t-Butyl perbenzoate, 3-chloropropionic acid (**1b**), and 3-bromopropionic acid (**1c**) were purchased from Aldrich Chemical Company, Inc. t-Butyl peracetate,[18] 3-chloro-2,2-dimethylpropionic acid (**1d**),[19] 3-chloro-2,2-diphenylpropionic acid (**1e**),[20] 3-chloro-2-methylpropionic acid (**1f**),[21] and *N*-phthaloylserine (**1g**)[22] were all prepared and purified by using literature procedures.

N-t-Butyl-3-chloropropionamide.—A mixture of the propionic acid (**1b**) (7.2 g, 67 mmol) and thionyl chloride (5.4 ml, 73 mmol) in dichloromethane (50 ml) was heated under reflux for 3 h, after which it was cooled and concentrated and the residue dissolved in dichloromethane (20 ml). t-Butylamine (11.6 ml, 110 mmol) was then added dropwise to the solution. After the mixture had been stirred at room temperature for 3 h, dichloromethane (50

ml) and water (50 ml) were added. The dichloromethane layer was separated, washed with water (2 × 50 ml), dried ($MgSO_4$), and concentrated under reduced pressure. The residue crystallized from light petroleum–ethyl acetate to give the title propionamide (6.4 g, 58%), m.p. 78—80 °C (Found: C, 51.6; H, 8.8; N, 8.4. $C_7H_{14}ClNO$ requires C, 51.4; H, 8.6; N, 8.6%); $\nu_{max.}$ 1 568, 1 635, and 3 260 cm^{-1}; *m/z* 165 (M^+, 21%), 163 (M^+, 73), 71 (100), 65 (11), and 63 (34); δ 1.36 (s, 9 H, Bu^t), 2.53 (t, *J* 6.5 Hz, 2 H, CH_2CO), 3.76 (t, *J* 6.5 Hz, 2 H, CH_2Cl), and 5.55 (br s, 1 H, NH).

1-*t-Butylazetidin-2-one* (**3a**).—A mixture of *N*-t-butyl-3-chloropropionamide (2.0 g, 12.2 mmol) and sodium iodide (5.0 g, 33.3 mmol) in butan-2-one (20 ml) was heated under reflux for 3 h, after which it was cooled and diluted with ether (150 ml). The resultant solution was filtered and the filtrate was concentrated under reduced pressure. The residue separated from light petroleum to give crude *N*-t-butyl-3-iodopropionamide (**2a**) (2.0 g) as an oil which was used without further purification; δ 1.36 (s, 9 H, Bu^t), 2.68 (t, *J* 6.5 Hz, 2 H, CH_2CO), 3.35 (t, *J* 6.5 Hz, 2 H, CH_2I), and 5.25 (br s, 1 H, NH). Sodium hydride (50% in oil; 950 mg, 20 mmol) was pre-washed with light petroleum (2 × 10 ml) and suspended in a mixture of dichloromethane and dimethylformamide (4:1; 500 ml). To this suspension a solution of the crude propionamide (**2a**) (2.0 g) in dichloromethane and dimethylformamide (4:1; 200 ml) was added dropwise over 6 h, under nitrogen. The solution was stirred for a further 30 min after which saturated aqueous ammonium chloride was added. The dichloromethane layer was separated, washed with saturated brine (5 × 100 ml) and water (2 × 100 ml), dried ($MgSO_4$), and concentrated under reduced pressure. The residual oil was chromatographed on silica and then distilled to give the title azetidin-2-one (**3a**) (80 mg, 5%), b.p. 77—78 °C/18 mmHg (block) (lit.,[23] 75 °C/3.5 mmHg); δ 1.36 (s, 9 H, Bu^t), 2.75 (m, 2 H, CH_2CO), and 3.17 (m, 2 H, CH_2N).

3-*Chloro*-N-*phenylpropionamide* (**2b**).—Treatment of the propionic acid (**1b**) (4.4 g, 41 mmol) with thionyl chloride (4.0 ml, 55 mmol) and then with aniline (7.7 g, 79 mmol), as described above for the preparation of *N*-t-butyl-3-chloropropionamide from the propionic acid (**1b**), thionyl chloride, and t-butylamine, afforded the title propionamide (**2b**) (6.1 g, 82%), m.p. 115—116 °C (lit.,[24] 115—116.5 °C); δ [$^2H_6$]acetone 2.86 (t, *J* 6.5 Hz, 2 H, CH_2CO), 3.90 (t, *J* 6.5 Hz, 2 H, CH_2N), and 6.80—7.80 (m, 6 H, ArH and NH).

1-*Phenylazetidin-2-one* (**3b**).—Treatment of the propionamide (**2b**) (1.0 g, 5.5 mmol) with sodium hydride (50% in oil; 328 mg, 6.8 mmol), as described above for the preparation of the azetidin-2-one (**3a**) from the propionamide (**2a**), gave the title azetidin-2-one (**3b**) (470 mg, 58%) as white crystals, m.p. 77—79 °C (lit.,[25] 77—78 °C); δ 3.08 (m, 2 H, CH_2CO), 3.60 (m, 2 H, CH_2N), and 7.20—7.40 (m, 5 H, ArH).

1-(4-*Methoxyphenyl*)*azetidin-2-one* (**3c**).—Treatment of the propionic acid (**1c**) (34.9 g, 228 mmol) with thionyl chloride (25 ml, 343 mmol) and then with 4-methoxyaniline (28 g, 228 mmol), as described above for the preparation of *N*-t-butyl-3-chloropropionamide from the propionic acid (**1b**), thionyl chloride, and t-butylamine, gave crude 3-bromo-*N*-(4-methoxyphenyl)propionamide (**2c**) (19.5 g, 33%) which was used without further purification, m.p. 132—135 °C; δ 2.94 (t, *J* 6 Hz, 2 H, CH_2CO), 3.70 (t, *J* 6 Hz, 2 H, CH_2Br), 3.80 (s, 3 H, OCH_3), and 6.70—7.50 (m, 5 H, ArH, and NH)].

Treatment of the propionamide (**2c**) (22.5 g, 87 mmol) with sodium hydride (80% in oil; 4.05 g, 135 mmol), as described above for the preparation of the azetid-1-in-2-one (**3a**) from the propionamide (**2a**), gave the title azetidin-2-one (**3c**) (8.9 g, 58%) as white crystals, m.p. 99—100 °C (lit.,[26] 97—98 °C); δ 3.05 (m, 2 H, CH_2CO), 3.60 (m, 2 H, CH_2N), 3.80 (s, 3 H, CH_3), and 6.80—7.40 (m, 4 H, ArH).

N-*t-Butyl*-3-*chloro*-2,2-*dimethylpropionamide* (**2d**).—Treatment of the propionic acid (**1d**) (9.0 g, 66 mmol) with thionyl chloride (5.4 ml, 74 mmol) and then with t-butylamine (11.6 ml, 110 mmol), as described above for the preparation of *N*-t-butyl-3-chloropropionamide from 3-chloropropionic acid (**1b**), thionyl chloride, and t-butylamine, gave the title propionamide (**2d**) (10.0 g, 79%), m.p. 78—79 °C (lit.,[27] 76.0—77.5 °C); δ(CCl_4) 1.20 (s, 6 H, 2 × CH_3), 1.32 (s, 9 H, Bu^t), 3.50 (s, 2 H, CH_2), and 5.30 (br s, 1 H, NH).

1-*t-Butyl*-3,3-*dimethylazetidin-2-one* (**3d**).—Treatment of the propionamide (**2d**) (1.8 g, 9.4 mmol) with sodium hydride (50% in oil; 1.1 g, 23 mmol), as described above for the preparation of the azetidin-2-one (**3a**) from the propionamide (**2a**), gave the title azetidin-2-one (**3d**) (748 mg, 51%), b.p. 60—62 °C/18 mmHg(block); *m/z* 155.1309 (M^+) [Calc for $C_9H_{17}NO$ (M^+) *m/z* 155.1310]; *m/z* 155 (M^+, 46%), 140 (12), 112 (100), 99 (38), 86 (37), 84 (37), and 70 (50); $\nu_{max.}$ 1 740 cm^{-1}; δ(CCl_4) 1.21 (s, 6 H, 2 × CH_3), 1.30 (s, 9 H, Bu^t), and 2.90 (2 H, s, CH_2).

1-*t-Butyl*-3,3-*diphenylazetidin-2-one* (**3e**).—Treatment of the propionic acid (**1e**) (1.8 g, 7.0 mmol) with thionyl chloride (1.0 ml, 14 mmol) and then with t-butylamine (1.9 ml, 18 mmol), as described above for the preparation of *N*-t-butyl-3-chloropropionamide from the propionic acid (**1b**), thionyl chloride, and t-butylamine, gave crude *N*-t-butyl-3-chloro-2,2-diphenylpropionamide (**2e**) (600 mg, 27%) as an oil which was used without further purification; δ 1.25 (s, 9 H, Bu^t), 4.40 (s, 2 H, CH_2Cl), 5.52 (br s, 1 H, NH), and 7.36 (s, 10 H, ArH).

Treatment of the propionamide (**2e**) (0.55 g, 1.7 mmol) with sodium hydride (50% in oil; 612 mg, 13 mmol), as described above for the preparation of the azetidin-2-one (**3a**) from the propionamide (**2a**), gave white crystals of the title azetidin-2-one (**3e**) (325 mg, 68%), m.p. 149—150 °C (Found: C, 81.9; H, 7.8; N, 4.9. $C_{19}H_{21}NO$ requires C, 81.7; H, 7.6; N, 5.0%); *m/z* 280 ($M^+ + 1$, 1%), 236 (9), 222 (12), 180 (100), and 166 (47); $\nu_{max.}$ 1 769 cm^{-1}; δ 1.36 (s, 9 H, Bu^t), 3.68 (s, 2 H, CH_2), and 7.10—7.40 (m, 10 H, ArH).

3-*Chloro*-N-(4-*methoxyphenyl*)-2-*methylpropionamide* (**2f**).—Treatment of the propionic acid (**1f**) (28.8 g, 235 mmol) with thionyl chloride (52 ml, 713 mmol) and then with 4-methoxyaniline (19 g, 155 mmol), as described above for the preparation of *N*-t-butyl-3-chloropropionamide from the propionic acid (**1b**), thionyl chloride, and t-butylamine, gave the title propionamide (**2f**) (27.7 g, 78%), m.p. 126—127 °C (Found: C, 58.0; H, 6.2; N, 6.2. $C_{11}H_{14}ClNO_2$ requires C, 58.0; H, 6.2; N, 6.2%); *m/z* 229 (M^+, 19%), 227 (M^+, 56), and 123 (100); $\nu_{max.}$ 1 643, 1 691, and 3 420 cm^{-1}; δ 1.31 (d, *J* 7 Hz, 3 H, CCH_3), 2.70 (m, 1 H, CHCO), 3.68 (t, *J* 6 Hz, 2 H, CH_2Cl), 3.80 (s, 3 H, OCH_3), and 6.80—7.80 (m, 5 H, ArH and NH).

1-(4-*Methoxyphenyl*)-3-*methylazetidin-2-one* (**3f**).—Treatment of the propionamide (**2f**) (20 g, 88 mmol) with sodium hydride (80% in oil; 4.1 g, 137 mmol), as described above for the preparation of the azetidin-2-one(**3a**) from the propionamide (**2a**), gave the title azetidin-2-one (**3f**) (13.8 g, 82%) as white crystals, m.p. 117—118 °C (Found: C, 69.3; H, 6.9; N, 7.4. $C_{11}H_{13}NO_2$ requires C, 69.1; H, 6.9; N, 7.3%); *m/z* 191 (M^+, 79%), 149 (70), and 135 (100); $\nu_{max.}$ 1 715 cm^{-1}; δ 1.42 (d, *J* 7 Hz, 3 H, CCH_3), 3.30 (m, 2 H, CH_2), 3.70 (m, 1 H, CH), 3.80 (s, 3 H, OCH_3), and 6.80—7.40 (m, 4 H, ArH).

N-(4-*Methoxyphenyl*)-N^a-*phthaloylserinamide* (**2g**).—A mixture of *N*-phthaloylserine (13.5 g, 57 mmol), 4-methoxy-

aniline (7.8 g, 63 mmol), and dicyclohexylcarbodi-imide (27 g, 131 mmol) in dichloromethane (330 ml) was stirred at room temperature for 18 h, after which it was filtered and the supernatant dissolved in ethyl acetate (700 ml). The resulting solution was washed with 10% aqueous sodium carbonate (3 × 350 ml), 0.1M HCl (120 ml), and brine (350 ml), dried ($MgSO_4$), and concentrated. The residue crystallized from ethyl acetate to give the title serinamide (**2g**) (9.7 g, 50%), m.p. 155—156 °C (Found: C, 63.7; H, 4.9; N, 8.4. $C_{18}H_{16}N_2O_5$ requires C, 63.5; H, 4.7; N, 8.2%); *m/z* 340 (M^+, 57%), 322 (14), 150 (32), and 143 (100); $\nu_{max.}$ 1 651, 1 698, 3 250, and 3 425 cm^{-1}; δ 3.53 (t, *J* 6 Hz, 1 H, OH), 3.73 (s, 3 H, CH_3), 4.20 (m, 2 H, CH_2), 5.04 (t, *J* 6 Hz, 1 H, CH), and 6.70—8.10 (m, 9 H, ArH and NH).

1-(4-*Methoxyphenyl*)-3-*phthalimidoazetidin*-2-*one* (**3g**).—Diethyl azodicarboxylate (4.3 ml, 27 mmol) was added dropwise to a solution of the serinamide (**2g**) (8.65 g, 25 mmol) and triphenylphosphine (7.1 g, 27 mmol) in tetrahydrofuran (300 ml) under nitrogen. The mixture was stirred for 17 h at room temperature, after which the reaction was quenched with water. The resultant precipitate was collected and recrystallized from ethyl acetate to give the title azetidin-2-one (**3g**) (4.6 g, 57%) as white crystals, m.p. 232—234 °C; *m/z* 322.0971 (M^+) [Calc. for $C_{18}H_{14}N_2O_4$ (M^+) *m/z* 322.0954]; *m/z* 322 (M^+, 12%), 149 (100), and 134 (22); $\nu_{max.}$ 1 710 and 1 760 cm^{-1}; δ 3.87 (s, 3 H, CH_3), 4.07 (m, 2 H, CH_2), 5.63 (m, 1 H, CH), and 6.90—8.10 (m, 8 H, ArH).

4-*Benzoyloxy*-1-*t-butylazetidin*-2-*one* (**4a**).—A solution of the azetidin-2-one (**3a**) (80 mg, 0.63 mmol), t-butyl perbenzoate (0.44 ml, 2.3 mmol), and cupric octanoate (10 mg, 0.02 mmol) in benzene (5 ml) was heated at reflux under nitrogen for 6 h. The solution was then washed with saturated aqueous sodium metabisulphite (3 × 25 ml), dried ($MgSO_4$), and concentrated under reduced pressure. The residue was chromatographed on silica to give the title azetidin-2-one (**4a**) (92 mg, 59%), m.p. 94—95 °C; *m/z* 247.1212 (M^+) [Calc. for $C_{14}H_{17}NO_3$ (M^+) *m/z* 247.1208]; *m/z* 247 (M^+, 3%), 232 (15), 219 (6), 204 (8), 105 (100), and 77 (92); $\nu_{max.}$ 1 720 and 1 740 cm^{-1}, δ 1.40 (s, 9 H, Bu^t), 2.81 (dd, *J* 1, 14 Hz, 1 H, *cis*-3-H), 3.33 (dd, *J* 4, 14 Hz, 1 H, *trans*-3-H), 6.34 (dd, *J* 1, 4, Hz, 1 H, 4-H), and 7.20—8.30 (m, 5 H, ArH). Unchanged (**3a**) (31 mg, 39%) was also recovered from the reaction mixture.

4-*Benzoyloxy*-1-*phenylazetidin*-2-*one* (**4b**).—Treatment of the azetidin-2-one (**3b**) (100 mg, 0.68 mmol) with t-butyl perbenzoate (0.39 ml, 2.1 mmol), as described above for the preparation of (**4a**) gave the title azetidin-2-one (**4b**) (97 mg, 54%), m.p. 104—106 °C (Found: C, 72.2; H, 5.1; N, 4.6. $C_{16}H_{13}NO_3$ requires C, 71.9; H, 4.9; N, 5.2%); *m/z* 267.0870 (M^+) [Calc. for $C_{16}H_{13}NO_3$ (M^+) *m/z* 267.0895]; *m/z* 267 (M^+, 13%), 223 (15), 161 (42), 145 (16), 105 (100), and 77 (53); $\nu_{max.}$ 1 720 and 1 765 cm^{-1}; δ 3.17 (dd, *J* 2, 16 Hz, 1 H, *cis*-3-H), 3.66 (dd, *J* 4, 16 Hz, 1 H, *trans*-3-H), 6.74 (dd, *J* 2, 4 Hz, 1 H, 4-H), and 7.10—8.30 (m, 10 H, ArH). Unchanged (**3b**) (27 mg, 27%) was also recovered from the reaction mixture.

4-*Benzoyloxy*-1-(4-*methoxyphenyl*)*azetidin*-2-*one* (**4c**).—Treatment of the azetidin-2-one (**3c**) (1.2 g, 6.8 mmol) with t-butyl perbenzoate (7.0 ml, 37 mmol), as described above for the preparation of (**4a**), gave the title azetidin-2-one (**4c**) (0.94 g, 46%), m.p. 135—136 °C (Found: C, 68.8; H, 5.1; N, 4.7. $C_{17}H_{15}NO_4$ requires C, 68.7; H, 5.1; N, 4.7%); *m/z* 297 (M^+, 20%), 175 (69), 149 (28), and 105 (100); $\nu_{max.}$ 1 700 and 1 740 cm^{-1}; δ 3.17 (dd, *J* 2, 16 Hz, 1 H, *cis*-3-H), 3.63 (dd, *J* 4, 16 Hz, 1 H, *trans*-3-H), 3.80 (s, 3 H, CH_3), 6.75 (dd, *J* 2, 4 Hz, 1 H, 4-H), and 6.80—8.30 (m, 9 H, ArH). Unchanged (**3c**) (0.61 g, 50%) was also recovered from the reaction mixture.

4-*Benzoyloxy*-1-*t-butyl*-3,3-*dimethylazetidin*-2-*one* (**4d**).—Treatment of the azetidin-2-one (**3d**) (100 mg, 0.65 mmol) with t-butyl perbenzoate (0.36 ml, 2 mmol), as described above for the preparation of (**4a**), gave the title azetidin-2-one (**4d**) (78 mg, 44%), m.p. 47—49 °C (Found: C, 69.7; H, 8.0; N, 4.8. $C_{16}H_{21}NO_3$ requires C, 69.8; H, 7.6; N, 5.0%); *m/z* 275 (M^+, 9%), 260 (17), 176 (56), 105 (100), and 77 (73); $\nu_{max.}$ 1 720 and 1 760 cm^{-1}; δ(CCl_4) 1.10 (s, 3 H, CH_3), 1.34 (s, 3 H, CH_3), 1.39 (s, 9 H, Bu^t), 5.88 (s, 1 H, CH), and 7.30—8.20 (m, 5 H, ArH).

Treatment of 1-*t-Butyl*-3,3-*diphenylazetidin*-2-*one* (**3e**) *with t-Butyl Perbenzoate*.—When the azetidin-2-one (**3e**) (100 mg, 0.36 mmol) was treated with t-butyl perbenzoate (0.8 ml, 4.3 mmol), as described above for the preparation of (**4a**), only unchanged starting material (**3e**) (93 mg, 93%) was recovered.

trans-4-*Benzoyloxy*-1-(4-*methoxyphenyl*)-3-*methylazetidin*-2-*one* (**4f**).—Treatment of the azetidin-2-one (**3f**) (1.0 g, 5.2 mmol) with t-butyl perbenzoate (7.0 ml, 35 mmol), as described above for the preparation of (**4a**), gave the title azetidin-2-one (**4f**) (752 mg, 46%), m.p. 98—100 °C (Found: C, 69.5; H, 5.5; N, 4.5. $C_{18}H_{17}NO_4$ requires C, 69.4; H, 5.5; N, 4.5%); *m/z* 311 (M^+, 4%), 189 (24), 149 (32), and 105 (100); $\nu_{max.}$ 1 720 and 1 760 cm^{-1}; δ 1.53 (d, *J* 7.5 Hz, 3 H, CCH_3), 3.40 (dq, *J* 0.9, 7.5 Hz, 1 H, 3-H), 3.78 (s, 3 H, OCH_3), 6.26 (d, *J* 0.9 Hz, 1 H, 4-H), and 6.80—8.20 (m, 9 H, ArH). Unchanged (**3f**) (287 mg, 29%) was also recovered from the reaction mixture. The ^1H n.m.r. spectrum of the crude reaction mixture showed that *cis*- and *trans*-(**4f**) were produced in the ratio *ca.* 1:3:*cis*-(**4f**), δ 1.27 (d, *J* 7.5 Hz, 3 H, CCH_3), 3.60 (m, 1 H, 3-H), 3.77 (s, 3 H, OCH_3), 6.62 (d, *J* 4.3 Hz, 1 H, 4-H), and 6.80—8.20 (m, 9 H, ArH).

trans-*Benzoyloxy*-1-(4-*methoxyphenyl*)-3-*phthalimidoazetidin*-2-*one* (**4g**).—Treatment of the azetidin-2-one (**3g**) (2.0 g, 6.2 mmol) with t-butyl perbenzoate (14 ml, 70 mmol), as described above for the preparation of (**4a**), gave the title azetidin-2-one (**4g**) (383 mg, 14%), m.p. 197—199 °C (Found: C, 67.5; H, 4.2; N, 6.1. $C_{25}H_{18}N_2O_6$ requires C, 67.9; H, 4.1; N, 6.3%); *m/z* 442 (M^+, 4%), 320 (4), 149 (15), and 105 (100); $\nu_{max.}$ 1 720, 1 780, and 1 790 cm^{-1}; δ 3.80 (s, 3 H, OCH_3), 5.55 (d, *J* 1.3 Hz, 1 H, 4-H), 6.86 (d, *J* 1.3 Hz, 1 H, 3-H), and 6.90—8.10 (m, 13 H, ArH). Unchanged (**3g**) (612 mg, 31%) was also recovered from the reaction mixture.

4-(2-*Bromoethoxy*)-1-(4-*methoxyphenyl*)*azetidin*-2-*one* (**5c**).—A mixture of the azetidin-2-one (**4c**) (3.0 g, 10 mmol), 2-bromoethanol (5.0 g, 70 mmol), and zinc acetate dihydrate (3.0 g, 13.7 mmol) in toluene (300 ml) was heated at reflux in a flask equipped with a Dean-Stark condenser for 7 h. The mixture was then cooled, washed twice with saturated aqueous sodium hydrogen carbonate, dried ($MgSO_4$), and concentrated. The residual oil was chromatographed on silica to give the title azetidin-2-one (**5c**) (1.73 g, 57%); *m/z* 299.0150 (M^+) [Calc. for $C_{12}H_{14}BrNO_3$ (M^+) *m/z* 299.0157]; *m/z* 301 (M^+, 8%), 299 (M^+, 8), 175 (10), 149 (100), and 134 (26); $\nu_{max.}$ 1 750 cm^{-1}; δ 3.20 (m, 2 H, 3-H), 3.50 (m, 2 H, CH_2Br), 3.83 (s, 3 H, OCH_3), 3.96 (m, 2 H, OCH_2), 5.60 (m, 1 H, 4-H), and 6.80—7.60 (m, 4 H, ArH).

When the reaction mixture was heated at reflux for <7 h, chromatography of the product mixture afforded, in addition to (**5c**), 4-acetoxy-1-(4-methoxyphenyl)azetidinone (**6c**), m.p. 100—101 °C (Found: C, 61.3; H, 5.5; N, 5.9. $C_{12}H_{13}NO_4$ requires C, 61.3; H, 5.6; N, 6.0%); *m/z* 235 (M^+, 38%), 193 (19), 175 (19), 149 (100), and 134 (40); $\nu_{max.}$ 1 750 cm^{-1}; δ 2.16 (s, 3 H, $OCOCH_3$), 3.06 (dd, *J* 2, 16 Hz, 1 H, *cis*-3-H), 3.53 (dd, *J* 4, 16 Hz, 1 H, *trans*-3-H), 3.84 (s, 3 H, OCH_3), 6.55 (dd, *J* 2, 4 Hz, 4-H), and 6.80—7.50 (m, 4 H, ArH).

trans-4-(2-*Bromoethoxy*)-1-(4-*methoxyphenyl*)-3-*methylazetidin*-2-*one* (**5f**).—Treatment of the azetidin-2-one (**4f**) (200 mg, 0.64 mmol) with 2-bromoethanol (400 mg, 5.6 mmol) and zinc acetate dihydrate (250 mg, 1.1 mmol), as described above for the preparation of the azetidin-2-one (**5c**), gave the title azetidin-2-one (**5f**) (68 mg, 33.7%), b.p. 110 °C/0.04 mmHg(block) (Found: C, 50.3; H, 5.2; N, 4.3. $C_{13}H_{16}BrNO_3$ requires C, 49.7; H, 5.1; N, 4.5%); *m/z* 315 (M^+, 32%), 313 (M^+, 32), 149 (100), and 134 (38); $\nu_{max.}$ 1 750 cm^{-1}; δ 1.38 (d, *J* 7 Hz, 3 H, CCH_3), 3.29 (dq, *J* 1.5, 7 Hz, 1 H, 3-H), 3.48 (t, *J* 6 Hz, 2 H, CH_2Br), 3.79 (s, 3 H, OCH_3), 3.91 (m, 2 H, OCH_2), 5.13 (d, *J* 1.5 Hz, 1 H, 4-H), and 6.80—7.50 (m, 4 H, ArH). The ^1H n.m.r. spectrum of the crude reaction mixture showed that *cis*- and *trans*-(**5f**) were produced in the ratio *ca.* 1:10: *cis*-(**5f**), δ 1.33 (d, *J* 7 Hz, 3 H, CCH_3), 3.40 (m, 1 H, 3-H), 3.54 (t, *J* 6 Hz, 2 H, CH_2Br), 3.79 (s, 3 H, OCH_3), 4.05 (m, 2 H, OCH_2), 5.36 (d, *J* 4 Hz, 1 H, 4-H), and 6.80—7.50 (m, 4 H, ArH).

trans-4-(2-*Bromoethoxy*)-1-(4-*methoxyphenyl*)-3-*phthalimidoazetidin*-2-*one* (**5g**).—Treatment of the azetidin-2-one (**4g**) (250 mg, 0.57 mmol) with 2-bromoethanol (800 mg, 6.4 mmol) and zinc acetate dihydrate (250 mg, 1.1 mmol), as described above for the preparation of the azetidin-2-one (**5c**), gave the title azetidin-2-one (**5g**) (92 mg, 37%) as white crystals, m.p. 58—59 °C (Found: C, 53.9; H, 3.9; N, 6.1. $C_{20}H_{17}BrN_2O_5$ requires C, 54.0; H, 3.9; N, 6.3%); *m/z* 446 (M^+, 4%), 444 (M^+, 4), 297 (42), 295 (42), 149 (100), and 134 (42); $\nu_{max.}$ 1 700 and 1 740 cm^{-1}; δ 3.50 (m, 2 H, CH_2Br), 3.80 (s, 3 H, OCH_3), 4.05 (m, 2 H, OCH_2), 5.50 (d, *J* 1.3 Hz, 1 H, 4-H), 5.80 (d, *J* 1.3 Hz, 1 H, 3-H), and 6.80—8.20 (m, 8 H, ArH).

4-*Acetoxy*-1-(4-*methoxyphenyl*)*azetidin*-2-*one* (**6c**).—Treatment of the azetidin-2-one (**3c**) (1.20 g, 6.8 mmol) with t-butyl peracetate (3.5 g, 27 mmol), as described above for the reaction of (**3c**) with t-butyl perbenzoate, gave the title azetidin-2-one (**6c**) (0.47 g, 29%), identical in all respects with the sample obtained as described above.

4-*Acetoxy*-1-*butyl*-3,3-*dimethylazetidin*-2-*one* (**6d**).—Treatment of the azetidin-2-one (**3d**) (100 mg, 0.65 mmol) with t-butyl peracetate (0.50 g, 3.8 mmol), as described above for the reaction of (**3d**) with t-butyl perbenzoate, gave the title azetidin-2-one (**6d**) (48 mg, 35%) as an oil, *m/z* 213.1371 (M^+) [Calc. for $C_{11}H_{19}NO_3$ (M^+) *m/z* 213.1365]; *m/z* 213 (M^+, 12%), 171 (38), 157 (22), and 127 (100), $\nu_{max.}$ 1 740 cm^{-1}; δ 1.11 (s, 3·H), 1.30 (s, 3 H), 1.37 (s, 9 H), 2.14 (s, 3 H), 5.75 (s, 1 H).

4-*Benzoyloxyazetidin*-2-*one* (**7c**).—To a solution of the azetidin-2-one (**4c**) (0.5 g, 1.7 mmol) in acetonitrile (20 ml) cooled to −10 °C, a solution of ceric ammonium nitrate (2.5 g, 4.6 mmol) in water (25 ml) was added dropwise. After 30 min at −10 °C, the mixture was diluted with water (110 ml) and extracted with ethyl acetate (3 × 25 ml). The organic extracts were combined, washed with 10% aqueous sodium sulphite and saturated brine, stirred over charcoal for 30 min, filtered, dried (Na_2SO_4), and concentrated. Chromatography of the residue afforded the title azetidin-2-one (**7c**) (169 mg, 53%) as colourless crystals from chloroform, m.p. 94—95 °C (lit.,[2] 93—94 °C).

4-(2-*Bromoethoxy*)*azetidin*-2-*one* (**8c**).—Treatment of the azetidin-2-one (**5c**) (140 mg, 0.41 mmol) with ceric ammonium nitrate (0.77 g, 1.4 mmol), as described above for the preparation of the azetidin-2-one (**7c**), gave the title azetidin-2-one (58 mg, 64%) as an oil, which had physical and spectral properties consistent with those reported previously.[28]

2-*Acetoxyazetidin*-2-*one* (**9c**).—Treatment of the azetidinone (**6c**) (200 mg, 0.85 mmol) with ceric ammonium nitrate (1.50 g, 2.7 mmol), as described above for the preparation of the azetidin-2-one (**7c**), gave the title azetidin-2-one (**9c**) (47 mg, 43%) as a low melting point solid, which had physical and spectral properties consistent with those reported previously.[2]

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References

1 See for example E. Arribas, C. Carreiro, and A. M. Valdeomillos, *Tetrahedron Lett.*, 1988, **29**, 1609; J. M. Odriozola, F. P. Cossio, and C. Palomo, *J. Chem. Soc., Chem. Commun.*, 1988, 809; R. P. Attrill, A. G. M. Barrett, P. Quayle, and J. Vander Westhuisen, *J. Org. Chem.*, 1984, **49**, 1679; K. Fujimoto, Y. Iwano, and K. Hirai, *Tetrahedron Lett.*, 1985, **26**, 89; W. F. Huffman, K. G. Holden, T. F. Buckley, J. G. Gleason, and L. Wu, *J. Am. Chem. Soc.*, 1977, **99**, 2352; T. N. Salzmann, R. W. Ratcliffe, B. G. Christensen, and F. A. Bouffard, *J. Am. Chem. Soc.*, 1980, **102**, 6161; M. M. Campbell and B. P. Connarty, *Heterocycles*, 1982, **19**, 1853; M. D. Bachi and A. Gross, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1157; M. Ihara, A. Nakayama, and K. Fukumoto, *Tetrahedron*, 1982, **38**, 2489.
2 K. Clauss, D. Grimm, and G. Prossel, *Liebigs Ann. Chem.*, 1974, 539.
3 For a review of the use of 4-acetoxyazetidin-2-one in synthesis see S. Mickel, *Aldrichimica Acta*, 1985, **18**, 95.
4 A. G. Brown, D. F. Corbett, and T. T. Howarth, *J. Chem. Soc., Chem. Commun.*, 1977, 359.
5 A. Arrieta, F. P. Cossio, J. M. Garcia, B. Lecea, and C. Palomo, *Tetrahedron Lett.*, 1988, **29**, 3129; D. M. Tschaen, L. M. Fuentes, J. E. Lynch, W. L. Laswell, R. P. Volante, and I. Shinkai, *ibid.*, p. 2779; G. I. Georg and J. Kant, *J. Org. Chem.*, 1988, **53**, 692.
6 D. R. Wagle, C. Garai, M. G. Monteleone, and A. K. Bose, *Tetrahedron Lett.*, 1988, **29**, 1649.
7 For a preliminary account of this work see C. J. Easton and S. G. Love, *Tetrahedron Lett.*, 1986, **27**, 2315.
8 M. Mori, K. Kagechika, K. Tohjima, and M. Shibasaki, *Tetrahedron Lett.*, 1988, **29**, 1409.
9 M. Mitzlaff, K. Warning, and H. Rehling, *Synthesis*, 1980, 315.
10 M. Okita, M. Mori, T. Wakamatsu, and Y. Ban, *Heterocycles*, 1985, **23**, 247.
11 M. L. M. Pennings and D. N. Reinhoudt, *J. Org. Chem.*, 1983, **48**, 4043; P. A. van Elburg and D. N. Reinhoudt, *Heterocycles*, 1987, **26**, 437.
12 I. L. Knunyants and N. P. Gambaryan, *Izv. Akad. Nauk. SSSR, Otdel. Khim. Nauk*, 1955, 1037; H. H. Wasserman, D. J. Hlasta, A. W. Tremper, and J. S. Wu, *Tetrahedron Lett.*, 1979, 549.
13 A. K. Bose, D. P. Sahu, and M. S. Manhas, *J. Org. Chem.*, 1981, **46**, 1229; M. J. Miller and P. G. Mattingly, *Tetrahedron*, 1983, **39**, 2563.
14 D. J. Rawlinson and G. Sosnovsky, *Synthesis*, 1972, 1.
15 M. Okita, T. Wakamatsu, and Y. Ban, *J. Chem. Soc., Chem. Commun.*, 1979, 749; M. Okita, T. Wakamatsu, M. Mori, and Y. Ban, *Heterocycles*, 1980, **14**, 1089; J. W. Pavlik and S. Tantayanon, *J. Am. Chem. Soc.*, 1981, **103**, 6755; J. C. Gramain, R. Remuson, and Y. Troin, *J. Chem. Soc., Chem. Commun.*, 1976, 194; for a review see T. Shono, *Tetrahedron*, 1984, **40**, 811.
16 K. D. Barrow and T. M. Spotswood, *Tetrahedron Lett.*, 1965, 3325.
17 D. R. Kronenthal, C. Y. Han, and M. K. Taylor, *J. Org. Chem.*, 1982, **47**, 2765.
18 P. D. Bartlett and R. R. Hiatt, *J. Am. Chem. Soc.*, 1958, **80**, 1398.
19 M. S. Kharasch and H. C. Brown, *J. Am. Chem. Soc.*, 1940, **62**, 925.
20 H. E. Zaugg, H. J. Glenn, R. J. Michaels, R. U. Schock, and L. R. Swett, *J. Am. Chem. Soc.*, 1957, **79**, 3912.
21 S. Groszkowski, J. Sienkiewicz, and L. Najman, *Farmacia*, 1967, **15**, 263.
22 G. G. L. Nefkens, G. I. Teser, and R. F. Nivard, *Recl. Trav. Chim. Pays-Bas*, 1960, **79**, 688.

23 H. H. Wasserman, B. H. Lipshutz, A. W. Tremper, and T. S. Wu, *J. Org. Chem.*, 1981, **46**, 2993.
24 R. Pohloudek-Fabini and E. Schroeph, *Pharmazie*, 1968, **23**, 561.
25 M. S. Manhas and S. J. Jeng, *J. Org. Chem.*, 1967, **32**, 1246.
26 S. Kano, T. Ebata, and S. Shibuya, *J. Chem. Soc., Perkin Trans. 1*, 1980, 2105.
27 R. A. Johnson and F. D. Greene, *J. Org. Chem.*, 1975, **40**, 2186.
28 P. H. Bentley and E. Hunt, *J. Chem. Soc., Perkin Trans. 1*, 1980, 2222.

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Exocyclic Bromination of *N*-Substituted β- and γ-Lactams

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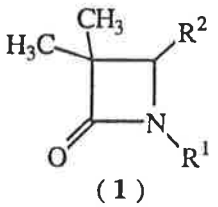
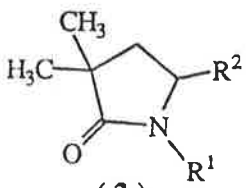
Regioselective halogenation of the *N*-susbtituted lactams (**3a**)-(**3d**) affords the corresponding exocyclic bromides (**4a**)-(**4d**). The procedure provides a novel alternative route to *N*-(α-haloalkyl)-substituted lactams, which are of particular interest in the synthesis of β-lactam antibiotics.

Reactions of *N*-substituted β- and γ-lactams at exocyclic carbon adjacent to amide nitrogen are complicated by competing reactions at the corresponding endocyclic position.[1-4] For example, the copper-catalysed reaction of (**1a**) with *t*-butyl perbenzoate afforded (**1b**) and (**1c**) as the primary products, in the ratio *ca.* 2:1,[1] while (**2a**) gave (**2b**) and (**2c**), in the ratio *ca.* 1:3.[2] Similar results were obtained through the electrochemical oxidation of lactams.[3,4] We envisaged that free-radical bromination could be affected regioselectively at the exocyclic position in lactams bearing activating substituents[5,6] at that position. Accordingly, we have investigated reactions of the azetidinones (**3a**) and (**3b**), and the pyrrolidinones (**3c**) and (**3d**), with *N*-bromosuccinimide. The lactams (**3a**)-(**3d**) used in this work were prepared by treatment of the corresponding 3-bromopropionamides and 4-chlorobutyramides with potassium hydroxide in the presence of a phase transfer catalyst.[7]



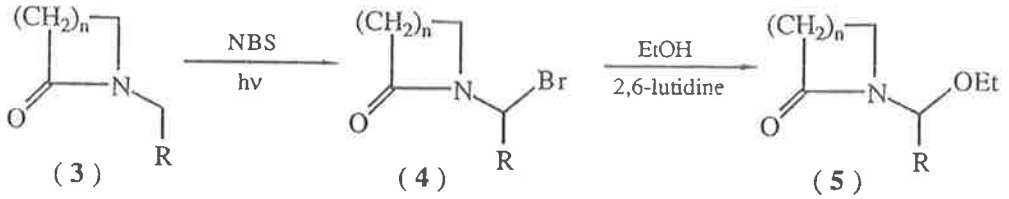
(**1**)



(**2**)

a; $R^1 = Me, R^2 = H$
b; $R^1 = CH_2OCOPh, R^2 = H$
c; $R^1 = Me, R^2 = OCOPh$

The β-lactam (**3a**) was treated with *N*-bromosuccinimide (1 equiv.) in a mixture of carbon tetrachloride and dichloromethane (5:1), at reflux under nitrogen for 0.25 hr, with reaction initiated by irradiation with a 250 W mercury lamp. The mixture was then cooled, filtered and concentrated to give only (**4a**), as determined by ^{1}H n.m.r. spectroscopic analysis [(300MHz, $CDCl_3$) δ 1.33 (3H, *t*, J 7.1 Hz), 3.01 (1H, *ddd*, J 15.6, 6.2, 3.5 Hz), 3.11 (1H, *ddd*, J 15.6, 5.9, 3.9 Hz), 3.56 (1H, *ddd*, J 6.5, 6.2, 3.9 Hz), 3.70 (1H, *ddd*, J 6.5, 5.9, 3.5 Hz), 4.27 (2H, *q*, J 7.1 Hz), 6.33 (1H, *s*)]. Similar treatment of (**3b**) gave only (**4b**) [^{1}H n.m.r. (300MHz, $CDCl_3$) δ 3.11 (1H, *ddd*, J 15.7, 6.0, 4.1 Hz), 3.21 (1H, *ddd*, J 15.7, 5.9, 4.0 Hz), 3.52 (1H, *ddd*, J 6.1, 6.0, 4.0 Hz), 3.56 (1H, *ddd*, J 6.1, 5.9, 4.1 Hz), 6.44 (1H, *s*)].



(3) (4) (5)

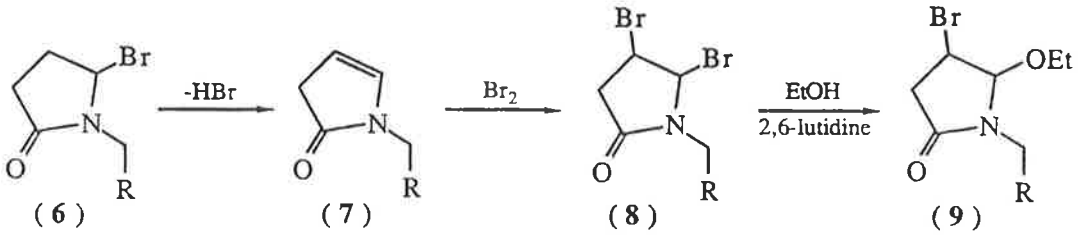
a; R = CO_2Et, n = 1
b; R = CN, n = 1
c; R = CO_2Me, n = 2
d; R = CN, n = 2

Scheme 1

Since the bromides (**4a**) and (**4b**) were not sufficiently stable for complete characterisation, they were converted to the corresponding ethers (**5a**) and (**5b**) through the addition of ethanol and 2,6-lutidine directly to crude reaction mixtures after cooling to room temperature. After purification by chromatography on silica, the ethers (**5a**) [^{1}H n.m.r. (300MHz, $CDCl_3$) δ 1.27 (3H, *t*, J 7.0 Hz), 1.32 (3H, *t*, J 7.2 Hz), 3.01 (1H, *ddd*, J 13.4, 4.9, 3.3 Hz), 3.07 (1H, *ddd*, J 13.4, 4.9, 3.9 Hz), 3.41 (1H, *ddd*, J 5.4, 4.9, 3.9 Hz), 3.46 (1H, *ddd*, J 5.4, 4.9, 3.3 Hz), 3.59 (1H, *dq*, J 9.5, 7.0 Hz), 3.68 (1H, *dq*, J 9.5, 7.0 Hz), 4.26 (2H, *q*, J 7.2 Hz), 5.35 (1H, *s*)] and (**5b**) [^{1}H n.m.r. (300MHz, $CDCl_3$) δ 1.27 (3H, *t*, J 7.0 Hz), 3.07 (1H, *ddd*, J 15.2, 5.5, 3.8 Hz), 3.13 (1H, *ddd*, J 15.2, 5.4, 3.9 Hz), 3.50 (1H, *ddd*, J 7.9, 5.5, 3.9 Hz), 3.52 (1H, *ddd*, J 7.9, 5.4, 3.8 Hz), 3.63 (1H, *dq*, J 9.3, 7.0 Hz), 3.67 (1H, *dq*, J 9.3, 7.0 Hz), 5.68 (1H, *s*)] were isolated in yields of 57 and 41%, based on (**3a**) and (**3b**), respectively, and were fully characterised. There was no evidence of ring substitution in the

reactions of (**3a**) and (**3b**) with *N*-bromosuccinimide. Presumably, the modest yields of (**5a**) and (**5b**) reflect the instability of the corresponding intermediate bromides (**4a**) and (**4b**).

Treatment of the γ-lactam (**3c**) with *N*-bromosuccinimide and subsequently with ethanol afforded, after chromatography of the reaction mixture on silica, the ether (**5c**) [37%; ^{1}H n.m.r. (300MHz, $CDCl_3$) δ 1.26 (3H, *t*, J 7.0 Hz), 2.10 (2H, *m*), 2.47 (2H, *t*, J 8.1 Hz), 3.37 (1H, *ddd*, J 9.8, 7.8, 6.5 Hz), 3.47 (1H, *ddd*, J 9.8, 7.8, 6.2 Hz), 3.57 (2H, *q*, J 7.0 Hz), 3.79 (3H, *s*), 5.75 (1H, *s*)] and the ring substitution product (**9a**) [9%; ^{1}H n.m.r. (300MHz, $CDCl_3$) δ 1.24 (3H, *t*, J 7.0 Hz), 2.76 (1H, *dd*, J 18.2, 2.9 Hz), 3.23 (1H, *ddd*, J 18.2, 7.4, 0.9 Hz), 3.61 (1H, *dq*, J 9.4, 7.0 Hz), 3.70 (1H, *dq*, J 9.4, 7.0 Hz), 3.77 (3H, *s*), 3.81 (1H, *dd*, J 17.6, 0.9 Hz), 4.24 (1H, *ddd*, J 7.4, 2.9, 1.5 Hz), 4.43 (1H, *d*, J 17.6 Hz), 5.21 (1H, *d*, J 1.5 Hz)]. Similar treatment of (**3d**) gave (**5d**) [26%; ^{1}H n.m.r. (300MHz, $CDCl_3$) δ 1.25 (3H, *t*, J 7.1 Hz), 2.15 (2H, *m*), 2.48 (2H, *t*, J 8.2 Hz), 3.60 (4H, *m*), 6.02 (1H, *s*)] and (**9b**) [8%; ^{1}H n.m.r. (300MHz, $CDCl_3$) δ 1.29 (3H, *t*, J 7.0 Hz), 2.76 (1H, *dd*, J 18.4, 2.1 Hz), 3.24 (1H, *ddd*, J 18.4, 7.2, 0.8 Hz), 3.71 (1H, *dq*, J 9.4, 7.0 Hz), 3.76 (1H, *dq*, J 9.4, 7.0 Hz), 4.07 (1H, *dd*, J 17.5, 0.8 Hz), 4.25 (1H, *ddd*, J 7.2, 2.1, 1.1 Hz), 4.51 (1H, *d*, J 17.5 Hz), 5.15 (1H, *d*, J 1.1 Hz)]. The production of (**9a**) and (**9b**) may be attributed to formation of the corresponding intermediate bromides (**6a**) and (**6b**), which react as shown in ***Scheme 2***.



(**6**) (**7**) (**8**) (**9**)

a; R = CO_2Me
b; R = CN

Scheme 2

The ratio of (**4c**) to (**8a**) in crude reaction mixtures was found to be *ca.* 3:1 as determined by ^{1}H n.m.r. spectroscopic analysis. The preferential reaction at the exocyclic position in the lactams (**3a**)-(**3d**) can be attributed to the relative ease of hydrogen atom abstraction from that position to give radicals stabilized by the combined resonance effects of the amido and alkoxycarbonyl or cyano substituents. Presumably the endocyclic methylenes adjacent to

nitrogen in the γ-lactams (**2a**), (**3c**) and (**3d**) are more reactive towards hydrogen atom abstraction than those in the corresponding β-lactams (**1a**), (**3a**) and (**3b**), due to the relative degrees of ring strain in the product radicals. Endocyclic substitution in the γ-lactams (**2a**), (**3c**) and (**3d**) is further favoured by the release of steric interactions between the C-4 and C-5 protons upon hydrogen atom abstraction from the endocyclic position.

Production of the α-bromo-2-oxoazetidineacetates (**4a**) and (**4b**) illustrates a novel attractive alternative procedure for the synthesis of *N*-(α-haloalkyl)-substituted lactams,[8] which have been used widely in the synthesis of β-lactam antibiotics.[9]

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REFERENCES:

1. Easton, C. J.; Love, S. G. *Tetrahedron Lett.* **1986**, *27*, 2315.
2. Easton, C. J.; Peters, S. C.; Love, S. G. *Heterocycles* **1988**, *27*, 2405.
3. Okita, M.; Mori, M.; Wakamatsu, T.; Ban, Y. *Heterocycles* **1985**, *23*, 247.
4. Okita, M.; Wakamatsu, T.; Ban, Y. *J Chem. Soc., Chem Commun.* **1979**, 749.
5. Viehe, H. G.; Janousek, Z.; Merenyi, R. *Acc. Chem. Res.* **1985**, *18*, 148.
6. Poutsma, M. L. In *Free Radicals* ; Kochi, J. K., Ed.; Wiley: New York, 1973, Vol. 2, p211.
7. Takahata, H.; Ohnishi, Y.; Takehara, H.; Tsuritani, K.; Yamazaki, T. *Chem. Pharm. Bull.* **1981**, *29*, 1063.
8. Scartazzini, R.; Peter, H.; Bickel, H.; Heusler, K.; Woodward, R. B. *Helv. Chim. Acta* **1972**, *55*, 408. Ernest, I.; Gosteli, J.; Greengrass, C. W.; Holick, W.; Jackman, D. E.; Pfaendler, H. R.; Woodward, R. B. *J. Am. Chem. Soc.* **1978**, *100*, 8214.
9. For examples see: Cama, L. D.; Christensen, B. G. *J. Am. Chem. Soc.* **1978**, *100*, 8006; Schmitt, S. M.; Johnston, D. B. R.; Christensen, B. G. *J. Org. Chem.* **1980**, *45*, 1135; Kametani, T.; Huang, S.-P.; Yokohama, S.; Suzuki, Y.; Ihara, M. *J. Am. Chem. Soc.* **1980**, *102*, 2060; Bachi, M. D.; Frolow, F.; Hoornaert, C. *J. Org. Chem.* **1983**, *48*, 1841; Crackett, P. H.; Pant, C. M.; Stoodley, R. J. *J. Chem. Soc., Perkin Trans I* **1984**, 2785; Smale, T. C.; Southgate, R. *J. Chem. Soc., Perkin Trans. I* **1985**, 2235.

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Monosubstitution of Symmetric Piperazine-2,5-dione Derivatives

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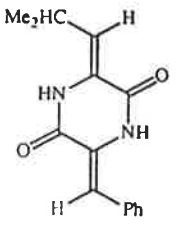
Abstract

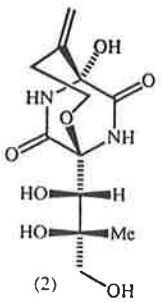
Bromination and methanolysis of derivatives of glycine anhydride affords the corresponding 3-bromo- and 3-methoxy-substituted piperazine-2,5-dione derivatives in a simple one-pot procedure.

Introduction

Piperazine-2,5-dione derivatives constitute a large and important class of naturally occurring compounds, many of which are biologically active. For example, albonoursin (1) has been isolated from *Streptomyces albus* var. *fungatus*, *Streptomyces noursei* and *Actinomyces tume-macerance*, and has been found to exhibit antibacterial and antitumour activity,[1] while bicyclomycin (2) has been obtained from *Streptomyces sapporonensis* and *Streptomyces aizunensis*, and has been shown to be a broad-spectrum antibiotic.[2] In the course of developing procedures for the synthesis of these compounds, Williams and Kwast[3] observed that the bromination of symmetric derivatives of glycine anhydride shows a strong tendency to give exclusively 3,6-dibrominated products in preference to the corresponding monobromides. As an example they reported that



(1)



(2)

[1] Brown, R., and Kelley, C., *Annu. Rep. N. Y. State Dep. Health, Albany*, 1957, 10; 1958, 47; 1960, 50; 1960, 52; 1961, 40; Rao, K. U., and Cullen, W. P., *J. Am. Chem. Soc.*, 1960, **82**, 1127; Rosenfeeld, G. S., Rostovtseva, L. I., Baikiam, V. M., Trakhtenbery, D. M., and Khokhlov, A. S., *Antibiotiki*, 1963, **8**, 201; Fukushima, K., Yazawa, K., and Arai, T., *J. Antibiot.*, 1973, **26**, 175.
[2] Miyoshi, T., Miyairi, N., Aoki, H., Kohsaka, M., Sakai, H., and Imanaka, H., *J. Antibiot.*, 1972, **25**, 569; Kamiya, T., Maeno, S., Hashimoto, M., and Mine, Y., *J. Antibiot.*, 1972, **25**, 576; Nishida, M., Mine, Y., and Matsubara, T., *J. Antibiot.*, 1972, **25**, 582; Nishida, M., Mine, Y., Matsubara, T., Goto, S., and Kuwahara, S., *J. Antibiot.*, 1972, **25**, 594; Miyamura, S., Ogasawara, N., Otsuka, H., Niwayama, S., Tanaka, H., Take, T., Uchiyama, T., Ochiai, H., Abe, K., Koizumi, K., Asao, K., Matsuki, K., and Hoshino, T., *J. Antibiot.*, 1972, **25**, 610; Miyamura, S., Ogasawara, N., Otsuka, H., Niwayama, S., Tanaka, H., Take, T., Uchiyama, T., Ochiai, H., *J. Antibiot.*, 1973, **26**, 479.
[3] Williams, R. M., and Kwast, A., *J. Org. Chem.*, 1988, **53**, 5785.

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treatment of (3a) with 0·9 equiv. of *N*-bromosuccinimide gave approximately 50% of the dibromide (5a) and 50% unreacted (3a). In stark contrast to that work, we have found that bromination of derivatives of glycine anhydride affords the corresponding monobromides. Methanolysis of the monobromides *in situ* affords ethers that are of interest in the synthesis of asymmetric piperazinediones.[3-5]

(3) (4) (5) (6) (7)

(a) R = $CH_2C_6H_4$-*p*-OMe (b) R = CH_2Ph (c) R = Me

Results and Discussion

In a typical experiment, (3a) was treated with *N*-bromosuccinimide (0·9 equiv.) in carbon tetrachloride at reflux under nitrogen for 0·5 h, with azobisisobutyronitrile to initiate the reaction. Analysis of the crude reaction mixture by ^{1}H n.m.r. spectroscopy showed the presence of (3a), the monobromide (4a) and the dibromide (5a), in the ratio *c.* 2:6:1. Similar treatment of (3b) gave a mixture of (3b), (4b) and (5b), in the ratio *c.* 1:6:1. The reaction of (3c) with *N*-bromosuccinimide was carried out in dichloromethane instead of carbon tetrachloride, in order to dissolve (3c), and gave a mixture of (3c), (4c) and (5c), in the ratio *c.* 5:15:1.

Since the monobromides (4a–c) were not sufficiently stable for isolation and purification, they were characterized by conversion into the corresponding ethers (6a–c), through the addition of methanol and triethylamine directly to crude reaction mixtures after cooling to 0°. Subsequent chromatography on silica gave (6a), (6b) and (6c), in yields of 41, 61 and 54%, based on (3a–c), respectively.

The dibromides (5a–c) were identified by comparison with authentic samples, prepared by treatment of (3a–c) with 2·1 equiv. of *N*-bromosuccinimide, and characterized as the corresponding ethers (7a–c). Only one diastereomer of each of the dibromides (5a–c) was detected, even by 300-MHz ^{1}H n.m.r. spectroscopy. The ethers (7a) and (7c) were isolated as mixtures of diastereomers, in the ratios 2·3:1 and 3:1, respectively. Only one diastereomer of the diether (7b) was detected, either in the crude reaction mixture or in the purified product.

These results show that in our hands the monobromides (4a–c) are produced through reaction of the corresponding substituted diketopiperazines (3a–c) with *N*-bromosuccinimide. While the earlier report[3] implied that the monobromides

[4] Williams, R. M., *Tetrahedron Lett.*, 1981, **22**, 2341.
[5] Williams, R. M., Anderson, O. P., Armstrong, R. W., Josey, J., Meyers, H., and Ericksson, C., *J. Am. Chem. Soc.*, 1982, **104**, 6092.

(4a–c) are much more reactive than (3a–c), our results show the converse. For the consecutive reactions

$$(3a) \xrightarrow{k_1} (4a) \xrightarrow{k_2} (5a) \quad \text{(i)}$$

the concentration of the monobromide (4a) reaches a maximum when

$$d[(4a)]/dt = k_1[(3a)][Br^\bullet] - k_2[(4a)][Br^\bullet] = 0 \quad \text{(ii)}$$

At this point

$$k_1/k_2 = [(4a)]/[(3a)] \quad \text{(iii)}$$

When the relative percentage concentrations of (3a), (4a) and (5a) were monitored as a function of the mole ratio of (3a) to *N*-bromosuccinimide, the monobromide (4a) reached a maximum concentration of over 70%, at which stage less than 10% of the starting material (3a) remained. On this basis the rate constant for the reaction of (3a) is at least seven times greater than that of (4a). Similar results were obtained for the reactions of (3b) and (3c).

Each of the reactions of (3a–c) was repeated at least five times, and the results were always consistent. There was no interconversion between the monobromides (4a–c) and the dibromides (5a–c) in carbon tetrachloride or dichloromethane, at room temperature or at reflux, unless both *N*-bromosuccinimide and azobisisobutyronitrile were present. Homogenous reactions of (3a) and (3b) carried out under dilute conditions gave similar results to those carried out in more concentrated solution, where all of the *N*-bromosuccinimide and the by-product succinimide did not dissolve. All of the reactions of (3c) with *N*-bromosuccinimide in dichloromethane were homogeneous.

In summary, we can offer no explanation for the disparity between our results and those reported previously;[3] however, the syntheses of (4a–c) and (6a–c) described above illustrate an alternative,[3–5] direct and simple one-pot procedure for the preparation of monosubstituted piperazine-2,5-dione derivatives. Ready access to these compounds should facilitate the synthesis of other substituted diketopiperazines.

Experimental

General experimental details have been reported previously.[6] 1,4-Di(*p*-methoxybenzyl)piperazine-2,5-dione (3a) and 1,4-dibenzylpiperazine-2,5-dione (3b) were prepared by alkylation of piperazine-2,5-dione.[7] 1,4-Dimethylpiperazine-2,5-dione (3c) was purchased from Sigma Chemical Company.

Bromination and Methanolysis of 1,4-Di(p-methoxybenzyl)piperazine-2,5-dione (3a)

A mixture of (3a) (1·03 g, 2·9 mmol), *N*-bromosuccinimide (1·08 g, 6·1 mmol) and azobisisobutyronitrile (*c.* 5 mg) in carbon tetrachloride (50 ml) was heated at reflux under nitrogen for 0·5 h; then it was cooled and filtered. The filtrate was concentrated in vacuum to give crude *3,6-dibromo-1,4-di(p-methoxybenzyl)piperazine-2,5-dione* (5a). ^{1}H n.m.r. δ 3·81, s, 6H; 3·96, d, *J* 14·5 Hz, 2H; 5·26, d, *J* 14·5 Hz, 2H; 5·87, s, 2H; 6·85–6·95, m, 4H; 7·20–7·25,

[6] Easton, C. J., and Peters, S. C., *Aust. J. Chem.*, 1990, **43**, 87.
[7] Sera, A., Itoh, K., Yamada, H., and Aoki, R., *Heterocycles*, 1984, **22**, 713.

m, 4H. Alternatively the filtrate was cooled to 0°, and methanol (20 ml) and triethylamine (1·0 ml, 7·2 mmol) were added. After stirring for 2 h at 0° the mixture was concentrated in vacuum. The residue was dissolved in chloroform, washed with water, dried (Na_2SO_4) and concentrated, to give an oil which was chromatographed on silica. Elution with a gradient of ethyl acetate/light petroleum gave *3,6-dimethoxy-1,4-di(*p*-methoxybenzyl)piperazine-2,5-dione* (7a) which crystallized from ethyl acetate/light petroleum as colourless needles of a 2·3:1 mixture of diastereomers (0·66 g, 55%), m.p. 108–115° (Found: C, 63·3; H, 6·4; N, 6·9. $C_{22}H_{26}N_2O_6$ requires C, 63·8; H, 6·3; N, 6·8%). ^{1}H n.m.r. δ 3·44, s, 0·7×6H; 3·49, s, 0·3×6H; 3·80, s, 0·7×6H; 3·81, s, 0·3×6H; 4·02, d, *J* 14 Hz, 0·7×2H; 4·06, d, *J* 14·5 Hz, 0·3×2H; 4·64, s, 0·3×2H; 4·78, s, 0·7×2H; 5·11, d, *J* 14·5 Hz, 0·3×2H; 5·24, d, *J* 14 Hz, 0·7×2H; 6·8–7·3, m, 8H. Mass spectrum: *m/z* 383 (7%), 382 (31), 121 (100).

When the reaction of (3a) was repeated with 0·9 equiv. of *N*-bromosuccinimide, concentration of the cooled and filtered reaction mixture afforded a crude mixture of (3a), *3-bromo-1,4-di(*p*-methoxybenzyl)piperazine-2,5-dione* (4a) [^{1}H n.m.r. δ 3·80, s, 3H; 3·81, s, 3H; 3.82, d, *J* 18 Hz, 1H; 3·91, d, *J* 14 Hz, 1H; 3·94, d, *J* 18 Hz, 1H; 4·26, d, *J* 14·5 Hz, 1H; 4·84, d, *J* 14·5 Hz, 1H; 5·18, d, *J* 14 Hz, 1H; 5·79, s, 1H; 6·6–6·9, m, 4H; 7·1–7·3, m, 4H] and (5a) in the ratio *c.* 2:6:1, as determined by analysis with ^{1}H n.m.r. spectroscopy. Alternatively treatment of the cooled and filtered reaction mixture with methanol and triethylamine, followed by chromatography, gave *3-methoxy-1,4-di(*p*-methoxybenzyl)piperazine-2,5-dione* (6a) as colourless needles from ethyl acetate/light petroleum (yield 41%), m.p. 96·5–97° (Found: C, 65·4; H, 6·2; N, 7·2. $C_{21}H_{24}N_2O_5$ requires C, 65·6; H, 6·3; N, 7·3%). ^{1}H n.m.r. δ 3·39, s, 3H; 3·78, d, *J* 18 Hz, 1H; 3·79, s, 6H; 4·02, d, *J* 18 Hz, 1H; 4·11, d, *J* 14·5 Hz, 1H; 4·33, d, *J* 14·5 Hz, 1H; 4·67, s, 1H; 4·68, d, *J* 14·5 Hz, 1H; 5·05, d, *J* 14·5 Hz, 1H; 6·8–6·9, m, 4H; 7·1–7·3, m, 4H. Mass spectrum: *m/z* 384 (2%), 121 (100).

Bromination and Methanolysis of 1,4-Dibenzylpiperazine-2,5-dione (3b)

The reactions of (3b) were carried out as described above for (3a).

Treatment of (3b) with 2·1 equiv. of *N*-bromosuccinimide afforded crude *1,4-dibenzyl-3,6-dibromopiperazine-2,5-dione* (5b) [^{1}H n.m.r. δ 4·03, d, *J* 14·6 Hz, 2H; 5·34, d, *J* 14·6 Hz, 2H; 5·90, s, 2H; 7·3–7·4, m, 10H], which reacted with methanol in the presence of triethylamine to give *1,4-dibenzyl-3,6-dimethoxypiperazine-2,5-dione* (7b) as colourless needles from ethyl acetate/light petroleum (yield 66%), m.p. 169–171·5° (Found: C, 67·6; H, 6·2; N, 8·0. $C_{20}H_{22}N_2O_4$ requires C, 67·8; H, 6·3; N, 7·9%). ^{1}H n.m.r. δ 3·46, s, 6H; 4·17, d, *J* 15 Hz, 2H; 4·69, s, 2H; 5·11, d, *J* 15 Hz, 2H; 7·25–7·35, m, 10H. Mass spectrum: *m/z* 322 (43%), 263 (60), 91 (100).

The reaction of (3b) with 0·9 equiv. of *N*-bromosuccinimide afforded a crude mixture of (3b), *1,4-dibenzyl-3-bromopiperazine-2,5-dione* (4b) [^{1}H n.m.r. δ 3·87, d, *J* 18 Hz, 1H; 3·94, d, *J* 14·5 Hz, 1H; 3·99, d, *J* 18 Hz, 1H; 4·32, d, *J* 14·5 Hz, 1H; 4·93, d, *J* 14·5 Hz, 1H; 5·24, d, *J* 14·5 Hz, 1H; 5·84, s, 1H; 7·2–7·4, m, 10H] and (5b), in the ratio *c.* 1:6:1. Treatment of the mixture with methanol and triethylamine, followed by chromatography, gave *1,4-dibenzyl-3-methoxypiperazine-2,5-dione* (6b) as colourless needles from ethyl acetate/light petroleum (yield 61%), m.p. 87·5–88° (Found: C, 70·1; H, 6·0; N, 8·4. $C_{19}H_{20}N_2O_3$ requires C, 70·4; H, 6·2; N, 8·6%). ^{1}H n.m.r. δ 3·41, s, 3H; 3·80, d, *J* 18 Hz, 1H; 4·06, d, *J* 18 Hz, 1H; 4·18, d, *J* 15 Hz, 1H; 4·38, d, *J* 14·5 Hz, 1H; 4·70, s, 1H; 4·76, d, *J* 14·5 Hz, 1H; 5·15, d, *J* 15 Hz, 1H; 7·2–7·4, m, 10H. Mass spectrum: *m/z* 293 (94%), 91 (100).

Bromination and Methanolysis of 1,4-Dimethylpiperazine-2,5-dione (3c)

The reactions of (3c) were carried out as described above for (3a), except that dichloromethane was used instead of carbon tetrachloride.

Treatment of (3c) with 2·1 equiv. of *N*-bromosuccinimide afforded crude *3,6-dibromo-1,4-dimethylpiperazine-2,5-dione* (5c) [^{1}H n.m.r. δ 3·10, s, 6H; 6·13, s, 2H], which reacted with methanol in the presence of triethylamine to give a 3:1 mixture of diastereomers of 3,6-dimethoxy-1,4-dimethylpiperazine-2,5-dione (7c) (68%), as a colourless oil with spectral properties consistent with those reported previously.[8]

[8] Nakatsuka, S., Sasaki, K., Yamaguchi, K., and Goto, T., *Chem. Lett.*, 1981, 695.

The reaction of (3c) with 0·9 equiv. of *N*-bromosuccinimide afforded a crude mixture of (3c), *3-bromo-1,4-dimethylpiperazine-2,5-dione* (4c) [^{1}H n.m.r. δ 3·01, s, 3H; 3·06, s, 3H; 3·92, d, J 18 Hz, 1H; 4·16, d, J 18 Hz, 1H; 6·02, s, 1H] and (5c), in the ratio *c.* 5:15:1. Treatment of the mixture with methanol and triethylamine, followed by chromatography, gave 3-methoxy-1,4-dimethylpiperazine-2,5-dione (6c) (54%) as a colourless oil with spectral properties consistent with those reported previously.[5]

Acknowledgment

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Majer (1981); Werner, Valent, Adelsköld & Švajlenová (1983).

References

AHMED, F. R., HALL, S. R., PIPPY, M. E. & HUBER, C. P. (1973). *NRC Crystallographic Programs for the IBM/360 System*. Accession Nos. 133–147 in *J. Appl. Cryst.* (1973), **6**, 309–346.

International Tables for X-ray Crystallography (1974). Vol. IV. Birmingham: Kynoch Press. (Present distributor Kluwer Academic Publishers, Dordrecht.)

PAVELČÍK, F., KRÄTSMÁR-ŠMOGROVIČ, J., ŠVAJLENOVÁ, O. & MAJER, J. (1981). *Collect. Czech. Chem. Commun.* **46**, 3186–3197.

UEKI, T., ASHIDA, T., SASADA, Y. & KAKUDO, M. (1969). *Acta Cryst.* B**25**, 328–335.

WERNER, P.-E., VALENT, A., ADELSKÖLD, V. & ŠVAJLENOVÁ, O. (1983). *Acta Chem. Scand. Ser. A*, **37**, 51–55.

Acta Cryst. (1990). C**46**, 1121–1122

Methyl (*E*)-3-Benzamido-2-bromoacrylate

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Abstract. $C_{11}H_{10}BrNO_3$, $M_r = 284{\cdot}1$, orthorhombic, $Pna2_1$, $a = 9{\cdot}915\ (1)$, $b = 17{\cdot}834\ (1)$, $c = 6{\cdot}442\ (1)$ Å, $V = 1139\ (1)$ Å^3, $Z = 4$, $D_x = 1{\cdot}657$ Mg m^{-3}, Mo $K\bar{\alpha}$ radiation, $\lambda = 0{\cdot}7107$ Å, $\mu = 3{\cdot}501$ mm^{-1}, $F(000) = 568$, $T = 295\ (2)$ K, $R = 0{\cdot}033$ for 1151 observed reflections. The structure investigation determines the stereochemistry of the title compound as *E*. Bond distances and angles are as expected. The non-phenyl portion of the molecule is planar [maximum deviation: 0·106 (5) Å] and forms a dihedral angle of 15·4° with the phenyl ring. There are no significant intermolecular contacts in the crystal lattice.

Experimental. A mixture of *N*-benzoyl-β-alanine methyl ester (0·3 g, 1·4 mmol), *N*-bromosuccinimide (0·26 g, 1·4 mmol) and 2,2′-azobisisobutyronitrile (*ca* 5 mg) in CCl_4 (15 ml) was heated for 2 h at reflux under N_2 whilst being irradiated with a 250 W Hg lamp. The filtrate was concentrated *in vacuo* and chromatographed on silica to give methyl (*E*)-3-benzamido-2-bromoacrylate (132 mg, 32%), isomer *A*, m.p. 371–372 K, found: C, 46·48; H, 3·53%; $C_{11}H_{10}BrNO_3$ requires C, 46·50; H, 3·54% and methyl (*Z*)-3-benzamido-2-bromoacrylate (62 mg, 15%), isomer *B*, m.p. 395·5–396·5 K, found: C, 46·51; H, 3·67%; $C_{11}H_{10}BrNO_3$ requires C, 46·50; H, 3·54%. Crystals of isomer *A* grown by slow evaporation of hexane into an ethyl acetate solution of the compound. Enraf–Nonius CAD-4F diffractometer controlled by a PDP8/A computer, graphite-monochromated Mo $K\bar{\alpha}$ radiation; ω:2θ-scan technique. Cell parameters on crystal 0·18 × 0·12 × 0·75 mm by least squares on 25 reflections ($8 \le \theta \le 12°$) (de Boer & Duisenberg, 1984). Analytical absorption correction applied; max. and min. transmission factors 0·710 and 0·520 (Sheldrick, 1976). Total of 2588 reflections ($1{\cdot}5 \le \theta \le 27{\cdot}5°$) measured in the range $0 \le h \le 12$, $-23 \le k \le 12$, $0 \le l \le 8$. No significant variation in the net intensities of three reference reflections ($2\bar{2}\bar{5}$, $2\bar{3}4$, $1\bar{4}\bar{2}$) measured every 7200 s. 1486 unique reflections (R_{int} 0·024) and 1151 satisfied $I \ge 2{\cdot}5\sigma(I)$. Structure solved by Patterson method, full-matrix least-squares refinement of 173 parameters based on *F* (Sheldrick, 1976). Anisotropic

Table 1. *Fractional atomic coordinates and* B_{eq} *values* (Å^2)

$B_{eq} = 8\pi^2(U_{11} + U_{22} + U_{33})/3$.

	x	*y*	*z*	B_{eq}
Br(2)	−0·06102 (4)	−0·05594 (3)	0	4·77
O(1)	−0·2244 (4)	0·0015 (3)	−0·3736 (8)	6·49
O(1′)	−0·3878 (5)	−0·0834 (3)	−0·3656 (7)	4·87
O(4)	−0·4316 (3)	−0·2594 (2)	0·1777 (6)	4·54
N(3)	−0·2455 (4)	−0·1872 (3)	0·1491 (7)	3·54
C(1)	−0·2761 (5)	−0·0516 (3)	−0·2937 (9)	3·54
C(1′)	−0·4478 (6)	−0·0519 (4)	−0·5501 (9)	5·41
C(2)	−0·2261 (5)	−0·0903 (3)	−0·1082 (8)	3·14
C(3)	−0·2894 (5)	−0·1479 (3)	−0·0213 (10)	3·15
C(4)	−0·3211 (5)	−0·2422 (3)	0·2438 (8)	3·27
C(41)	−0·2616 (5)	−0·2767 (3)	0·4353 (7)	3·11
C(42)	−0·1609 (5)	−0·2417 (3)	0·5468 (8)	3·55
C(43)	−0·1157 (6)	−0·2729 (4)	0·7321 (10)	4·62
C(44)	−0·1690 (6)	−0·3382 (4)	0·7993 (10)	5·01
C(45)	−0·2665 (7)	−0·3750 (4)	0·6880 (11)	5·21
C(46)	−0·3127 (5)	−0·3445 (3)	0·5021 (15)	4·44

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Table 2. *Selected interatomic distances* (Å) *and bond angles* (°)

C(1)—O(1)	1·195 (6)	C(1)—O(1′)	1·327 (6)
O(1′)—C(1′)	1·443 (7)	C(1)—C(2)	1·466 (7)
C(2)—Br(2)	1·882 (5)	C(2)—C(3)	1·328 (7)
C(3)—N(3)	1·372 (7)	N(3)—C(4)	1·377 (7)
C(4)—O(4)	1·214 (5)	C(4)—C(41)	1·499 (7)

C(1)—O(1′)—C(1′)	117·7 (5)	O(1)—C(1)—O(1′)	123·1 (5)
O(1)—C(1)—C(2)	125·4 (5)	O(1′)—C(1)—C(2)	111·5 (5)
C(1)—C(2)—Br(2)	116·3 (4)	C(1)—C(2)—C(3)	123·2 (5)
Br(2)—C(2)—C(3)	120·4 (4)	C(2)—C(3)—N(3)	125·6 (5)
C(3)—N(3)—C(4)	123·0 (4)	N(3)—C(4)—O(4)	121·0 (5)
N(3)—C(4)—C(41)	116·2 (4)	O(4)—C(4)—C(41)	122·7 (5)

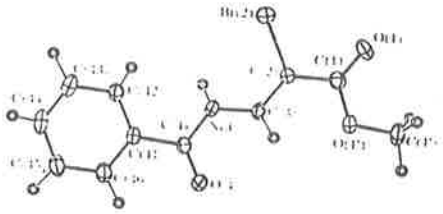

Fig. 1. Molecular structure and numbering scheme for $C_{11}H_{10}BrNO_3$. H atoms are labelled according to the atom to which they are bonded (Johnson, 1971).

thermal parameters for non-H atoms and isotropic thermal parameters for H atoms which were located from a difference map except for methyl H atoms which were included at their calculated positions (C—H 0·97 Å) and refined with a common isotropic thermal parameter. At convergence $R = 0{\cdot}033$, $wR = 0{\cdot}033$, $w = 1{\cdot}89/[\sigma^2(F) + 0{\cdot}0003F^2]$, $S = 1{\cdot}97$, $(\Delta/\sigma)_{max} \leq 0{\cdot}001$, $\Delta\rho_{max} = 0{\cdot}41$, $\Delta\rho_{min} = -0{\cdot}46$ e Å^{-3}; no extinction correction. Scattering factors for H, C, N and O given in *SHELX*76 (Sheldrick, 1976) and those for neutral Br corrected for f' and f'' from *International Tables for X-ray Crystallography* (1974). All calculations on a SUN4/280 computer system. Atomic parameters are given in Table 1, selected parameters in Table 2* and the numbering scheme used is shown in Fig. 1, drawn with *ORTEP* (Johnson, 1971) at 25% probability ellipsoids.

Related literature. Owing to similarities in the spectroscopic data for isomers *A* and *B* it was necessary to characterize one of these by X-ray methods to determine the stereochemistry as *E* or *Z*. The structure determination of the title compound forms part of a wider study of the synthesis of halogenated amino acid derivatives (Burgess, Easton & Hay, 1989).

The Australian Research Council is thanked for support.

* Lists of structure factors, anisotropic thermal parameters, H-atom parameters, all bond distances and angles, and mean-plane data have been deposited with the British Library Document Supply Centre as Supplementary Publication No. SUP 52564 (9 pp.). Copies may be obtained through The Technical Editor, International Union of Crystallography, 5 Abbey Square, Chester CH1 2HU, England.

References

BOER, J. L. DE & DUISENBERG, A. J. M. (1984). Enraf–Nonius CAD-4F diffractometer software update, February 1984. Groningen and Utrecht, The Netherlands.

BURGESS, V. A., EASTON, C. J. & HAY, M. P. (1989). *J. Am. Chem. Soc.* **111**, 1047–1052.

International Tables for X-ray Crystallography (1974). Vol. IV, pp. 99, 149. Birmingham: Kynoch Press. (Present distributor Kluwer Academic Publishers, Dordrecht.)

JOHNSON, C. K. (1971). *ORTEP*II. Report ORNL-3794, revised. Oak Ridge National Laboratory, Tennessee, USA.

SHELDRICK, G. M. (1976). *SHELX*76. Program for crystal structure determination. Univ. of Cambridge, England.

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A New Synthesis of Cyclodextrin Dimers

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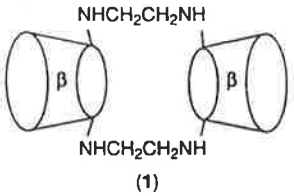
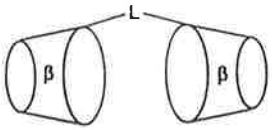
The synthesis and characterization of the linked cyclodextrins (**6a–c**) is reported.

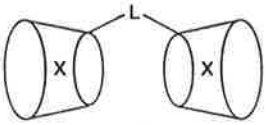
A number of cyclodextrin dimers have been synthesized in order to investigate the co-operative binding properties of covalently linked cyclodextrins.[1–7]† In 1979, Tabushi *et al.*[1] reported the synthesis of the tetramine (**1**). Subsequently, Harada and co-workers[2] described the formation of the diesters (**2a**) and (**2b**), and Fujita and co-workers reported the preparation of the disulphides (**3a**) and (**3b**),[3] and (**3c**).[4] During the course of the work described in this paper,[5] Breslow *et al.*[6] reported the synthesis of the disulphide (**2c**) and the diester (**3d**), and referred to an earlier synthesis in their laboratories of (**2d**).[7]



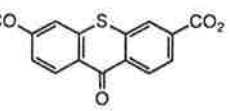
(1)



(2)
a; L = $OCOCH_2CH_2CO_2$
b; L = $OCOCH_2CH_2CH_2CO_2$
c; L = S_2
d; L = OCO-*p*-Ph-CO_2



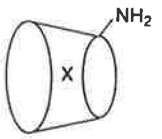
(3)
a; X = α; L = S_2
b; X = β; L = S_2
c; X = γ; L = S_2
d; X = β; L = OCO



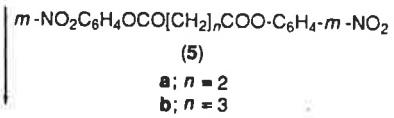
There are a number of limitations, however, associated with the synthesis, purification, and storage of the dimers (**1**)–(**3**), and their use to form inclusion complexes. For example, the yields reported for the preparation of (**1**), (**2a**), and (**2b**) were only 2.6, 0.5, and 0.5%, respectively, based on β-cyclodextrin, and no yields were reported for the synthesis of (**2c**) or (**3a–d**). Only (**2d**) was obtained in a yield of 10%, based on α-cyclodextrin. In this report we describe the synthesis and characterization of a new class of cyclodextrin dimers. We have prepared the diamides (**6a–c**) in yields of 19, 14, and 13%, respectively, based on the corresponding unmodified cyclodextrins.

Experimental

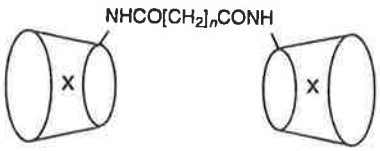
^{13}C NMR spectra were recorded in D_2O, using dioxane (δ 67.8) as the internal reference. ^1H NMR were recorded in $[^2H]_6$-DMSO, using tetramethylsilane as the internal reference. HPLC was carried out on a Waters Carbohydrate Analysis Column (3.9 mm × 30 cm), using water–acetonitrile (1:2 v/v) as eluant and a flow rate of 1.5 ml min^{-1}. Under these conditions α- and β-cyclodextrin each had R_T 6.0 min. Each of the diamides (**6a–c**) gave satisfactory microanalytical data and was fully characterized.



(4)
a; X = α
b; X = β



(5)
a; *n* = 2
b; *n* = 3



(6)
a; X = α; *n* = 2
b; X = β; *n* = 2
c; X = β; *n* = 3

The succinate and glutamate diesters (**5a**) and (**5b**) were prepared by treatment of the corresponding diacids with *m*-nitrophenol and *N,N'*-dicyclohexylcarbodi-imide in ethyl acetate.[2,8] The amines (**4a**) [δ_C 42.8 (t, C-6^)] and (**4b**) [δ_C 41.3 (t, C-6^)] were prepared from α- and β-cyclodextrin, in respective yields of 20 and 21%, through modification of the procedure of Melton and Slessor for the preparation of (**4a**).[9] A mixture of the amine (**4a**) and the succinate diester (**5a**) (0.5 equiv.) in pyridine was set aside at room temperature for 5 d, and then it was concentrated under reduced pressure. Residual pyridine was removed from the crude product mixture by co-distillation with water *in vacuo*, after which precipitation from water–acetone (1:8 v/v) gave a 94% yield of the cyclodextrin

† A truncated cone is commonly used[1–7] to represent the torus of a cyclic D-glucose polymer containing either six (α-cyclodextrin), seven (β-cyclodextrin) or eight (γ-cyclodextrin) anhydroglucose units joined by α-1,4-glucosidic linkages. A substituent drawn at the narrow end of the cone indicates that it replaces one of the C-6 hydroxy groups in the cyclodextrin, while a substituent drawn at the wide end of the cone indicates that it replaces either a C-2 or C-3 hydroxy group.

dimer (**6a**), as a white powder [HPLC, R_T 19.0 min; δ_H 2.29 (4 H, s, CH_2CO), and 7.8 (2 H, br s, NH); δ_C 32.2 (t, CH_2CO), 41.4 (t, C-6^), 61.3 (t), 61.6 (t), 71.5 (d), 72.9 (d), 73.2 (d), 74.3 (d), 74.5 (d), 82.4 (d), 84.3 (d), 102.6 (d), and 175.9 (s, CO)]. Similar treatment of the amine (**4b**) with (**5a**) and (**5b**) gave (**6b**) [68% yield; HPLC, R_T 33.5 min; δ_H 2.07 (4 H, s, CH_2CO) and 7.6 (2 H, br s, NH); δ_C 31.9 (t, CH_2CO), 41.0 (t, C-6^), 61.2 (t), 71.2 (d), 72.8 (d), 73.0 (d), 74.0 (d), 82.0 (d), 84.0 (d), 102.8 (d), and 175.5 (s, CO)] and (**6c**) [60% yield; HPLC, R_T 20.5 min; δ_H 1.75 (2 H, m, $CH_2CH_2CH_2$), 2.15 (4 H, m, CH_2CO), and 7.65 (2 H, br s, NH); δ_C 22.7 (t, $CH_2CH_2CH_2$), 35.9 (t, CH_2CO), 41.0 (t, C-6^), 61.0 (t), 61.3 (t), 71.1 (d), 72.6 (d), 73.0 (d), 74.0 (d), 82.0 (d), 84.1 (d), 102.8 (d), and 176.7 (s, CO)], respectively. This approach is suitable for the preparation of substantial quantities of (**6a–c**) and has been used to prepare over 50 g of (**6b**).

Conclusions

The reactions to give dimers (**6a–c**) demonstrate the greater nucleophilicity of the primary amino substituents compared to the primary and secondary hydroxy groups in compounds (**4a**) and (**4b**). The diamides (**6a–c**) are more resistant to hydrolysis than esters such as (**2a**), (**2b**), (**2d**), and (**3d**), therefore they are easier to purify and more stable on storage. We expect that the methodology described above for the preparation of (**6a–c**) can be used to produce diamides that are tailored to form high-stability inclusion complexes with specific substrates. These studies are continuing in our laboratories.

Acknowledgements

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References

1 I. Tabushi, Y. Kuroda, and K. Shimokawa, *J. Am. Chem. Soc.*, 1979, **101**, 1614.
2 A. Harada, M. Furue, and S.-I. Nozakura, *Polymer J.*, 1980, **12**, 29.
3 K. Fujita, S. Ejima, and T. Imoto, *J. Chem. Soc., Chem. Commun.*, 1984, 1277.
4 K. Fujita, S. Ejima, and T. Imoto, *Chem. Lett.*, 1985, 11.
5 S. F. Lincoln, J. H. Coates, C. J. Easton, S. J. van Eyk, B. M. May, P. Singh, M. A. Stile, and M. L. Williams, Internat. Pat. Appl. PCT/AU 89/00359; Australian Provisional Pat. Appl. PJ 0165.
6 R. Breslow, N. Greenspoon, T. Guo, and R. Zarzycki, *J. Am. Chem. Soc.*, 1989, **111**, 8296.
7 Y. Chao, Ph.D. Dissertation, Columbia University, New York, 1972.
8 A. Harada, M. Furue, and S. Nozakura, *Macromolecules*, 1976, **9**, 705.
9 L. D. Melton and K. N. Slessor, *Carbohydrate Res.*, 1971, **18**, 29.

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SYNTHESIS OF HOMOCHIRAL HYDROXY-α-AMINO ACID DERIVATIVES

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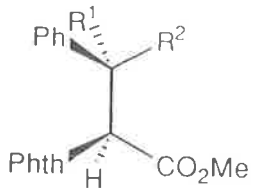
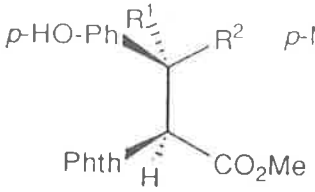
Summary: Treatment of N-phthaloyl-α-amino acid methyl esters with N-bromosuccinimide, followed by reaction with silver nitrate in aqueous acetone, affords homochiral hydroxy-α-amino acid derivatives, the stereochemistry of which is predetermined by that of the starting amino acids.

Hydroxy-substituted α-amino acids have attracted considerable attention due mainly to their physiological activity, either in the free form or as components of peptides[1]. They have also been used as enzyme inhibitors[2] and in synthesis[3]. Although a number of elegant methods have been developed for the stereocontrolled synthesis of these compounds[4], most suffer the disadvantage that they are indirect, and many are only enantioselective rather than enantiospecific. In this report we describe a synthesis of the homochiral hydroxy-α-amino acid derivatives (**1d**), (**2d**), (**4c**) and (**5c**), which is diastereoselective in the cases of (**1d**) and (**2d**). The method involves the direct substitution of derivatives of proteinogenic amino acids and the absolute stereochemistry of the products is defined by that of the starting materials. There has been one other report[5] of the direct hydroxylation of a tyrosine derivative, but that procedure was not generally applicable, even to the reaction of the corresponding phenylalanine derivative.

The amino acid derivative (**1a**) was prepared by treatment of (*S*)-phenylalanine with phthalic anhydride[6], followed by esterification with acidified methanol. Reaction of (**1a**) with N-bromosuccinimide in refluxing carbon tetrachloride under nitrogen, with reaction initiated by irradiation with a 250-W mercury lamp, gave a 1:1 mixture the diastereomeric bromides (**1b**) and (**1c**) in quantitative yield[7]. Treatment of the mixture with silver nitrate (1.5 equiv.) in water/acetone (2:3) at room temperature for 24 h gave a 93% yield of a 5:1 mixture of the diastereomers of the β-hydroxyphenylalanine derivative (**1d**) and (**1e**), which were separated either by reverse-phase chromatography or by fractional crystallization from dichloromethane/hexane. The major diastereomer (**1d**) had m.p. 185-186 °C, $[\alpha]_D^{16}$ -67.0° (*c* 0.006, ethanol). ^{1}H NMR ($CDCl_3$) δ 3.86 (s, 3H), 5.13 (d, *J* 10.4 Hz, 1H), 5.51 (d, *J* 4.6 Hz, 1H), 5.71 (dd, *J* 4.6, 10.4 Hz, 1H), 7.24 (m, 5H), 7.70 (m, 2H) and 7.79 (m, 2H)[8]. The minor diastereomer (**1e**) had m.p. 110-111 °C. ^{1}H NMR ($CDCl_3$) δ 3.79 (s, 3H), 4.34 (d, *J* 2.3 Hz, 1H), 5.02 (d, *J* 8.4 Hz, 1H), 5.52 (dd, *J* 2.3, 8.4 Hz, 1H), 7.26 (m, 5H), 7.68 (m, 2H) and 7.75 (m, 2H). The relative and absolute stereochemistry of (**1d**) was determined by X-ray crystallographic analysis (Figure 1)[9]. The absolute stereochemistry of (**1d**)



(1)



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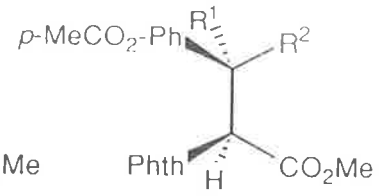
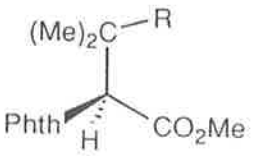
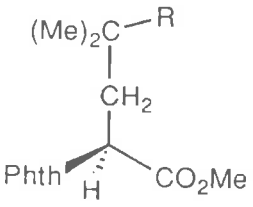


(3)

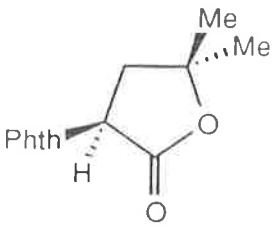
a) $R^1 = R^2 = H$
b) $R^1 = H$; $R^2 = Br$
c) $R^1 = Br$; $R^2 = H$
d) $R^1 = H$; $R^2 = OH$
e) $R^1 = OH$; $R^2 = H$



(4)



(5)



(6)

a) R = H
b) R = Br
c) R = OH

is predetermined by that of (S)-phenylalanine, and the diastereoselectivity observed in the production of (**1d**) can be attributed to nucleophilic attack from the less hindered face of the intermediate carbocation (Figure 2).

The procedure used in the preparation of (**1d**) is suitable for the preparation of a range of hydroxy-α-amino acid derivatives. The amino acid derivatives (**2a**), (**4a**) and (**5a**) were prepared from the corresponding (S)-amino acids, as described above for the preparation of (**1a**). Treatment of (**2a**) with acetic anhydride gave the acetate (**3a**), which reacted with N-bromosuccinimide to give a 1:1 mixture of the diastereomeric bromides (**3b**) and (**3c**). Reaction of the mixture with silver nitrate in aqueous acetone, followed by hydrolysis with aqueous methanol in the presence of *p*-toluenesulphonic acid, gave a 6:1 mixture of the diastereomers of the β-hydroxytyrosine derivative (**2d**) [m.p. 200-202 °C, $[\alpha]_D^{16}$ -70.7°

(c0.004, ethanol), ^{1}H NMR ($CDCl_3$) δ 3.85 (s, 3H), 4.76 (s, 1H), 5.02 (d, J 10.3 Hz, 1H), 5.44 (d, J 4.8 Hz, 1H), 5.64 (dd, J 4.8, 10.3 Hz, 1H), 6.71 (d, 8.6 Hz, 2H), 7.19 (d, 8.6 Hz, 2H), 7.72 (m, 2H) and 7.80 (m, 2H)] and (**2e**) [^{1}H NMR ($CDCl_3$) δ 3.78 (s, 3H), 4.27 (d, J 2.1 Hz, 1H), 4.84 (s, 1H), 4.97 (d, J 8.6 Hz, 1H), 5.49 (dd, J 2.1, 8.6 Hz, 1H), 6.65 (d, 8.6 Hz, 2H), 7.20 (d, 8.6 Hz, 2H), 7.69 (m, 2H) and 7.76 (m, 2H)].

Similar reactions of the derivatives of valine (**4a**) and leucine (**5a**) with N-bromosuccinimide gave the corresponding bromides (**4b**) and (**5b**), which reacted with silver nitrate in aqueous acetone to give the β-hydroxyvaline derivative (**4c**) [m.p. 79-80 °C, $[\alpha]_D^{16}$ -49.3° (c0.005, ethanol), ^{1}H NMR ($CDCl_3$) δ 1.31 (s, 3H), 1.53 (s, 3H), 3.77 (s, 3H), 4.41 (br. s, 1H), 4.91 (s, 1H), 7.80 (m, 2H) and 7.91 (m, 2H)] and the γ-hydroxyleucine derivative (**5c**) [m.p. 71-72 °C, $[\alpha]_D^{16}$ -25.9° (c0.005, ethanol), ^{1}H NMR ($CDCl_3$) δ 1.24 (s, 3H), 1.31 (s, 3H), 1.70 (br. s, 1H), 2.38 (dd, J 8.8, 15.1 Hz, 1H), 2.50 (dd, J 4.0, 15.1 Hz, 1H), 3.73 (s, 3H), 5.15 (dd, J 4.0, 8.8 Hz, 1H), 7.74 (m, 2H) and 7.86 (m, 2H)], respectively. The production of (**4c**) and (**5c**) occurred without racemization. Only one enantiomer of the hydroxyvaline derivative (**4c**) was detected when analysed by ^{1}H NMR spectroscopy in the presence of the chiral shift reagent $Eu(hfbc)_3$[10], under conditions which resolved the enantiomers of a corresponding racemic sample prepared from (R,S)-valine. Treatment of the γ-hydroxyleucine derivative (**5c**) with 2,2,2-trifluoroethanol gave the known (S)-lactone (**6**)[11].

The synthesis of (**1d**), (**2d**), (**4c**) and (**5c**) illustrates a complementary method[4,5] for the stereocontrolled synthesis of homochiral hydroxy-α-amino acid derivatives. Using this procedure the stereochemistry of the starting amino acids is retained in the products. The regioselectivity of the hydroxylation is determined by that of the bromination of the N-phthaloylamino acid derivatives, which in turn reflects the stability of the corresponding intermediate radicals. The procedure is suitable for the

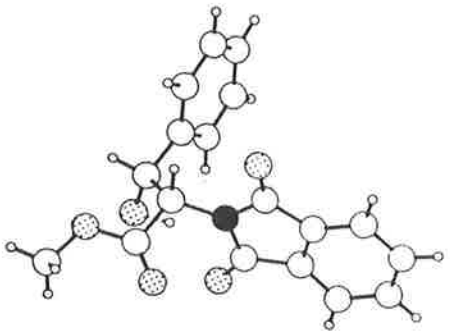


Figure 1. Molecular structure of (**1d**)

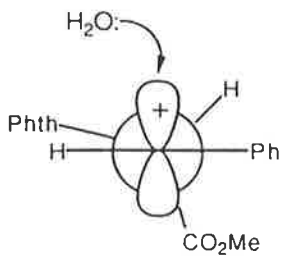


Figure 2. Diastereoselective reaction to give (**1d**)

preparation of free hydroxy amino acids, as illustrated by the deprotection of (**1d**) on treatment with a 2:1 mixture of 6N hydrochloric acid and acetic acid, at reflux for 5 h, to give the known[1,2] (2S,3R)-3-phenylserine

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References and Notes

1. For examples see: D. H. Williams, *Acc. Chem. Res.*, **1984**, *17*, 364; C. M. Harris, H. Kopecka and T. M. Harris, *J. Am. Chem. Soc.*, **1983**, *105*, 6915; S. D. Jolad, J. J. Hoffmann, S. J. Torrance, R. M. Wiedhopf, J. R. Cole, S. K. Arora, R. B. Bates, R. L. Gargiulo and G. R. Kriek, *J. Am. Chem. Soc.*, **1977**, *99*, 8040; and references cited therein.
2. I. Cenci di Bello, P. Dorling, L. Fellows and B. Winchester, *FEBS Lett.*, **1984**, *176*, 61.
3. M. J. Miller, *Acc. Chem. Res.*, **1986**, *19*, 49; D. M. Floyd, A. W. Fritz, J. Pluscec, E. R. Weaver and C. M. Cimarusti, *J. Org. Chem.*, **1982**, *47*, 5160.
4. For selected references see: G. Guanti, L. Banfi, E. Narisano and C. Scolastico, *Tetrahedron*, **1988**, *44*, 3671; D. Seebach, E. Juaristi, D. D. Miller, C. Schickli and T. Weber, *Helv. Chim. Acta*, **1987**, *70*, 237; A. V. Rama Rao, T. G. Murali Dhar, D. Subhas Bose, T. K. Chakraborty and M. K. Gurjar, *Tetrahedron*, **1989**, *45*, 7361; A.V. Rama Rao, J. S. Yadav, S. Chandrasekhar and C. Srinivas Rao, *Tetrahedron Lett.*, **1989**, *30*, 6769; M. E. Jung and Y. H. Jung, *Tetrahedron Lett.*, **1989**, *30*, 6637; D. A. Evans, E. B. Sjogren, A. E. Weber and R. E. Conn, *Tetrahedron Lett.*, **1987**, *28*, 39; C.-Q. Sun and D. H. Rich, *Tetrahedron Lett.*, **1988**, *29*, 5205; N. Kurokawa and Y. Ohfune, *J. Am. Chem. Soc.*, **1986**, *108*, 6041; D. A. Evans and A. E. Weber, *J. Am. Chem. Soc.*, **1987**, *109*, 7151; R. C. Roemmele and H. Rapoport, *J. Org. Chem.*, **1989**, *54*,1866; and references quoted therein.
5. K. Shimamoto and Y. Ohfune, *Tetrahedron Lett.*, 1988, *29*, 5177.
6. J. C. Sheehan, D. W. Chapman and R. W. Roth, *J. Am. Chem. Soc.*, **1952**, *74*, 3822.
7. C. J. Easton, E. W. Tan and M. P. Hay, *J. Chem. Soc., Chem. Commun.*, **1989**, 385.
8. All new compounds gave satisfactory ^{1}H NMR, IR, high resolution mass spectral and microanalytical data.
9. Molecular structure of (**1d**); trigonal space group, $P3_221$, a = 11.810(7), c = 19.556(7) Å^3, R =0.053 for 1077 reflections.
10. H. L. Goering, J. N. Eikenberrry, G. S. Koermer and C. J. Lattimer, *J. Am. Chem. Soc.*, **1974**, *96*, 1493.
11. S. Clarke, R. C. Hider and D. I. John, *J. Chem. Scc., Perkin Trans. 1*, **1973**, 230.
12. K. Vogler, *Helv. Chim. Acta*, **1950**, *33*, 2111.

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Synthesis and Molecular Structure of Stable Derivatives of (*E*)- and (*Z*)-Dehydrophenylalanine

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Abstract

Bromination of the phenylalanine derivative (1) affords the bromides (2) and (3), which react by stereospecific *anti*-elimination to give the isomeric pair of stable dehydrophenylalanine derivatives (4) and (5). The molecular structures of (4) and (5), which have been determined by X-ray crystallographic analysis, correlate with their lack of interconversion and the unusual stability of (5).

Introduction

α,β-Dehydrophenylalanine derivatives occur widely in nature as constituents of peptides, many of which have interesting physiological properties.[1] They are also of interest in the synthesis of other phenylalanine derivatives,[2–7] particularly through asymmetric hydrogenation,[4–7] and have been used as structural variants to probe structure–activity relationships in peptides.[8,9] Although a number of procedures have been reported for the synthesis of dehydrophenylalanine derivatives,[1] many afford the (*Z*)-isomer exclusively and significant quantities of the (*E*)-isomer have been produced only in a limited number of cases.[1,6,10] Even in those cases the (*E*)-isomer has often proved unstable and isomerized to give the more stable (*Z*)-isomer.[6,10]

In this report we describe a procedure for the stereocontrolled synthesis of the stable dehydrophenylalanine derivatives (4) and (5). Their molecular structures have been determined by X-ray crystallographic analysis, and

[1] Schmidt, U., Lieberknecht, A., and Wild, J., *Synthesis*, 1988, 159, and references cited therein.
[2] Samarenko, V. Y., Markova, N. R., and Passet, B. V., *Zh. Org. Khim.*, 1982, **18**, 139.
[3] Cativiela, C., Diaz de Villegas, M. D., Mayoral, J. A., and Melendez, E., *J. Org. Chem.*, 1985, **50**, 3167.
[4] Glaser, R., and Geresh, S., *Tetrahedron*, 1979, **35**, 2381.
[5] Onuma, K.-I., Ito, T., and Nakamura, A., *Bull. Chem. Soc. Jpn*, 1980, **53**, 2016.
[6] Vineyard, B. D., Knowles, W. S., Sabacky, M. J., Bachman, G. L., and Weinkauff, D. J., *J. Am. Chem. Soc.*, 1977, **99**, 5946.
[7] Fuganti, C., Grasselli, P., Malpezzi, L., and Casati, P., *J. Org. Chem.*, 1986, **51**, 1126.
[8] Shimohigashi, Y., Costa, T., and Stammer, C. H., *FEBS Lett.*, 1981, **133**, 269.
[9] Shimohigashi, Y., English, M. L., Stammer, C. H., and Costa, T., *Biochem. Biophys. Res. Commun.*, 1982, **104**, 583.
[10] Brocklehurst, K., Price, H. S., and Williamson, K., *J. Chem. Soc., Chem. Commun.*, 1968, 884.

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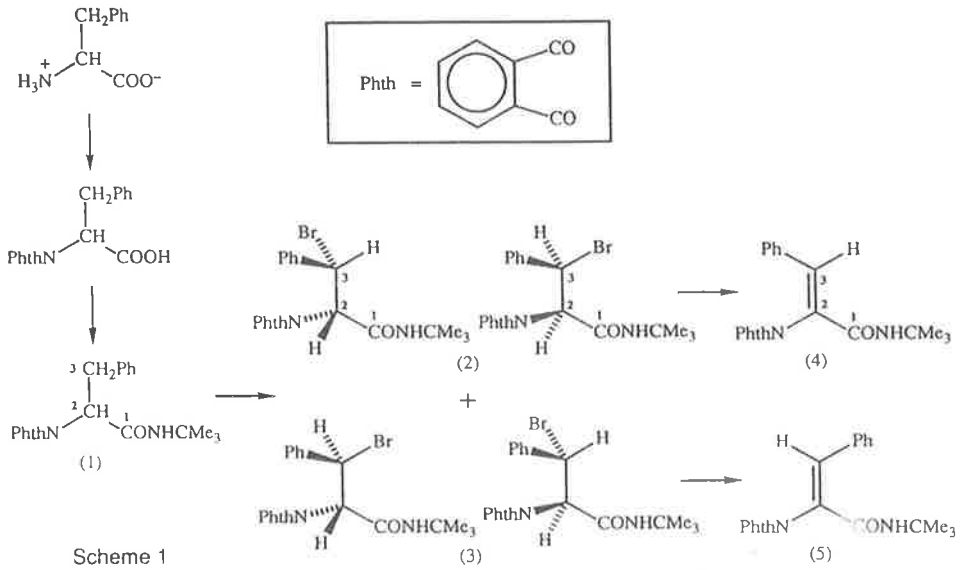
the relationship between their structures and their chemical behaviour and spectroscopic properties has been examined.

Results and Discussion

The (*Z*)-dehydrophenylalanine derivative (4) and the corresponding (*E*)-isomer (5) were synthesized as outlined in Scheme 1. A mixture of phthalic anhydride and (*RS*)-phenylalanine was heated at 150° to give *N*-phthaloylphenylalanine.[11] Treatment of *N*-phthaloylphenylalanine with thionyl chloride and a catalytic amount of pyridine gave the corresponding acid chloride, which gave *N*-t-butyl-N^2-phthaloylphenylalaninamide (1)* when treated with t-butylamine. Reaction of (1) with *N*-bromosuccinimide in a refluxing mixture of carbon tetrachloride/dichloromethane (3 : 1) under nitrogen, with reaction initiated by irradiation with a 250-W mercury lamp,[12] gave a 1 : 1 mixture of the diastereomeric *β*-bromophenylalanine derivatives (2) and (3), in quantitative yield. Attempts to separate (2) and (3) by fractional crystallization or by chromatography on silica were only partly successful; however, recrystallization of the mixture from a 1 : 1 mixture of light petroleum/propan-2-ol resulted in the formation of crystals of two distinct sizes and shapes. After separation of the crystal types on 0·25 and 0·60 mm mesh sieves, the small colourless crystals were recrystallized from light petroleum/propan-2-ol to give (2) in 41% yield, while recrystallization of the pale yellow granules gave (3) in 39% yield.



Scheme 1

* The carbon atoms of the aliphatic amino acid chain in phenylalanine are usually designated *α* (for the atom attached to the amino and carboxy groups) and *β* (for the atom attached to the phenyl ring): $PhC^{\beta}H_2C^{\alpha}H(NH_2)CO_2H$. In this paper, however, numerical locants will be used for the sake of convenience [see structure (1) (Scheme 1), and Fig. 1].

[11] Sheehan, J. C., Chapman, D. W., and Roth, R. W., *J. Am. Chem. Soc.*, 1952, **74**, 3822.
[12] Easton, C. J., Tan, E. W., and Hay, M. P., *J. Chem. Soc., Chem. Commun.*, 1989, 385.

Treatment of the bromide (2) with the 18-crown-6 complex of potassium fluoride in acetonitrile[13] at reflux for 0·5 h gave the (*Z*)-dehydrophenylalanine derivative (4) in 84% yield. Similar treatment of the diastereomeric bromide (3) gave the (*E*)-dehydrophenylalanine derivative (5) in 57% yield. The reactions of (2) and (3) appear to be stereospecific and most probably involve *anti*-elimination. On this basis the bromide (2) may be assigned the (2*RS*,3*RS*)-stereochemistry, and (3) the (2*RS*,3*SR*)-stereochemistry.

The molecular structures of the dehydrophenylalanine derivatives (4) and (5), as determined by X-ray crystallographic analysis, are illustrated in Fig. 1,

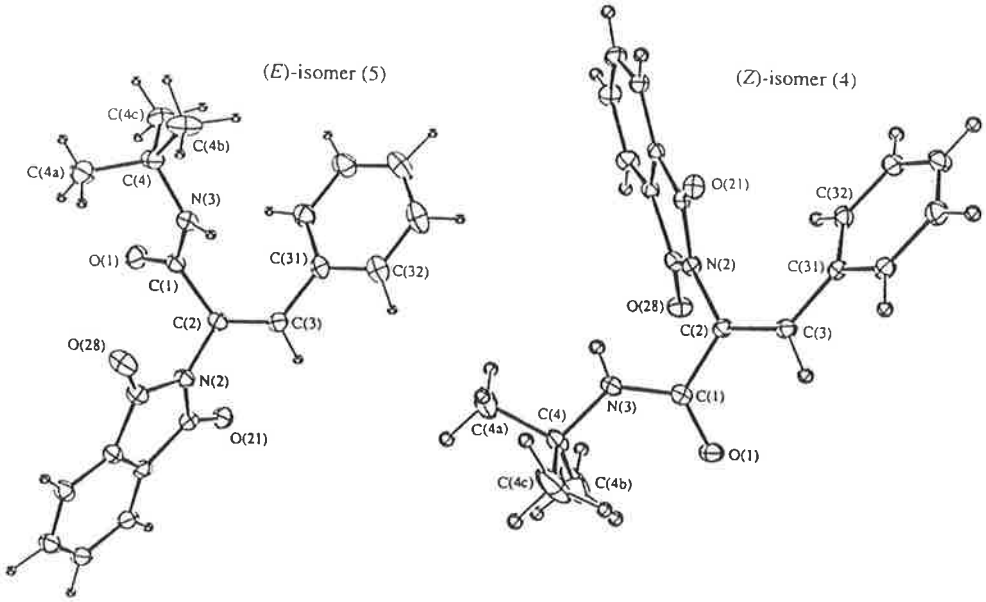


Fig. 1. Molecular structure and crystallographic numbering scheme employed for (*E*)-*N*-t-butyl-N^2-phthaloyldehydrophenylalaninamide (5) and (*Z*)-*N*-t-butyl-N^2-phthaloyldehydrophenylalaninamide (4).

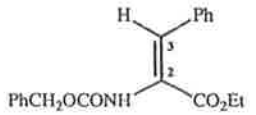
Table 1. Selected interatomic parameters for (*Z*)-*N*-t-butyl-N^2-phthaloyldehydrophenylalaninamide (4) and (*E*)-*N*-t-butyl-N^2-phthaloyldehydrophenylalaninamide (5)

Atoms	Bond lengths (Å) (5)	(4)	Atoms	Bond angles (degrees) (5)	(4)
C(1)–O(1)	1·216(4)	1·236(3)	O(1)–C(1)–N(3)	124·9(3)	124·2(2)
C(1)–N(3)	1·340(5)	1·337(3)	O(1)–C(1)–C(2)	119·8(3)	120·1(2)
C(1)–C(2)	1·505(5)	1·502(3)	N(3)–C(1)–C(2)	115·3(3)	115·7(2)
C(2)–N(2)	1·428(4)	1·431(3)	C(1)–C(2)–N(2)	113·5(3)	117·1(2)
C(2)–C(3)	1·331(5)	1·336(3)	C(1)–C(2)–C(3)	126·8(3)	120·3(2)
N(3)–C(4)	1·467(5)	1·480(3)	N(2)–C(2)–C(3)	119·5(3)	122·5(2)
C(3)–C(31)	1·475(5)	1·462(3)	C(2)–C(3)–C(31)	127·3(4)	129·8(2)
			C(2)–C(3)–H(3)	119(2)	116(1)
			C(31)–C(3)–H(3)	114(2)	114(1)

[13] Liotta, C. L., and Harris, H. P., *J. Am. Chem. Soc.*, 1974, **96**, 2250.

and selected bond lengths and bond angles are listed in Table 1. The structures are truly molecular, there being no significant intermolecular interactions in either crystal lattice. As can be seen from Table 1 there are no significant differences in bond lengths describing the two isomers. There are some significant differences in bond angles, however, notably the angles about C(2). The C(1)–C(2)–C(3) angle of 126·8(3)° in the (*E*)-isomer (5) is greater than the comparable angle of 120·3(2)° in the (*Z*)-isomer (4) owing to the proximity of the phenyl group to the *N*-alkylamido residue; the remaining angles about C(2) are contracted in (5) to accommodate the expansion in the C(1)–C(2)–C(3) angle. The lack of coplanarity of the four critical residues around C(2)–C(3) indicates that there is little if any extended conjugation in either (4) or (5). In summary, on the basis of the results of the X-ray crystallographic analysis the only apparent differences defining the two molecules may be explained in terms of steric effects.

The crystal structures of several other dehydrophenylalanine derivatives have been reported.[7,14–21] Interestingly in all but one example[14] the compounds were found to have the (*Z*)-configuration and showed little or no extended conjugation. In the case of (6), the single (*E*)-dehydrophenylalanine derivative hitherto reported, there was found to be substantial conjugation between the carbamate residue and C(2)–C(3). This difference probably arises from the relative facility of the carbamate residue to reorganize the π-electron density in (6), compared to the amido substituents in each of the (*Z*)-dehydrophenylalanine derivatives.



(6)

There are no molecular structure data for the (*Z*)-isomer corresponding to (6), and the isomers (4) and (5) structurally characterized in this study are the first such pair available. The data for (5) establish that extended conjugation is not a prerequisite for synthesis of (*E*)-dehydrophenylalanine derivatives. The steric effects evident from a comparison of (4) and (5) may reflect why the (*Z*)-isomers of dehydrophenylalanine derivatives seem to predominate, but the extent of this correlation is not clear.

There has been a number of reports of the isomerization of dehydrophenylalanine derivatives under neutral and acidic conditions.[14,22,23] For example,

[14] Nitz, T. J., Holt, E. M., Rubin, B., and Stammer, C. H., *J. Org. Chem.*, 1981, **46**, 2667.
[15] Brocklehurst, K., Bywater, R. P., Palmer, R. A., and Patrick, R., *J. Chem. Soc., Chem. Commun.*, 1971, 632.
[16] Ajo, D., Casarin, M., Granozzi, G., and Buseti, V., *Tetrahedron*, 1981, **37**, 3507.
[17] Ajo, D., Buseti, V., and Granozzi, G., *Tetrahedron*, 1982, **38**, 3329.
[18] Ajo, D., Buseti, V., Ottenheim, H. C. J., and Plate, R., *Acta Crystallogr., Sect. C*, 1984, **40**, 324.
[19] Buseti, V., Ajo, D., and Casarin, M., *Acta Crystallogr., Sect. C*, 1984, **40**, 1245.
[20] Buseti, V., Ajo, D., and Vittandi, A., *Acta Crystallogr., Sect. C*, 1986, **42**, 1178.
[21] Glowka, M. L., Gilli, G., Bertolasi, V., and Makowski, M., *Acta Crystallogr., Sect. C*, 1987, **43**, 1403.
[22] Poisel, H., and Schmidt, U., *Chem. Ber.*, 1975, **108**, 2547.
[23] Srinivasan, A., Stephenson, R. W., and Olsen, R. K., *J. Org. Chem.*, 1977, **42**, 2256.

the (*E*)-dehydrophenylalanine derivative (6) isomerized to the corresponding (*Z*)-isomer on heating in chloroform and, more rapidly, on standing in neat trifluoroacetic acid.[14] In contrast both (4) and (5) were stable under these conditions. The stability of (4) and (5) to acid presumably reflects the lack of extended conjugation and the steric hindrance to direct protonation of the double bond in each molecule.

Finally, it has been possible to confirm an empirical spectroscopic rule for the determination of configuration of dehydrophenylalanine derivatives. Srinivasan, Richards and Olsen[24] reported that in compounds with the (*Z*)-configuration the ^{1}H n.m.r. chemical shift of the vinyl proton shows a downfield shift when the solvent is changed from chloroform to trifluoroacetic acid, whereas the converse is observed with the (*E*)-isomers. In accord with this observation, the ^{1}H n.m.r. chemical shift of the vinyl proton in (4) moves 0·15 ppm downfield from δ 7·59 in chloroform to 7·74 in trifluoroacetic acid, whereas that of (5) moves 0·17 ppm upfield from δ 7·05 in chloroform to 6·88 in trifluoroacetic acid.

Experimental

General experimental details have been reported previously.[25]

N-*t-Butyl*-N^2-*phthaloylphenylalaninamide (1)*

Thionyl chloride (24 g, 0·2 mol) and pyridine (0·2 ml) were added to a suspension of *N*-phthaloylphenylalanine[11] (20·0 g, 68 mmol) in carbon tetrachloride (200 ml). The mixture was heated at reflux for 2 h; then it was cooled and concentrated under reduced pressure. The residual oil was suspended in carbon tetrachloride (200 ml), and t-butylamine (12·4 g, 170 mmol) was added. The resultant mixture was heated at reflux for 1 h; then it was cooled and concentrated under reduced pressure. The residue was dissolved in ethyl acetate; the solution was washed with dilute aqueous sodium carbonate and water, then dried and concentrated under reduced pressure. The residual solid was recrystallized from light petroleum/ethyl acetate to give N-*t-butyl*-N^2-*phthaloylphenylalaninamide* (1) as colourless crystals (20·6 g, 87%), m.p. 194–195° (Found: C, 72·0; H, 6·3; N, 8·1. $C_{21}H_{22}N_2O_3$ requires C, 72·0; H, 6·3; N, 8·0%). ^{1}H n.m.r. ($CDCl_3$) δ 1·30, s, 9H; 3·48, dd, *J* 10·1, 14·0 Hz, 1H; 3·56, dd, *J* 6·7, 14·0 Hz, 1H; 4·99, dd, *J* 6·7, 10·1 Hz, 1H; 5·82, br s, 1H; 7·18, m, 5H; 7·75, m, 4H. ν_{max} 3300, 1720, 1660, 1510 cm^{-1}. Mass spectrum: *m/z* 350 (M).

(2RS,3RS)-*3-Bromo*-N-*t-butyl*-N^2-*phthaloylphenylalaninamide (2) and* (2RS,3SR)-*3-Bromo*-N-*t-butyl*-N^2-*phthaloylphenylalaninamide (3)*

N-t-Butyl-*N*^{2}-phthaloylphenylalaninamide (1) (20·0 g, 57 mmol) was dissolved in dichloromethane/carbon tetrachloride (1 : 3, 200 ml). *N*-Bromosuccinimide (12·0 g, 67·5 mmol) was added and the mixture was heated at reflux for 2 h, while it was irradiated with a 250-W mercury lamp. The resultant solution was allowed to cool, then it was filtered; the filtrate was washed with water, then dried and concentrated under reduced pressure to give a 1 : 1 mixture of (2*RS*,3*RS*)- and (2*RS*,3*SR*)-3-bromo-*N*-t-butyl-*N*^{2}-phthaloylphenylalaninamide (2) and (3) (24·5 g, 100%).

[24] Srinivasan, A., Richards, K. D., and Olsen, R. K., *Tetrahedron Lett.*, 1976, 891.
[25] Easton, C. J., and Peters, S. C., *Aust. J. Chem.*, 1990, **43**, 87.

A sample of the mixture (15·0 g) was recrystallized from light petroleum/propan-2-ol (1:1, 500 ml) to give two types of crystals. These were separated according to size by using 0·25 and 0·60 mm mesh sieves. The smaller, colourless, needle-shaped crystals were recrystallized from light petroleum/propan-2-ol to give (*2*RS,*3*RS)-*3-bromo*-N-*butyl*-N^2-*phthaloylphenylalaninamide* (2) (6·10 g, 41%), m.p. 199–200° (Found: C, 58·8; H, 4·9; N, 6·6. $C_{21}H_{21}BrN_2O_3$ requires C, 58·8; H, 4·9; N, 6·5%). ^{1}H n.m.r. ($CDCl_3$) δ 1·03, s, 9H; 5·28, d, J 11·8 Hz, 1H; 5·85, br s, 1H; 6·25, d, J 11·8 Hz, 1H; 7·60, m, 5H; 7·95, m, 4H. ν_{max} 3375, 1775, 1705, 1530 cm^{-1}. Mass spectrum: *m/z* 430, 428 (M).

The larger, pale yellow, granular crystals were recrystallized from light petroleum/propan-2-ol to give (*2*RS,*3*SR)-*3-bromo*-N-*t-butyl*-N^2-*phthaloylphenylalaninamide* (3) (5·84 g, 39%), m.p. 213–213·5° (Found: C, 58·5; H, 5·0; N, 6·8. $C_{21}H_{21}BrN_2O_3$ requires C, 58·8; H, 4·9; N, 6·5%). ^{1}H n.m.r. ($CDCl_3$) δ 1·43, s, 9H; 5·32, d, J 11·5 Hz, 1H; 6·04, d, J 11·5 Hz, 1H; 6·50, br s, 1H; 7·40, m, 5H; 7·75, m, 4H. ν_{max} 3350, 1700, 1530 cm^{-1}. Mass spectrum: *m/z* 430, 428 (M).

(Z)-N-*t-Butyl*-N^2-*phthaloyldehydrophenylalaninamide (4)*

A solution of 18-crown-6 in acetonitrile (0·15 M, 25 ml) was treated with anhydrous potassium fluoride (0·11 g, 2·3 mmol), and stirred vigorously at reflux for 0·5 h. (*2RS,3RS*)-3-Bromo-*N*-t-butyl-*N*^{2}-phthaloylphenylalaninamide (2) (0·50 g, 1·2 mmol) was then added; the mixture was stirred at reflux for a further 0·5 h, then it was cooled and concentrated under reduced pressure. The residue was suspended in ethyl acetate; the suspension was washed with water, then dried and concentrated under reduced pressure. Recrystallization of the solid residue from ethyl acetate/light petroleum afforded (Z)-N-*t-butyl*-N^2-*phthaloyldehydrophenylalaninamide* (4) (0·36 g, 84%) as colourless crystals, m.p. 214–215° (Found: C, 72·6; H, 5·8; N, 8·0%; M$^+$, 248·1487. $C_{21}H_{20}N_2O_3$ requires C, 72·4; H, 5·8; N, 8·0%; M$^+$, 348·1474). ^{1}H n.m.r. ($CDCl_3$) δ 1·40, s, 9H; 5·97, br s, 1H; 7·25, m, 5H; 7·59, s, 1H; 7·80, m, 4H. ^{1}H n.m.r. (CF_3COOH) δ 7·74, s, 1H. ν_{max} 3350, 1780, 1720, 1660, 1630, 1525 cm^{-1}.

(E)-N-*t-Butyl*-N^2-*phthaloyldehydrophenylalaninamide (5)*

Treatment of (*2RS,3SR*)-3-bromo-*N*-t-butyl-*N*^{2}-phthaloylphenylalaninamide (3) with the 18-crown-6 complex of potassium fluoride in acetonitrile, as described above for the preparation of (4) from (2), gave (5) as colourless *plates* in 57% yield, m.p. 185–186° (Found: C, 72·5; H, 5·8; N, 8·0%; M$^+$, 348·1467. $C_{21}H_{20}N_2O_3$ requires C, 72·4; H, 5·8; N, 8·0%; M$^+$, 348·1474). ^{1}H n.m.r. ($CDCl_3$) δ 1·22, s, 9H; 5·50, br s, 1H; 7·05, s, 1H; 7·40, m, 5H; 7·85, m, 4H. ^{1}H n.m.r. (CF_3COOH) δ 6·88, s, 1H. ν_{max} 3420, 1715, 1660, 1510 cm^{-1}.

Crystallography

Intensity data for (4) and (5) were measured at 293 and 210 K, respectively, on an Enraf–Nonius CAD4F diffractometer fitted with graphite-monochromatized Mo Kα radiation (λ 0·7107 Å). The ω:2θ scan technique was employed to measure data up to a maximum Bragg angle of 22·5° in each case. The data were corrected for Lorentz and polarization effects but not for absorption. Relevant crystal data are collected in Table 2.

The structures were solved by direct methods,[26] and each was refined by a full-matrix least-squares procedure based on F.[26] Non-hydrogen atoms in both models were refined with anisotropic thermal parameters, and in (5) all hydrogen atoms were refined isotropically. For (4) the methyl hydrogen atoms were included in the model at their calculated positions, and the remaining hydrogen atoms were refined isotropically as for (5). After the inclusion of a weighting scheme of the form $w = k/[\sigma^2(F)+g(F)^2]$, the refinements were continued until convergence. Final refinement details are listed in Table 2. The analysis of variance showed no special features in any of the refinements; this indicated that appropriate weighting schemes had been applied in each case. Fractional atomic coordinates are listed in Table 3,

[26] Sheldrick, G. M., SHELX76, Program for Crystal Structure Determination, Cambridge University, U.K., 1976.

Table 2. Crystal data and refinement details for (*Z*)-*N*-t-butyl-N^2-phthaloyldehydrophenylalaninamide (4) and (*E*)-*N*-t-butyl-N^2-phthaloyldehydrophenylalaninamide (5)

	(5)	(4)		(5)	(4)
Formula	$C_{21}H_{20}N_2O_3$	$C_{21}H_{20}N_2O_3$	D_c (g cm^{-3})	1·246	1·276
Mol. wt	348·4	348·4	$F(000)$	1472	736
Crystal system	orthorhombic	monoclinic	μ (cm^{-1})	0·49	0·50
Space group	*Pbca*	$P2_1/c$	No. of data collected	2800	3546
a (Å)	37·444(4)	14·934(1)	No. of unique data	2397	3198
b (Å)	13·564(2)	11·249(2)	No. of unique reflections		
c (Å)	7·313(1)	10·811(2)	used with $I \geq 2{\cdot}5\sigma(I)$	1337	1972
α (degrees)	90	90	R	0·040	0·041
β (degrees)	90	92·79(1)	k	1·0	0·14
γ (degrees)	90	90	g	0·016	0·021
V (Å^3)	3714·2	1814·0	R_w	0·043	0·048
Z	8	4	Residual ρ_{max} (e Å^{-3})	0·13	0·36

Table 3. Fractional atomic coordinates for (*Z*)-*N*-t-butyl-N^2-phthaloyldehydrophenylalaninamide (4) and (*E*)-*N*-t-butyl-N^2-phthaloyldehydrophenylalaninamide (5)

Atom	(*Z*)-Isomer (4)			(*E*)-Isomer (5)		
	10^4x	10^4y	10^4z	10^4x	10^4y	10^4z
O(1)	2229(1)	8023(2)	4055(2)	−1148(1)	795(2)	2107(4)
O(21)	4136(1)	6184(1)	700(1)	−979(1)	−1467(2)	−1636(4)
O(28)	1645(1)	4943(2)	2659(2)	−503(1)	−754(3)	3968(4)
N(2)	2902(1)	5823(2)	1879(2)	−826(1)	−1103(2)	1367(4)
N(3)	1792(1)	7820(2)	2021(2)	−1329(1)	64(3)	4735(4)
C(1)	2311(2)	7564(2)	3028(2)	−1213(1)	61(3)	3003(5)
C(2)	3048(2)	6682(2)	2844(2)	−1170(1)	−943(3)	2153(4)
C(3)	3818(2)	6753(2)	3522(2)	−1421(1)	−1631(3)	1980(5)
C(4)	1016(2)	8640(2)	1944(2)	−1396(1)	938(3)	5865(5)
C(4a)	560(3)	8471(4)	668(3)	−1052(1)	1522(6)	6085(11)
C(4b)	361(2)	8321(5)	2900(4)	−1530(2)	560(5)	7695(7)
C(4c)	1343(3)	9906(3)	2087(5)	−1684(1)	1567(4)	4993(8)
C(21)	3480(2)	5605(2)	905(2)	−756(1)	−1324(3)	−478(5)
C(22)	3104(2)	4545(2)	247(2)	−358(1)	−1354(2)	−631(5)
C(23)	3416(2)	3939(2)	−762(2)	−145(1)	−1519(3)	−2135(6)
C(24)	2907(2)	2983(2)	−1194(3)	223(1)	−1484(3)	−1836(7)
C(25)	2130(2)	2637(2)	−648(2)	367(1)	−1286(3)	−150(6)
C(26)	1829(2)	3235(2)	377(2)	151(1)	−1103(3)	1331(6)
C(27)	2334(2)	4192(2)	811(2)	−217(1)	−1146(3)	1055(5)
C(28)	2204(2)	4988(2)	1885(2)	−511(1)	−966(3)	2380(6)
C(31)	4615(2)	6000(2)	3508(2)	−1799(1)	−1562(3)	2546(5)
C(32)	4578(2)	4775(2)	3282(2)	−1963(1)	−2394(4)	3260(5)
C(33)	5344(2)	4095(3)	3324(2)	−2313(1)	−2356(5)	3840(7)
C(34)	6161(2)	4602(3)	3583(2)	−2504(1)	−1507(5)	3661(7)
C(35)	6219(2)	5814(3)	3824(2)	−2352(1)	−691(5)	2914(8)
C(36)	5452(2)	6497(2)	3798(2)	−1997(1)	−712(4)	2332(6)

and the numbering schemes employed are shown in Fig. 1 which was drawn with ORTEP[27] at 15% probability ellipsoids. Scattering factors were as incorporated in the SHELX76 program,[26] and refinement was performed on a Sun4/280 computer. Complete lists of bond distances and angles, thermal parameters, hydrogen atom coordinates and structure factor tables have been deposited with the Australian Journal of Chemistry, P.O. Box 89, East Melbourne, Vic. 3002.

Acknowledgment

This work was supported by grants from the Australian Research Council.

[27] Johnson, C. K., ORTEP-II, Report ORNL-2794, Oak Ridge National Laboratory, Tennessee, U.S.A., 1971.

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Cyclodextrin Inclusion Complexes of Two Non-Steroidal Antiinflammatory Drugs and of an Analgesic Drug

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Abstract

U.v.–visible spectrophotometric studies of the interactions of Naproxen, Ibuprofen and Panadol with α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin and dimethyl β-cyclodextrin enable the determination of stability constants of inclusion complexes when their formation gives rise to appreciable spectral changes. The magnitudes of the stability constants are discussed in terms of the relative sizes and the chemical natures of the cyclodextrins and the included molecules.

Introduction

Inclusion complexes are chemical species consisting of two associated molecules in which one of the molecules, the 'host', forms or possesses a cavity into which it can admit a 'guest' molecule, this resulting in a stable association involving only non-covalent bonds.[1]

α-, β- and γ-cyclodextrins, cyclic (1→4)-linked oligomers containing six, seven and eight glucopyranose units respectively, which contain cavities approximately in the shape of truncated right cones of depth 790–800 pm and with terminal radii of 470–520, 600–640 and 750–830 pm respectively, are capable of acting as hosts for a wide variety of guest molecules which are characterized by the possession of well defined hydrophobic moieties.[2–6]

Non-steroidal antiinflammatory drugs and analgesic drugs, in common with many orally administered drugs, contain both hydrophobic and hydrophilic moieties and are thus potentially suitable for inclusion as guests within some of the cyclodextrins.[3,4] Furthermore, it has been proposed that the known deleterious effects of non-steroidal antiinflammatory drugs on the epithelium of the gastro-intestinal tract may be ameliorated by their inclusion within a cyclodextrin molecule, resulting in a reduced concentration of free drug in the gastro-intestinal tract.[3,4] However, as has been pointed out by Habon

[1] Cramer, F., *Rev. Pure Appl. Chem.*, 1955, **5**, 143.
[2] Bender, M. L., and Komiyama, M., 'Cyclodextrin Chemistry' (Springer: Berlin 1978).
[3] Saenger, W., *Angew. Chem., Int. Ed. Engl.*, 1980, **19**, 344.
[4] Szejtli, J., 'Cyclodextrins and Their Inclusion Complexes' (Akademio Kaido: Budapest 1982).
[5] Tabushi, I., *Acc. Chem. Res.*, 1982, **15**, 66.
[6] Clarke, R. J., Coates, J. H., and Lincoln, S. F., *Adv. Carbohydr. Chem. Biochem.*, 1988, **46**, 205.

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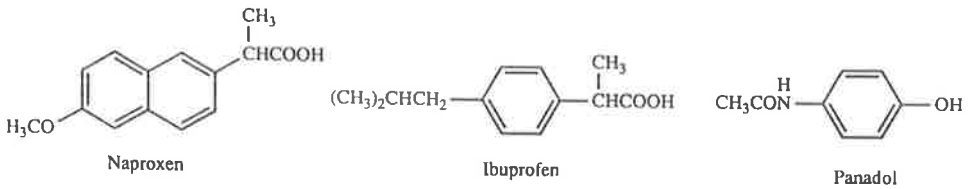
et al.,[7] inclusion within a cyclodextrin will only be effective in reducing the concentration of free drug if the inclusion complex stability constant is sufficiently large.

Here we report determinations of the stability constants of some inclusion complexes formed between a number of cyclodextrins and the three drugs Naproxen, Ibuprofen and Panadol.



Naproxen Ibuprofen Panadol

Experimental

On inclusion of a molecule within a cyclodextrin, the u.v.–visible spectrum is usually altered, since on inclusion the solvation shell of the molecule is partly or wholly replaced by the cyclodextrin molecule, this leading to altered solute/environment interactions.[4–6] The measurements reported here involve determining the spectrum of each drug alone and in the presence of increasing concentrations of a cyclodextrin. Two approaches can be used. When the change in molar absorption coefficient on inclusion is large, conventional spectrophotometry is employed and the spectrum of a solution of drug mixed with cyclodextrin in an appropriate buffer is measured against a buffer reference solution. When the change in molar absorption coefficent is small, difference spectrophotometry is employed to allow the use of higher drug concentrations, without causing the difference in absorbance between the reference and sample beams to be greater than the range the instrument will permit. In this technique, spectrophotometer cells each with two identical cell compartments in series are used; both cell compartments in the sample beam contain a solution made up of equal volumes of cyclodextrin and drug solutions mixed together, whereas in the reference beam unmixed drug and cyclodextrin solutions are separately placed in each compartment. All solutions are made up in an appropriate buffer solution.

For the formation of a single 1:1 species between a cyclodextrin molecule and a drug molecule the necessary equilibrium and conservation equations are as follows:

$$\mathbf{D} + \mathbf{C} \rightleftharpoons \mathbf{DC}$$

$$K = [\mathbf{DC}]/([\mathbf{D}][\mathbf{C}])$$

$$[\mathbf{D}] = [\mathbf{D}]_0 - [\mathbf{DC}] \qquad [\mathbf{C}] = [\mathbf{C}]_0 - [\mathbf{DC}]$$

where $[\mathbf{D}]_0$ and $[\mathbf{C}]_0$ are the initial concentrations of drug **D** and cyclodextrin **C** respectively in the reaction mixture. [**D**] and [**C**] are the equilibrium concentrations of **D** and **C**, and [**DC**] is the equilibrium concentration of the drug–cyclodextrin inclusion complex **DC**. K is the equilibrium constant for the formation of the inclusion complex. [**D**] and [**C**] were expressed in terms of [**DC**], substituted into the expression for the equilibrium constant, and the resulting quadratic equation was solved for [**DC**], the concentration of the inclusion complex at equilibrium, expressed in terms of $[\mathbf{D}]_0$, $[\mathbf{C}]_0$ and K.

The equations describing the absorbance are as follows. For conventional spectrophotometry the absorbances A_S and A_r of the sample and reference cells respectively, are given by:

$$A_S = l \times (\epsilon_{\mathbf{D}}[\mathbf{D}] + \epsilon_{\mathbf{C}}[\mathbf{C}] + \epsilon_{\mathbf{DC}}[\mathbf{DC}]) \qquad A_r = 0$$

[7] Habon, I., Fritsch, S., and Szejtli, J., *Pharmazie*, 1984, **39**, 830.

For difference spectrophotometry:

$$A_S = 2\,l' \times (\epsilon_D[\mathbf{D}] + \epsilon_C[\mathbf{C}] + \epsilon_{DC}[\mathbf{DC}]) \qquad A_r = l' \times (\epsilon_D[\mathbf{D}^R]_0 + \epsilon_C[\mathbf{C}^R]_0)$$

For both methods the observed absorbance A_{obs} is given by:

$$A_{obs} = A_S - A_r$$

where l is the path length of the cells used in conventional spectrophotometry; l' is the path length of the cell compartments used in difference spectrophotometry; $[\mathbf{D}^R]_0$ and $[\mathbf{C}^R]_0$ are respectively the concentrations of drug and cyclodextrin in each of the reference cell compartments, these concentrations having values close to $2[\mathbf{D}]_0$ and $2[\mathbf{C}]_0$ respectively; and ϵ_D, ϵ_C and ϵ_{DC} are the molar absorption coefficients of the drug, the cyclodextrin and the inclusion complex, respectively.

The values of ϵ_D and ϵ_C were determined in separate experiments in which a series of solutions of known concentration of each compound were made up in the appropriate buffer and their absorbances determined. Linear regression of the absorbance against the concentration gave the molar absorption coefficient for each compound.

A non-linear regression of A_{obs} (for wavelengths in the vicinity of maximum values of A_{obs}) against $[\mathbf{D}]_0$ and $[\mathbf{C}]_0$, in which estimates of K and ϵ_{DC} were iterated until a solution was found, was used to determine the best-fit values of K and ϵ_{DC} at each wavelength. The values of K varied significantly and smoothly from one wavelength to another, therefore an average value of K was calculated in which each K included was weighted inversely to its standard error. The K values included in the average were chosen so as to be representative of K values derived from as many absorption maxima as possible, this being consistent with having an acceptably small standard error for the average value of K. To validate the difference spectra method, idealized spectra with molar absorption coefficient and wavelength ranges similar to those found for drug–cyclodextrin mixtures were simulated by using appropriate equations to simulate vibronic spectra for the drug and the drug–cyclodextrin inclusion compound, and by using an assumed value for the equilibrium constant (K). Random variations were imposed on the simulated data which were then fitted in a similar manner to the experimental data. It was found that when A_{obs} values were small, variations in K occurred as a function of wavelength similar to those observed experimentally. Averaging the values of K, weighted as described above, returned a value of K close to that assumed in the simulation.

The drugs and the cyclodextrins were stored in evacuated desiccators over P_2O_5. Spectra were recorded by using a Zeiss DMR 10 double-beam spectrophotometer with a constant temperature cell block (±0·1 K) at 298·2 K. Spectral data were recorded digitally and analysed on a Sun 3/60 workstation. Phosphate buffer, pH 6·90, ionic strength 0·10, was prepared from ultra-pure MILLI-Q water.

Results and Discussion

Difference spectra were obtained for the formation of inclusion complexes between Naproxen [(*S*)-2-(6-methoxy-2-naphthyl)propionic acid] and α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin and dimethyl β-cyclodextrin [heptakis(2,6-di-*O*-methyl)-β-cyclodextrin], and their stability constants were determined as described above. Similar measurements were made for Ibuprofen [2-(4-(2-methylpropyl)phenyl)propionic acid] and Panadol [*N*-(4-hydroxyphenyl)acetamide]. The results are summarized in Table 1.

General Characteristics of the Difference Spectra

The difference spectra obtained for Naproxen for eight of 16 concentrations of β-cyclodextrin studied are shown in Fig. 1. It is apparent from the spectra that the spectrum of Naproxen included in β-cyclodextrin differs from that of Naproxen itself. The difference spectrum method, using wavelengths at which

Table 1. Inclusion stability constants (with standard error) determined in phosphate buffer at pH 6·90, ionic strength 0·10 and temperature 298·2 K

Drug	Cyclo-dextrin	Wavelength range(s) (nm)	No. of spectra	K (mol^{-1} dm^3)
Naproxen	α	340–333, 294–288[A]	17	16±1
Naproxen	β	339–334, 293–289[A]	16	670±40
Naproxen	γ	340–334, 293–288[A]	23	120±10
Naproxen	dimethyl β	340–331, 289–285[A]	20	510±80
Ibuprofen	α	[B]		
Ibuprofen	β	277–274[C]	18	2900±500
Ibuprofen	γ	[B]		
Ibuprofen	dimethyl β	277–275[C]	16	9100±500
Panadol	α	[B]		
Panadol	β	267–250[A]	18	130±10
Panadol	γ	[B]		
Panadol	dimethyl β	259–255[A]	19	83±3

[A] Spectra sampled at 1 nm wavelength intervals.
[B] Change in spectra too small to use.
[C] Spectra sampled at 0·5 nm wavelength intervals.

there is sufficient change in A_{obs}, allows determination of stability constants for such systems. Fig. 2 shows, for the Naproxen system, a typical plot of A_{obs} against concentration of β-cyclodextrin. The characteristic shape of the plot, showing the saturation of the cavities of the cyclodextrin molecules with molecules of the drug, is apparent. Fitting of the data points to the model outlined above allows calculation of the inclusion association constant and the molar absorption coefficient of the inclusion complex, from which values the smooth curve is then calculated.

The molar absorption coefficients of Naproxen and of the Naproxen–β-cyclodextrin complex, the latter calculated for wavelengths where the spectral

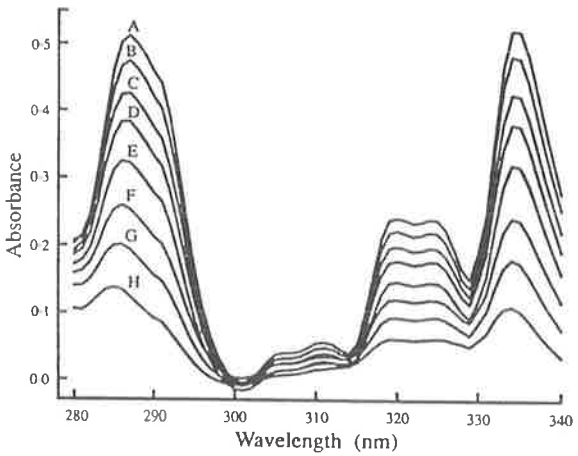


Fig. 1. Difference spectra measured at 1 nm intervals for solutions of Naproxen ($4·45\times10^{-4}$ mol dm^{-3}) in the presence of increasing concentrations of β-cyclodextrin (H, $0·331\times10^{-3}$; G, $0·643\times10^{-3}$; F, $0·971\times10^{-3}$; E, $1·78\times10^{-3}$; D, $2·45\times10^{-3}$; C, $3·26\times10^{-3}$; B, $5·24\times10^{-3}$; A, $7·71\times10^{-3}$ mol dm^{-3}) at 298·2 K in 0·10 ionic strength phosphate buffer at pH 6·90. Each cell compartment had a path length of 1·00 cm.

change was sufficiently large to allow satisfactory fitting of the difference spectra data, are shown in Fig. 3. Results were obtained in a similar way for the other two drugs studied in the presence of a number of cyclodextrins.

Naproxen

As shown in Table 1, values of K were obtained for inclusion compounds formed between Naproxen, which at pH 6·90 exists as the anion, and four cyclodextrins. The largest value of K (670±40 mol^{-1} dm^3) was obtained for

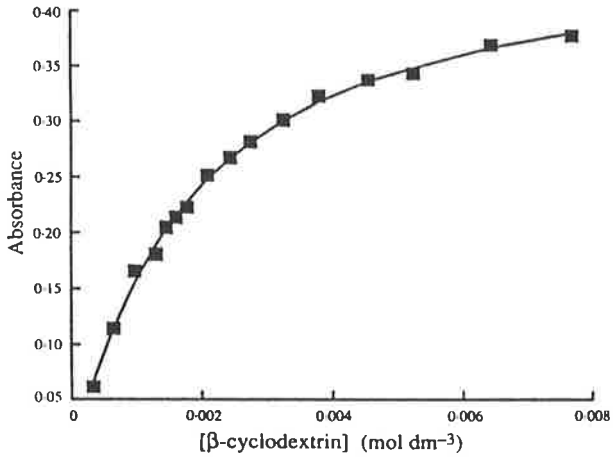


Fig. 2. Absorbance at 338 nm for solutions of Naproxen ($4·45\times10^{-4}$ mol dm^{-3}) containing increasing concentrations of β-cyclodextrin in 0·10 ionic strength phosphate buffer, pH 6·90 at 298·2 K.

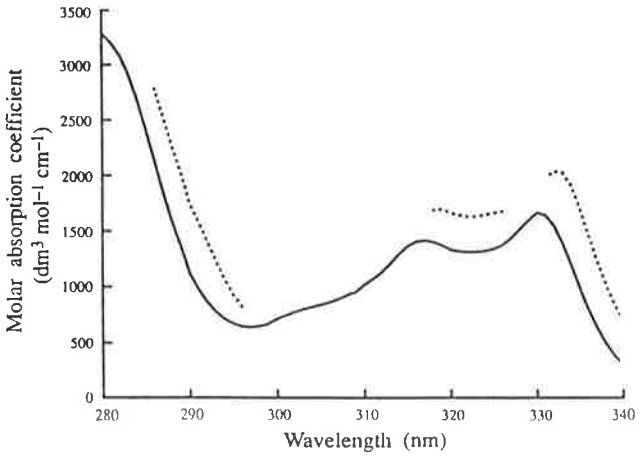


Fig. 3. The experimentally determined u.v.–visible spectrum of Naproxen (full line) together with the spectrum of the Naproxen–β-cyclodextrin inclusion complex (dotted line) calculated by the non-linear fitting program at wavelengths where the spectral deviation was sufficiently large.

β-cyclodextrin, which implies that, for the four cyclodextrin–Naproxen inclusion compounds studied, the closest geometrical fit occurs between β-cyclodextrin and Naproxen.[3-6]

Examination of Corey–Pauling–Koltun (CPK) space-filling molecular models confirms that the Naproxen molecule can penetrate the α-cyclodextrin annulus to only a small extent, leaving much of the naphthalene moiety exposed to solvent water, and leading to a small value of K (16±1 mol^{-1} dm^3). The internal diameter of the β-cyclodextrin annulus, however, is just great enough to allow the naphthalene moiety to reside inside with the methoxy and the carboxylate groups protruding from either end, thus simultaneously allowing the naphthalene moiety the minimum exposure to the solvent water, and the charged carboxylate group to avoid inclusion in the hydrophobic cyclodextrin cavity.

The K value for dimethyl β-cyclodextrin (510±80 mol^{-1} dm^3) is less than that for β-cyclodextrin, but since their internal diameters are identical there must be similar fit between the naphthalene moiety and the annulus. Methylation effectively extends the length of the cyclodextrin cone which may prevent the carboxylate protruding as far into the solvent water; also the interaction of the protruding carboxylate group with the methylated OH groups is less favourable than with the hydroxy groups of β-cyclodextrin itself. Both these effects would tend to decrease the stability of the dimethyl β-cyclodextrin complex compared with the β-cyclodextrin complex.

γ-Cyclodextrin has a much smaller K value (120±10 mol^{-1} dm^3) which is consistent with the looseness of fit between the naphthalene moiety and the inside of the γ-cyclodextrin annulus. Closeness of fit is important for the development of a strong van der Waals–London dispersion force interaction between the cyclodextrin and its guest.[8,9]

Ibuprofen

The hydrophobic moiety of Ibuprofen comprises a benzene ring substituted with a 2-methylpropyl group *para* to a propionic acid group; the latter is present as the carboxylate group at pH 6·90. Examination of CPK models shows that both substituents are probably too large to enter the α-cyclodextrin annulus, making the formation of an inclusion compound unlikely. This conclusion is consistent with the vanishingly small spectral perturbation observed with α-cyclodextrin.

On the other hand the hydrophobic moiety of Ibuprofen fits readily into the β-cyclodextrin annulus and according to the CPK models would allow the propionate ion moiety to project into the domain of the peripheral hydroxy groups and the surrounding solvent water molecules. Ibuprofen, in contrast to Naproxen, has a hydrophobic moiety which does not carry a methoxy group, and whose dimensions are such that it can be almost completely encapsulated within the β-cyclodextrin annulus. Furthermore, its shape results in a close fit between the hydrophobic moiety and the β-cyclodextrin annulus, which leads

[8] Cromwell, W. C., Byström, K. Eftink, M. R., *J. Am. Chem. Soc.*, 1985, **89**, 362.
[9] Tabushi, I., Kiyosuke, Y., Sugimoto, T., and Yamamura, K., *J. Phys. Chem.*, 1978, **100**, 916.

to a stability constant (K 2900±500 mol^{-1} dm^3) much greater than that for Naproxen.

γ-Cyclodextrin produces only a very small change in the Ibuprofen spectrum, which may be due either to a small association constant or a small perturbation of the molecular environment, both consequent on the disparity in size between the cyclodextrin and the hydrophobic moiety of the Ibuprofen.[8,9]

Ibuprofen forms a much more stable inclusion complex with dimethyl β-cyclodextrin than with β-cyclodextrin itself. Methylation of the 2-OH and the 6-OH groups of β-cyclodextrin leads to an increase in length of its hydrophobic annulus. Examination of CPK models shows that the hydrophobic moiety can be completely contained within the annulus with a close fit, whilst the charged carboxylate group penetrates into the solvent water. This molecular arrangement would lead to the high stability constant (K 9100±500 mol^{-1} dm^3) observed, compared with that of β-cyclodextrin, because of the opportunity for total inclusion of the hydrophobic moiety in the dimethyl β-cyclodextrin annulus simultaneously with the penetration of the charged propionate group into the solvent water.

Panadol

The perturbation of the u.v.–visible spectrum of Panadol on the addition of α-cyclodextrin was too small to allow determination of the stability constant by this method. This observation is consistent with the solubility studies of Lin *et al.*[10] in which a value of K 3·53 mol^{-1} dm^3 was obtained for the stability constant for Panadol–α-cyclodextrin association. Examination of CPK models shows that complete inclusion of the hydrophobic benzene ring into the α-cyclodextrin cavity is not possible, thus leading to energetically unfavourable interactions between the un-included part of the benzene ring and solvent water. Interaction between Panadol and both β-cyclodextrin and dimethyl β-cyclodextrin gave rise to absorbance changes large enough to allow determination of stability constants K 130 and 83 mol^{-1} dm^3 respectively. Examination of CPK models suggests that the benzene moiety of the Panadol molecule can be totally included within either cyclodextrin, and in the case of β-cyclodextrin the polar hydroxy and amide groups could interact with the hydroxy groups of the cyclodextrin itself or with solvent water. In the case of dimethyl β-cyclodextrin the possibility of the polar groups of Panadol interacting with hydroxy groups of the cyclodextrin and with solvent water is reduced since there are fewer hydroxy groups present and the length of the hydrophobic cone is greater. γ-Cyclodextrin has no effect on the u.v.–visible spectrum of Panadol presumably because the benzene ring of Panadol is small compared with the internal diameter of the γ-cyclodextrin annulus, this leading to a very small tendency for inclusion complex formation.

Conclusion

The results described here show clearly that, for the three drugs studied, the stability constants for inclusion vary systematically with the size of the cyclodextrin annuli, β-cyclodextrin showing the greatest stability constant for

[10] Lin, S.-Y., Yang, J.-C., and Kawashima, Y., *J. Taiwan Pharm. Assoc.*, 1984, **36**, 24.

the complexation of unmodified cyclodextrins with each of the three drugs. Methylation of β-cyclodextrin leads to a decrease in the stability constant compared with unmodified β-cyclodextrin for Naproxen and Panadol, and an increase in stability constant for Ibuprofen. There are specific effects leading to high stability constants for certain host–guest combinations, the greatest effect being shown in the interaction of Ibuprofen with dimethyl β-cyclodextrin which gives rise to a value of K 9100 mol^{-1} dm^3. Further work is being carried out in this laboratory on the specific modification of cyclodextrins in order to enhance the stability of pharmacologically important inclusion complexes.

Acknowledgments

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Chiral Differentiation in the Deacylation of 6^A-*O*-{2-[4-(2-Methylpropyl)phenyl]propanoyl}-β-cyclodextrin

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In 0.1 mol dm^{-3} sodium carbonate buffer at pH 11.5 the pseudo first-order rate constants for the hydrolysis of the diastereoisomers of the title compound to give Ibuprofen {2-[4-(2-methylpropyl)phenyl]propanoic acid} and β-cyclodextrin are 2.97 × 10^{-5} s^{-1} and 3.16 × 10^{-4} s^{-1}, with the diastereoisomer derived from (*R*)-Ibuprofen being the most susceptible to hydrolysis.

Interest in the differentiation between the enantiomers of Ibuprofen {2-[4-(2-methylpropyl)phenyl]propanoic acid}[1,2] stems from the fact that, although the drug is currently administered as a racemate, the physiological activity of (*S*)-Ibuprofen is much greater than that of the (*R*)-enantiomer.[3] In this report we describe the synthesis of the Ibuprofen prodrug 6^A-*O*-{2-[4-(2-methylpropyl)phenyl]propanoyl}-β-cyclodextrin **1**.[4] The diastereoisomers of **1** are distinguishable by HPLC and ^{1}H NMR spectroscopy, and at pH 11.5 there is a greater than tenfold difference between the relative rates of their hydrolysis. Acylation and deacylation reactions of cyclic oligomers of D-glucopyranose, or cyclodextrins, have been studied extensively as models of covalent catalysis by enzymes.[5] There have been several reports of the enantioselective acylation of cyclodextrins.[5–7] This selectivity has been attributed to the inherent chirality of the cyclodextrins and their ability to form diastereoisomeric inclusion complexes with chiral guests, and is analogous to the chiral discrimination characteristic of enzymes such as α-chymotrypsin.[7] Both the acylation and deacylation of enzymes have been shown to be stereoselective but to the best of our knowledge this is the first report of chiral differentiation in the deacylation of a cyclodextrin derivative.

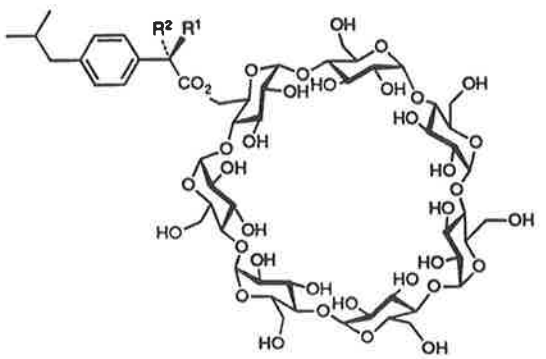
Treatment of 6^A-*O*-(4-methylphenylsulphonyl)-β-cyclodextrin[8] with the caesium salt[9] of (*RS*)-Ibuprofen (0.9 equiv.) in *N*,*N*-dimethylformamide at 100 °C for 24 h gave a 59% yield of the regiospecifically monosubstituted cyclodextrin derivative **1**, after chromatography of the crude product on Sephadex G-15 using 85% aqueous acetonitrile as eluent. The diastereoisomers of **1** were produced in approximately equal quantities, as determined by HPLC analysis,† and samples of each diastereoisomer were separated by preparative HPLC. One diastereoisomer **1a** had an HPLC retention time of 0.32 relative to β-cyclodextrin, and a ^{1}H NMR spectrum [300 MHz, $(CD_3)_2SO$] with resonances at δ 7.27 and 7.17 (dd, J_{AB} 8 Hz, 4H), 2.51 (d, *J* 8 Hz, 2H), 1.90 (m, 1H), 1.46 (d, *J* 7 Hz, 3H) and 0.95 (d, *J* 7 Hz, 6H), attributable to protons of the Ibuprofen moiety. The other diastereoisomer **1b** had an HPLC retention time of 0.38 relative to β-cyclodextrin. The resonances for the protons of the Ibuprofen moiety in the ^{1}H NMR spectrum of **1b** were very similar to those of **1a**, except that the aromatic protons of **1b** gave rise to resonances at δ 7.21 and 7.12 (dd, J_{AB} 8 Hz). The diastereoisomers of **1** also gave satisfactory elemental analysis and FAB mass spectral data.

In order to assign the absolute stereochemistry of the diastereoisomers of **1**, the synthesis was repeated using a sample of the (*R*)-enantiomer of Ibuprofen obtained by incubation of the methyl ester of Ibuprofen with horse liver acetone powder.[2] That product was identical in all respects to **1b**.

Each of the diastereoisomers of **1** hydrolysed to give Ibuprofen and β-cyclodextrin. The reactions were studied by HPLC analysis† and the released Ibuprofen was isolated then analysed by ^{1}H NMR spectroscopy. The rate of hydrolysis of **1a** was significantly slower than that of **1b** across the pH range from 1.3 to 13.0. For example, the pseudo first-order rate constant for the hydrolysis of **1a** on incubation at 37 °C in 0.1 mol dm^{-3} sodium carbonate buffer[10] at pH 11.5 was 2.97 × 10^{-5} s^{-1} (calculated from nine data points over four half-lives with r^2 = 0.9968: r = linear correlation coefficient), corresponding to a half-life of 6.48 h, while the rate constant for the hydrolysis of **1b** was 3.16 × 10^{-4} s^{-1} (calculated from seven data points over four half-lives with r^2 = 0.9978), corresponding to a half-life of 0.61 h.

The rates of hydrolysis of **1a** and **1b** are sufficiently different to be exploited in a modest kinetic resolution of Ibuprofen. A 1:1 mixture of the diastereoisomers of **1** was incubated at 37 °C in 0.1 mol dm^{-3} sodium carbonate buffer at pH 11.5 for 2 h. The reaction was then terminated by adjusting the pH to 2.0 with hydrochloric acid and the acidified solution was extracted with diethyl ether. In this way (*R*)-Ibuprofen was obtained in approximately 70% enantiomeric excess, as shown by conversion to its methyl ester through treatment with methanol that had been pretreated with thionyl chloride, and analysis of the methyl ester by ^{1}H NMR spectroscopy in the presence of the chiral shift reagent Eu(hfc)$_3$.[2]

The diastereoselectivity observed in the hydrolysis of **1** can be attributed to the proximity of the Ibuprofen and cyclodextrin moieties. The ^{1}H NMR spectra of **1a** and **1b** indicate that the chemical environment of the aromatic protons in **1a** differs from that in **1b**. On this basis it seems likely that the chiral differentiation results from intramolecular inclusion of the Ibuprofen moiety in the annulus of the cyclodextrin, but the specific cause of the discrimination remains an enigma. Nevertheless these results show that the deacylation of cyclodextrin derivatives, as well as the acylation,[5–7] can exhibit diastereoselectivity analogous to that displayed by enzymes. In fact the extent of the chiral differentiation



1a R^1 = H, R^2 = Me
1b R^1 = Me, R^2 = H

† Analytical and preparative HPLC was carried out using a Waters Carbohydrate Analysis column (3.9 × 300 mm) with 70% aqueous acetonitrile as eluent. Under these conditions β-cyclodextrin had a retention time of 38 min.

observed in the hydrolysis of **1** is similar to that reported for the deacylation step in the hydrolysis of esters catalysed by α-chymotrypsin.[7]

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References

1 For examples see: G. Castaldi, S. Cavicchioli, C. Giordano and F. Uggeri, *J. Org. Chem.*, 1987, **52**, 3018; G. Parrinello and J. K. Stille, *J. Am. Chem. Soc.*, 1987, **109**, 7122; W. D. Lubell and H. Rapoport, *J. Am. Chem. Soc.*, 1988, **110**, 7447; S.-H. Wu, Z.-W. Guo and C. J. Sih, *J. Am. Chem. Soc.*, 1990, **112**, 1990.
2 M. Ahmar, C. Girard and R. Bloch, *Tetrahedron Lett.*, 1989, **30**, 7053.
3 For an example see: S. Dawling and P. Crome, *Clin. Pharmacokinet.*, 1989, **17**, 236.
4 S. F. Lincoln, J. H. Coates, C. J. Easton, S. J. van Eyk, B. M. May, P. Singh, M. A. Stile and M. L. Williams, Internat. Pat. Appl. PCT/AU 89/00359.
5 For a review see: M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer-Verlag, New York, 1978.
6 F. Cramer and W. Dietsche, *Chem. Ber.*, 1959, **92**, 1739; K. Flohr, R. M. Paton and E. T. Kaiser, *J. Chem. Soc., Chem. Commun.*, 1971, 1621; G. L. Trainor and R. Breslow, *J. Am. Chem. Soc.*, 1981, **103**, 154; Y. Ihara, E. Nakanishi, M. Nango and J. Koga, *Bull. Chem. Soc. Jpn.*, 1986, **59**, 1901.
7 K. Flohr, R. M. Paton and E. T. Kaiser, *J. Am. Chem. Soc.*, 1975, **97**, 1209.
8 Y. Chao, Ph.D. Thesis, Columbia University, 1972; M. Shiono, T. Shibanuma and T. Mukaiyama, *Chem. Lett.*, 1976, 1041.
9 Z. Blum, *Acta Chem. Scand.*, 1989, **43**, 248.
10 *Biochemists' Handbook*, ed. C. Long, Spon, London, 1961.

J. CHEM. SOC. FARADAY TRANS., 1991, 87(17), 2699–2703

Chiral Molecular Recognition: A ^{19}F Nuclear Magnetic Resonance Study of the Diastereoisomer Inclusion Complexes formed between Fluorinated Amino Acid Derivatives and α-Cyclodextrin in Aqueous Solution

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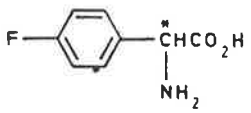
A ^{19}F NMR spectroscopic study (282.35 MHz) of the formation of diastereoisomeric inclusion complexes by fluorinated amino acid derivatives and α-cyclodextrin (αCD) in 10% aqueous D_2O solution yields the apparent stability constants K_R and $K_S/dm^3\ mol^{-1} = 7.7 \pm 0.3$ and 8.2 ± 0.3 for protonated α-(*p*-fluorophenyl)glycine (1 + H), 21.5 ± 0.4 and 22.5 ± 0.4 for deprotonated α-(*p*-fluorophenyl)glycine (1 − H), 14.4 ± 0.1 and 14.6 ± 0.1 for *N*-acetyl-α-(*p*-fluorophenyl)glycine (2), 13.1 ± 0.5 and 14.1 ± 0.5 for deprotonated *N*-acetyl-α-(*p*-fluorophenyl)-glycine (2 − H), and 12.4 ± 0.3 and 10.6 ± 0.4 for deprotonated *N*-(*p*-fluorobenzoyl)valine (3 − H), where the first and second of each pair of values refers to the diastereoisomer formed between αCD and the *R* and *S* enantiomer, respectively. The chemical shifts of the *R*-amino acid derivative · αCD inclusion complexes are upfield from those of their *S* analogues for deprotonated *N*-(*p*-fluorobenzoyl)valine (3 − H), deprotonated α-(*p*-fluorophenyl) glycine (1 − H), and deprotonated *N*-acetyl-α-(*p*-fluorophenyl)glycine (2 − H), but this relationship is reversed for protonated α-(*p*-fluorophenyl)glycine (1 + H) and *N*-acetyl-α-(*p*-fluorophenyl)glycine (2 + H). In the case of the *N*-(*p*-fluorobenzoyl)valine · αCD inclusion complex (3 · αCD) the chemical shift difference between the diastereo-isomers formed with the *R* and *S* enantiomers is too small for quantitative analysis and accordingly a composite $K_{R,S}/dm^3\ mol^{-1} = 8.3 \pm 0.3$ was determined. The factors causing the variations in apparent stability constants and chemical shifts are discussed.

Chiral molecular recognitition is a well established feature of biological chemistry, and is currently the subject of increasing attention in areas as diverse as electronic energy-transfer processes,[1] chromatography[2,3] and drug interaction studies.[4,5] The ability of the chiral α-1,4-linked cyclic oligomers of D-glucopyranose, or cyclodextrins (CDs), to act as host molecules in the formation of inclusion complexes with a wide range of guest molecules is well established,[6–14] and renders them very suitable for chiral recognition studies. As CDs only exist as D enantiomers, two diastereoisomeric inclusion complexes may be formed with a racemic guest molecule. In some cases the formation of such diastereoisomers leads to a physical discrimination between the guest enantiomers based on their chirality as indicated by the partial resolution of racemic guests by the preferential precipitation of one diastereoisomer inclusion complex,[15–17] and also by the chromatographic separation of enantiomers on columns in which the stationary phase consists of CDs bonded to silica.[2,3] Chiral discrimination by CDs has also been directly observed in solution for propranolol where the formation of diastereoisomeric inclusion complexes with βCD and γCD results in separate ^1H NMR spectra for the propranolol enantiomers.[18] However, the quantitative aspects of such chiral discrimination in solution have been little studied.[12] Accordingly we have investigated the chiral discrimination exhibited by αCD† in its complexation of the chiral guest species *N*-(*p*-fluorobenzoyl)valine (3), α-(*p*-fluorophenyl)glycine (1), and *N*-acetyl-α-(*p*-fluorophenyl)glycine (2) which offer an opportunity to test the effects of (*a*), the proximity of the chiral centre to the *p*-fluorophenyl moiety; (*b*), the size of the chiral centre; and (*c*), the variation in charge of the guest species resulting from changes in the position of protonic equilibria, through observation of the variation of the ^{19}F chemical shift of the guest F substituent which other studies[10–12] have shown to be particularly sensitive to changes in environment.



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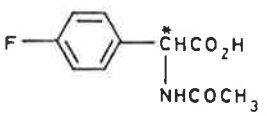

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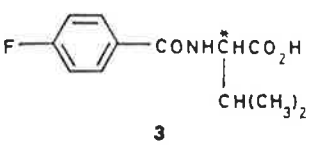

3

* indicates chiral centre

Experimental

αCD (Sigma) was dried to constant weight and stored over P_2O_5 in a vacuum desiccator prior to use. All other reagents

† αCD was chosen because of its high solubility in water (145 g dm^{-3}) by comparison to βCD (18.5 g dm^{-3}), and because its small annular diameter (470–520 pm) substantially decreases the possibility of the inclusion of guest species as dimers which occurs with γCD (diameter = 750–830 pm) and which might obscure observation of chiral interactions.

were of analytical grade. (2*RS*)-*N*-(*p*-Fluorobenzoyl)valine and the corresponding (2*S*)- and (2*R*)-valine derivatives were prepared by reaction of the corresponding free amino acids with *p*-fluorobenzoyl chloride.[19,20] (2*RS*)-α-(*p*-Fluorophenyl)-glycine was prepared by condensation of *p*-fluorobenzaldehyde with chloroform and ammonia[21,22] and resolved by treatment of the corresponding *N*-acetyl derivative with hog renal acylase 1.[23] The *N*-acetyl derivatives of (2*RS*)- and (2*S*)-α-(*p*-fluorophenyl)glycine were prepared through reaction with acetic anhydride.[24]

The pK_a values of the amino acid derivatives were determined by standard titrimetric methods in aqueous solutions of the amino acid derivative (10^{-3} mol dm^{-3}) and KCl (10^{-1} mol dm^{-3}) thermostatted at 298.2 K, using a Metrohm Dosimat E665 titrimator and an Orion SA 720 potentiometer. Appropriate water titration corrections were made. Buffer solutions (KCl–HCl, KH_2PO_4–Na_2HPO_4, and glycine–NaOH) used to buffer the amino acid derivative–αCD solutions used in the ^{19}F NMR studies were prepared as in the literature.[25]

The ^{1}H-broad-band decoupled ^{19}F NMR spectra were recorded on a Bruker CXP 300 spectrometer at 282.35 MHz locked on the deuterium frequency of D_2O. The fluorinated amino acid derivative and αCD in 10% aqueous D_2O solutions were contained in 5 mm NMR tubes. Chemical shifts were measured from a 2% CF_3CO_2Na–D_2O external reference solution. An average of 2000 transients were accumulated in a 8192 point data base for each solution, and the solution temperature (295.5 K) was controlled by a Bruker B-VT1000 variable temperature unit to within ±0.3 K.

Results

The singlet ^{19}F resonances of the protonated glycine (**1** + H) (pK_a 2.3), *N*-acetylglycine (**2**) (pK_a 2.8) and valine (**3**) (pK_a 3.4), and their deprotonated analogues exhibit substantial upfield shifts and split into symmetrical doublets with increasing concentration of αCD, as shown in Fig. 1–3, consistent with the formation of two diastereoisomers (in the case of the valine **3**, the splitting was insufficient for separate analysis of the data from the *R* and *S* enantiomers). The glycine **1** (pK_a 8.8) was insufficiently soluble to study by ^{19}F NMR spectroscopy. Identification of the diastereoisomers was made both by the measurement of the ^{19}F chemical shift for the pure *S*-amino acid derivative in the presence of αCD, and by the addition of pure *S*-amino acid derivative to a solution of the racemic amino acid derivative in the presence of αCD and observing which component of the ^{19}F doublet

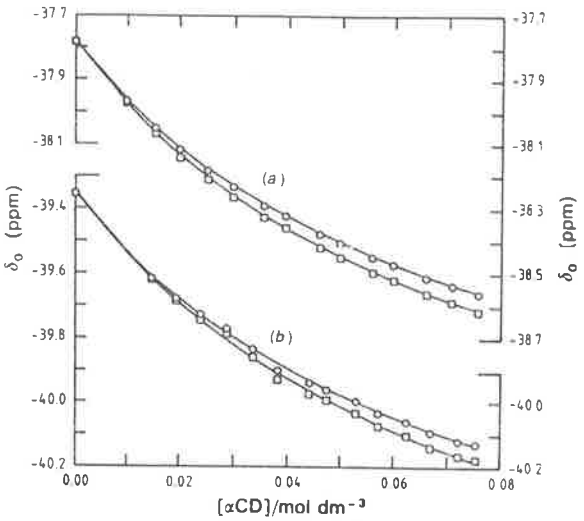

Fig. 2 The variation of ^{19}F δ_{obs} for (*a*), the racemic *N*-acetylglycine **2** (0.982×10^{-3} mol dm^{-3}, pH 1.3) ○, *R*; □, *S*; and (*b*), the racemic deprotonated *N*-acetylglycine (**2** – H) (1.01×10^{-3} mol dm^{-3}, pH 6.9) ○, *S*; □, *R*; in the presence of αCD at $I = 0.100$ and 295.5 K. The amino acid derivative enantiomer in the diastereoisomer formed with αCD is identified by *R* or *S*. The solid curves represent the least-squares best fit of the δ_{obs} data to eqn. (2)

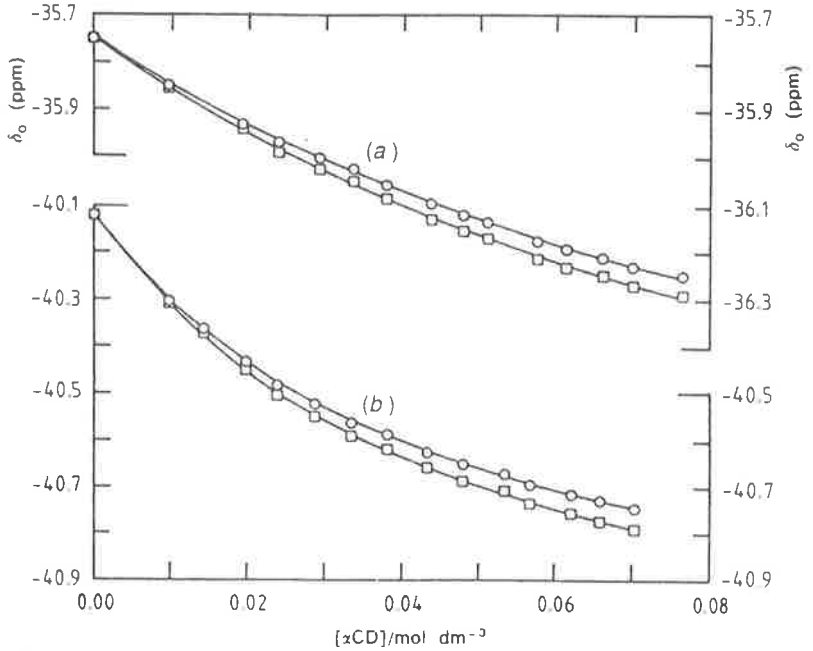

Fig. 1 The variation of ^{19}F δ_{obs} for (*a*), the racemic protonated glycine (**1** + H) (1.00×10^{-3} mol dm^{-3}, pH 1.3) ○, *R*; □, *S*; and (*b*), the racemic deprotonated glycine (**1** – H) (1.02×10^{-3} mol dm^{-3}, pH 10.8) ○, *S*; □, *R*; in the presence of αCD at $I = 0.100$ and 295.5 K. The amino acid derivative enantiomer in the diastereoisomer formed with αCD is identified by *R* or *S*. The solid curves represent the least-squares best fit of the δ_{obs} data to eqn. (2)

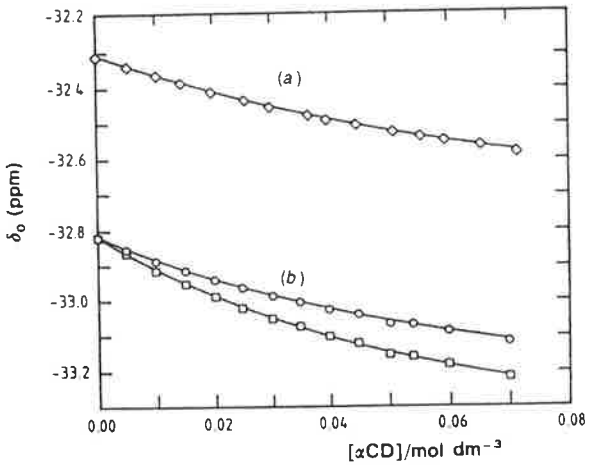

Fig. 3 The variation of ^{19}F δ_{obs} for (*a*), the racemic valine **3** (1.02×10^{-3} mol dm^{-3}, pH 1.3) ◇, *R*, *S*; and (*b*), the racemic deprotonated valine (**3** − H) (0.990×10^{-3} mol dm^{-3}, pH 6.9) ○, *S*; □, *R*; in the presence of αCD at $I = 0.100$ and 295.5 K. The amino acid derivative enantiomer in the diastereoisomer formed with αCD is identified by *R* or *S*. The solid curves represent the least-squares best fit of the δ_{obs} data to eqn. (2)

increased in intensity. The variation of the ^{19}F chemical shift with αCD concentration for each enantiomer (E) is consistent with the formation of a 1:1 diastereoisomeric inclusion complex (E · αCD) according to eqn. (1) and is given by eqn. (2), where δ_{obs} is the environmentally averaged shift of E and E · αCD under the fast exchange conditions applying to these systems, and δ_F and δ_R or δ_S (depending on which enantiomer is complexed) are the chemical shifts of E and E · αCD, respectively.[10–12] The δ_{obs} data for the *R* enantiomer (*RE*) were fitted to eqn. (2) (an equivalent equation was used for *SE*) using a non-linear regression analysis, and the derived apparent stability constants, K_R and K_S, {where for the *RE*, $K_R = [RE \cdot \alpha CD][RE]^{-1}[\alpha CD]^{-1}$, and an equivalent expression holds for *SE*} and δ_F, δ_R and δ_S, appear in Table 1.

$$E + \alpha CD \underset{}{\overset{K_R \text{ or } K_S}{\rightleftharpoons}} E \cdot \alpha CD \quad (1)$$

$$\delta_{obs} = (\delta_F[RE] + \delta_R[RE \cdot \alpha CD])/([RE] + [RE \cdot \alpha CD]) \quad (2)$$

The formation of guest · $(\alpha CD)_2$ complexes has been reported for other guests in some cases, as indicated by a biphasic variation of the substrate chemical shift.[10,11] No evidence for such a stoichiometry was obtained in this study.

Discussion

The thermodynamic discrimination between the enantiomers of a given amino acid derivative is small, and in some cases is insignificant within experimental error (Table 1). Nevertheless, the separate resonances observed for the diastereoisomers (Fig. 1–3) indicate a significant difference in the magnetic environment of the ^{19}F nuclei of the diastereoisomers formed with a given amino acid derivative, and thereby a difference in the interaction between αCD and the *R* and *S* enantiomers, arising from their opposite chiralities. The difference in the chemical shift of the F substituent of the amino acid derivative in the solvated state, δ_F, and the complexed state, δ_R and δ_S, arises from a combination of the change in the local environment of the F substituent and the overall environmental change of the amino acid derivative on complexation. Corey–Pauling–Koltun (CPK) models show that the *p*-fluorophenyl moieties of the amino acid derivatives can enter the αCD annulus from either end, but that steric constraints position the F substituent in the hydrophobic section of the annulus in the vicinity of the ether linkages. This also results in the inclusion of a substantial portion of the phenyl ring in the annulus with the amino acid substituent in the vicinity of the hydroxy groups of αCD. (CPK models also show that, should the amino acid substituent reside wholly within the αCD annulus, steric interactions are likely to minimise the entry of the phenyl ring into the annulus, but this orientation seems less likely as crystallographic X-ray studies of αCD inclusion complexes with a range of aromatic guests show a substantial portion of the aromatic moiety to be within the αCD annulus.[26–29]) The resulting solvation change of the F substituent is probably a

Table 1 Apparent stability constants and ^{19}F chemical shifts of α-cyclodextrin–amino acid derivative diastereomers in 10% aqueous D_2O at $I = 0.100$ and 295.5 K

amino acid derivative (charge)	pH	buffer or electrolyte	K_R and K_S [a] /dm^3 mol^{-1}	δ_R and δ_S [a,b] (ppm)	$\Delta\delta$ (ppm)
1 + H (+1)	1.3	KCl–HCl	K_R, 7.7 ± 0.3 K_S, 8.2 ± 0.3	δ_R, −37.13 ± 0.03 δ_S, −37.19 ± 0.04 δ_F, −35.75 ± 0.004	$\delta_R - \delta_F$, −1.38 ± 0.03 $\delta_S - \delta_F$, −1.44 ± 0.04 $\delta_R - \delta_S$, 0.06 ± 0.04
1 − H (−1)	10.8	glycine–NaOH	K_R, 21.5 ± 0.4 K_S, 22.5 ± 0.4	δ_R, −41.24 ± 0.01 δ_S, −41.14 ± 0.01 δ_F, −40.12 ± 0.004	$\delta_R - \delta_F$, −1.12 ± 0.01 $\delta_S - \delta_F$, −1.02 ± 0.01 $\delta_R - \delta_S$, −0.10 ± 0.01
2 (0)	1.3	KCl–HCl	K_R, 14.4 ± 0.1 K_S, 14.6 ± 0.1	δ_R, −39.28 ± 0.01 δ_S, −39.37 ± 0.01 δ_F, −37.78 ± 0.004	$\delta_R - \delta_F$, −1.50 ± 0.01 $\delta_S - \delta_F$, −1.59 ± 0.01 $\delta_R - \delta_S$, 0.09 ± 0.01
2 − H (−1)	6.9	KH_2PO_4–Na_2HPO_4	K_R, 13.1 ± 0.5 K_S, 14.1 ± 0.5	δ_R, −41.02 ± 0.04 δ_S, −40.85 ± 0.03 δ_F, −39.36 ± 0.004	$\delta_R - \delta_F$, −1.66 ± 0.04 $\delta_S - \delta_F$, −1.49 ± 0.03 $\delta_R - \delta_S$, −0.17 ± 0.04
3 (0)[c]	1.3	KCl–HCl	$K_{R,S}$, 8.3 ± 0.3	$\delta_{R,S}$, −33.03 ± 0.02 δ_F, −32.31 ± 0.004	$\delta_{R,S} - \delta_F$, −0.72 ± 0.02
3 − H (−1)	6.9	KH_2PO_4–Na_2HPO_4	K_R, 12.4 ± 0.3 K_S, 10.6 ± 0.4	δ_R, −33.67 ± 0.01 δ_S, −33.51 ± 0.02 δ_F, −32.82 ± 0.004	$\delta_R - \delta_F$, −0.85 ± 0.01 $\delta_S - \delta_F$, −0.69 ± 0.02 $\delta_R - \delta_S$, −0.16 ± 0.02

[a] Errors represent one standard deviation derived from a least-squares fit of the data to eqn. (2), except for δ_F where the error represents the digital resolution of the spectrum. [b] Chemical shifts referenced to external 2% CF_3CO_2Na in D_2O which was assigned a shift of zero. Thus the more negative the value the further is δ upfield from the reference. [c] At the highest αCD concentration studied (0.065 mol dm^{-3}) the observed $\delta_R - \delta_S = 0.021 \pm 0.004$ ppm, which is insufficient for a reliable derivation of K_R and K_S and as a consequence a composite $K_{R,S}$ is derived.

major cause of the variation in chemical shift resulting from formation of the diastereoisomers. However, the overall environmental changes of the amino acid derivatives vary as a consequence of the different sizes of the amino acid substituents. Thus the amino acid substituents of the glycine **1** and the *N*-acetylglycine **2** are in close proximity to either the primary or secondary hydroxy groups of αCD, depending on the orientation of the amino acid derivative in the αCD annulus (see below). By comparison, the amino acid and propyl substituents of the valine **3** protrude further from the αCD complex, and the overall environmental changes experienced by both the valine **3** and its deprotonated analogue (**3** − H) are proportionately less, which is consistent with the smaller change in chemical shift characterizing these species on complexation.

For the diastereoisomers formed with the three negatively charged deprotonated amino acid derivatives $|\delta_R| > |\delta_S|$, whereas for those formed with the positively charged protonated glycine (**1** + H) and the zero charged *N*-acetylglycine **2**, $|\delta_R| < |\delta_S|$ (Table 1). This suggests that the change in charge produces a reorientation of the enantiomers inside the αCD annulus. The dipole moment of αCD is estimated to be 13.5 D,† with the positive and negative ends of the dipole adjacent to the rings of primary and secondary hydroxy groups, respectively, delineating the narrow and wide ends of the αCD annulus.[30,31] It has been observed that the dipole moments of *p*-nitrophenol, *p*-hydroxybenzoic acid, and benzoic acid are antiparallel to the direction of the dipole moment of αCD in their inclusion complexes such that the nitro and carboxylic acid substituents of the guests are in the vicinity of the αCD primary hydroxy groups.[30] As the localised charge of the carboxylate group of a negatively charged amino acid derivative enantiomer dominates the orientation of its dipole moment, is is probable that the enantiomer will be oriented with its F substituent near the hydrophobic region of the annulus and its carboxylate group hydrogen-bonded to a primary hydroxy group of αCD in the diastereoisomer. Thus the interaction between the negatively charged enantiomers and αCD may be subdivided into: (*a*) the interaction of the *p*-fluorophenyl moiety and αCD; (*b*) hydrogen bonding between the carboxylate group at the chiral centre and the primary hydroxy groups of αCD; and (*c*) the interaction of the other groups at the chiral centre with the primary hydroxy groups of αCD. For a given deprotonated amino acid derivative it is the arrangement of the groups involved in interaction (*c*) which determines the enantiomer discrimination resulting in the observation that $|\delta_R| > |\delta_S|$.

In addition to the reversal of the relative chemical shifts alluded to above, the modulus $|\delta_R - \delta_S|$ is greater for the diastereoisomers formed by the deprotonated amino acids ($|\delta_R| > |\delta_S|$) than for those formed by the protonated glycine (**1** + H) and the *N*-acetylglycine (**2**) ($|\delta_R| < |\delta_S|$). This suggests a change in effectiveness of the chiral interactions with change in enantiomer charge. The localised positive charge on the amine function of the protonated glycine (**1** + H) will probably reverse the direction of its dipole moment and its orientation in the αCD annulus, by comparison with that of its negatively charged analogue. On this basis, the interaction between the protonated glycine (**1** + H) and αCD may be subdivided into: (*a*) the inclusion of the *p*-fluorophenyl moiety in the hydrophobic region of the annulus of αCD; (*b*) hydrogen bonding between the ammonium group at the chiral centre and the secondary hydroxy groups of αCD; and (*c*) the interaction of the other groups at the chiral centre with the secondary hydroxy groups of αCD, resulting in $|\delta_R| < |\delta_S|$.

† 1 D = 3.335 64 × 10^{-30} C m.

The direction of the dipole moment of the *N*-acetylglycine **2** is uncertain and thus the deduction of the orientation of the diastereoisomers formed with αCD on this basis is also uncertain. Nevertheless, it is likely that chiral discrimination results from (*a*) the inclusion of the *p*-fluorophenyl moiety in the annulus of αCD; and (*b*) interaction of the two polar groups at the chiral centre with hydroxy groups of αCD. In the case of the valine **3**, inclusion of the *p*-fluorobenzoyl moiety in the αCD annulus leaves only one other polar group at the chiral centre to interact with the hydroxy groups of αCD, with the result that there is less chiral discrimination than is the case for the *N*-acetylglycine **2**.

The driving force for the complexation of an aromatic substrate by a CD arises from a combination of changes in solvation of the substrate and the CD resulting from complexation, and hydrophobic, van der Waals, hydrogen bonding and dipolar interactions between the substrate and the CD in varying degrees. The interplay of these factors has been adequately discussed elsewhere,[6–9] and only those aspects which may explain the variations observed in this study are considered here. Despite the small, and in some cases insignificant, thermodynamic discrimination between the amino acid derivative enantiomers by αCD, the variation of K_R and K_S can be interpreted in terms of interactions within the diastereoisomers for amino acid derivatives of similar charge. Hence, as the interaction of the *p*-fluorophenyl moiety and αCD, and hydrogen bonding between the carboxylate group at the chiral centre and the primary hydroxy groups of αCD probably make similar contributions to the stability in complexes of all three deprotonated amino acid derivatives, the diminution of K_R and K_S for these substrates in the sequence: glycine (**1** − H) ≫ *N*-acetylglycine (**2** − H) > valine (**3** − H) is largely attributable to the differing characteristics of the other groups at the chiral centre. Thus, the dipolar interaction of the amino group of glycine **1** with primary hydroxy groups of αCD is greater than the interaction of the acetamido group of *N*-acetylglycine **2**, which is in turn greater than that of the propyl group of valine **3**.

This conclusion, that dipolar interactions constitute a significant component of the binding force in the complexes studied here, in addition to their importance in complex orientation, is similar to that reached on the basis of data for the inclusion of *p*-hydroxybenzoate, *p*-nitrobenzoate and *m*-hydroxybenzoate by αCD.[32] The K_R and K_S values for complexation of the deprotonated amino acid derivative enantiomers are comparable to the *K* values obtained for the complexation by αCD of *p*-hydroxybenzoate (11.5, 298.2 K), *p*-nitrobenzoate (27.4, 303.2 K), and *m*-hydroxybenzoate (6.8, 293.2 K).

We are grateful to the Australian Research Council and the University of Adelaide for supporting this research.

References

1 D. H. Metcalf, W. S. Snyder, J. N. Demas and F. S. Richardson, *J. Am. Chem. Soc.*, 1990, **112**, 5681.
2 W. L. Hinze, T. E. Rieh, D. W. Armstrong, W. DeMond, A. Alak and T. Ward, *Anal. Chem.*, 1985, **57**, 237.
3 D. W. Armstrong, T. Ward, R. D. Armstrong and T. E. Beesley, *Science*, 1986, **232**, 1132.
4 A. Rubin, M. P. Knadler, P. P. Ho, L. D. Bechtol and R. L. Wole, *J. Pharm. Sci.*, 1985, **74**, 82.
5 J. A. Hamilton and L. Chen, *J. Am. Chem. Soc.*, 1988, **110**, 4379.
6 J. Szejtli, *Cyclodextrins and Their Inclusion Complexes*, Akademiai Kiado, Budapest, 1982.
7 M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer, New York, 1977.

8 R. J. Clarke, J. H. Coates and S. F. Lincoln, *Adv. Carbohyd. Chem. Biochem.*, 1989, **46**, 205.
9 W. Saenger, *Inclusion Compounds*, 1984, **2**, 231.
10 I. M. Brereton and T. M. Spotswood, S. F. Lincoln and E. H. Williams, *J. Chem. Soc., Faraday Trans. 1*, 1984, **80**, 3147.
11 D. L. Pisaniello, S. F. Lincoln and J. H. Coates, *J. Chem. Soc., Faraday Trans. 1*, 1985, **81**, 1247.
12 N. J. Smith, T. M. Spotswood and S. F. Lincoln, *Carbohydr. Res.*, 1989, **192**, 9.
13 S. F. Lincoln, A. M. Hounslow, J. H. Coates and B. G. Doddridge, *J. Chem. Soc., Faraday Trans. 1*, 1987, **83**, 2697.
14 S. F. Lincoln, A. M. Hounslow, J. H. Coates, R. P. Villani and R. L. Schiller, *J. Inclusion Phenom.*, 1988, **6**, 183.
15 F. Cramer and W. Dietsche, *Chem. Ber.*, 1959, **92**, 378.
16 M. Mikolajczyk, J. Drabowicz and F. Cramer, *J. Chem. Soc., Chem. Commun.*, 1971, 317.
17 M. Mikolajczyk and J. Drabowicz, *J. Am. Chem. Soc.*, 1978, **100**, 2510.
18 D. Greatbanks and R. Pickford, *Magn. Reson. Chem.*, 1987, **25**, 208.
19 M. P. Spratt, Y. Meng and H. C. Dorn, *Anal. Chem.*, 1985, **57**, 76.
20 J. H. Coates, D. R. Coghlan, C. J. Easton, B. F. Hoskins, S. F. Lincoln and E. R. T. Tiekink, *Z. Krist.*, 1989, **188**, 131.
21 E. L. Compere and D. A. Weinstein, *Synthesis*, 1977, 852.
22 D. Landini, F. Montanari and F. Rolla, *Synthesis*, 1979, 26.
23 J. E. Baldwin and T. S. Wan, *Tetrahedron*, 1981, **37**, 1589.
24 A. Schouteen, Y. Christidis and G. Mattioda, *Bull. Soc. Chim. Fr.*, 1978, 248.
25 *Biochemists' Handbook*, ed. C. Long, Spon, London, 1961, p. 30, 32, 36.
26 K. Harata, *Bull. Chem. Soc. Jpn.*, 1975, **48**, 2409.
27 K. Harata, *Bull. Chem. Soc. Jpn.*, 1976, **49**, 1493.
28 K. Harata, *Bull. Chem. Soc. Jpn.*, 1976, **49**, 2066.
29 K. Harata, *Bull. Chem. Soc. Jpn.*, 1977, **50**, 1416.
30 M. Kitagawa, H. Hoshi, M. Sakurai, Y. Inoue and R. Chujo, *Carbohydr. Res.*, 1987, **163**, c1.
31 M. Sakurai, M. Kitagawa, H. Hoshi, Y. Inoue and R. Chujo, *Chem. Lett.*, 1988, 895.
32 R. I. Gelb, L. M. Schwartz, B. Cardelino, H. S. Fuhrman, R. F. Johnson and D. A. Laufer, *J. Am. Chem. Soc.*, 1981, **103**, 1750.

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Regioselective Functionalization of *N*-Phthaloyl-Substituted Amino Acid and Peptide Derivatives

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The free-radical reactions of a range of amino acid derivatives with *N*-bromosuccinimide are described. The products and relative rates of these reactions indicate that the α-position of an *N*-phthaloyl-substituted α-amino acid derivative is much less reactive than that of a corresponding *N*-acyl amino acid derivative toward hydrogen atom transfer. This is attributed to the proactive effects of acylamino and carboxyl substituents, in contrast to the counteractive effects of phthalimido and carboxyl groups. The reactions exemplify procedures for the regiocontrolled functionalization of amino acid and peptide derivatives.

Introduction

Hydrogen atom transfer reactions of *N*-acyl α-amino acid derivatives generally favor formation of α-carbon-centered radicals.[1] These radicals are resonance stabilized by the combined action of an electron-releasing amido substituent and an electron-withdrawing carboxy substituent, and they may be classified as captodative,[2] merostabilized,[3] or push–pull-stabilized[4] radicals. Amido-, carboxy-substituted radicals have been identified in proteins upon irradiation[5] and are thought to be intermediates in the photoalkylation[6] and carboxylation[7] of peptides.

(1) For a review of the formation of α-carbon-centered radicals in reactions of amino acid derivatives, see: Easton, C. J. *Advances in Detailed Reaction Mechanisms*; Coxon, J. M., Ed.; JAI Press: Greenwich, CT, 1991; Vol. 1, p 83.

(2) Viehe, H. G.; Merenyi, R.; Stella, L.; Janousek, Z. *Angew. Chem., Int. Ed. Engl.* **1979**, *18*, 917. Viehe, H. G.; Janousek, Z.; Merenyi, R. *Acc. Chem. Res.* **1985**, *18*, 148.

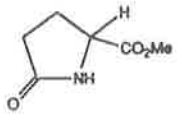
(3) Baldock, R. W.; Hudson, P.; Katritzky, A. R.; Soti, F. *Heterocycles* **1973**, *1*, 67. Baldock, R. W.; Hudson, P.; Katritzky, A. R.; Soti, F. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1422.

(4) Balaban, A. T. *Rev. Roum. Chim.* **1971**, *16*, 725.

(5) Henrikson, T.; Melo, T. B.; Saxebol, G. *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic Press: New York, 1976; Vol. 2, p 213.

(6) Elad, D.; Sperling, J. *J. Chem. Soc. C* **1969**, 1579. Elad, D.; Schwarzberg, M.; Sperling, J. *J. Chem. Soc., Chem. Commun.* **1970**, 617. Sperling, J.; Elad, D. *J. Am. Chem. Soc.* **1971**, *93*, 967. Sperling, J.; Elad, D. *J. Am. Chem. Soc.* **1971**, *93*, 3839. Schwarzberg, M.; Sperling, J.; Elad, D. *J. Am. Chem. Soc.* **1973**, *95*, 6418.

Other examples of their generation include the formation and dimerization of the radical **2** on irradiation of a mixture of methyl pyroglutamate (**1**) and di-*tert*-butyl peroxide,[8] and the oxidation of **3a** to give the diperoxide **3b**, presumably via **4a** and **4b**.[9]



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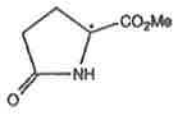

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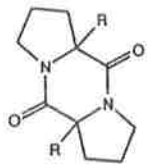

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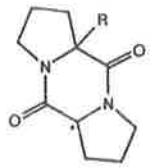
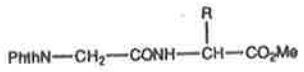


4

a) R = H
b) R = OOH

Consistent with this general trend, reactions of *N*-acyl α-amino acid derivatives with *N*-bromosuccinimide (NBS) proceed via formation of the corresponding α-carbon-centered radicals.[10,11] In direct contrast, however, formation of α-carbon-centered radicals from *N*-phthaloyl-substituted amino acid derivatives is strongly disfavored.[12] The extent of this effect is reflected in the regiospecific reactions of **5a–8a** to give the corresponding bromides **5b–8b**. In this report we describe the reactions of NBS with a range of amino acid derivatives, chosen to examine the cause of the contrasting effects of the *N*-phthaloyl and *N*-acyl substituents and to further illustrate the exploitation of those effects in the regioselective functionalization of amino acid and peptide derivatives.



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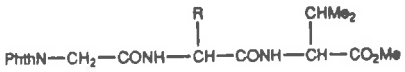

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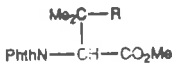

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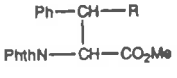
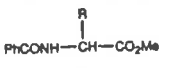


8

a) R = H
b) R = Br

Results and Discussion

To investigate the contrasting effects of *N*-phthaloyl and *N*-acyl substituents on the formation of α-carbon-centered radicals in reactions of α-amino acid derivatives, reactions of the methyl esters of *N*-benzoylglycine (**9a**), *N*-phthaloylglycine (**10a**), *N*-phthaloyl-β-alanine (**11a**), and *N*-benzoyl-β-alanine (**12**) with NBS were investigated and compared. The β-alanine derivatives **11a** and **12**, which have the phthalimido and benzamido substituents spacially separated from the methoxycarbonyl group, were chosen to examine the relative effects of these substituents acting individually, while the glycine derivatives **9a** and **10a** were selected to study the effects of the benzamido and phthalimido substituents in combination with the methoxycarbonyl group.



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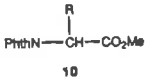

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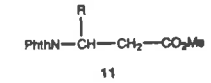
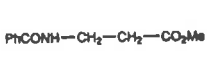


11

a) R = H
b) R = Br



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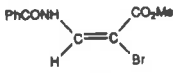

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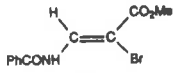

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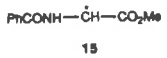

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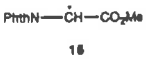

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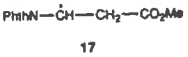

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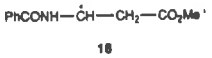


18

The reactions of **9a** and **11a** with NBS (1 equiv) in carbon tetrachloride gave the corresponding bromides **9b** and **11b**. Similar treatment of **12** gave a mixture of the α,β-dehydro-β-alanine derivatives **13** and **14** and unreacted starting material, while with excess NBS (2 equiv) all of the starting material was consumed. The reaction of **9a** was complete in 0.25 h, whereas the reactions of **11a** and **12** required 2 h for complete reaction. The reaction of **10a** to give **10b** was much less efficient, requiring excess NBS (2 equiv) and a reaction time of 48 h for 50% conversion. This qualitative comparison of the reactivity of **9a**, **10a**, **11a**, and **12** with NBS is reflected in the relative rates of reaction of the amino acid derivatives, which were determined in competitive experiments using, for example, equimolar mixtures of **9a** and **12**, **10a** and **11a**, and **11a** and **12**. Aliquots of crude reaction mixtures were easily and conveniently analyzed, by ^{1}H NMR spectroscopy, for consumption of starting materials and formation of products. In the competitive reaction of **9a** and **12** there was no evidence for any reaction of **12** until reaction of **9a** to give **9b** appeared to be complete. Similarly, with mixtures of **10a** and **11a**, and **11a** and **12**, **11a** reacted to the exclusion of **10a**, and **12** reacted to the exclusion of **11a**, within the limits of detection by ^{1}H NMR spectroscopy. From these experiments a conservative estimate for the relative rates of reaction of **9a** and **12**, **12** and **11a**, and **11a** and **10a** is 10:1 in each case.

The products of treatment of **9a**, **10a**, **11a**, and **12** with NBS indicate that the reactions involve formation of the corresponding radicals **15–18**, with subsequent bromine incorporation. In the reaction of **12**, subsequent elimination of hydrogen bromide, bromine addition, and hydrogen bromide elimination afford **13** and **14**. The regioselectivity of hydrogen atom transfer from **12** can be deduced from the greater relative rate of reaction of **12** compared to **11a** and the regioselectivity of reaction of **11a**. Reaction at the

(7) Wheelan, P.; Kirsch, W. M.; Koch, T. H. *J. Org. Chem.* **1989**, *54*, 4360.
(8) Obata, N.; Niimura, K. *J. Chem. Soc., Chem. Commun.* **1977**, 238.
(9) Schmidt, U.; Hausler, J.; Ohler, E.; Poisel, H. *Prog. Chem. Org. Nat. Prod.* **1979**, *37*, 286.
(10) Easton, C. J.; Hay, M. P. *J. Chem. Soc., Chem. Commun.* **1985**, 425. Easton, C. J.; Hay, M. P.; Love, S. G. *J. Chem. Soc., Perkin Trans. 1* **1988**, 265.
(11) Easton, C. J.; Hay, M. P. *J. Chem. Soc., Chem. Commun.* **1986**, 55. Burgess, V. A.; Easton, C. J.; Hay, M. P. *J. Am. Chem. Soc.* **1989**, *111*, 1047.
(12) Easton, C. J.; Tan, E. W.; Hay, M. P. *J. Chem. Soc., Chem. Commun.* **1989**, 385.

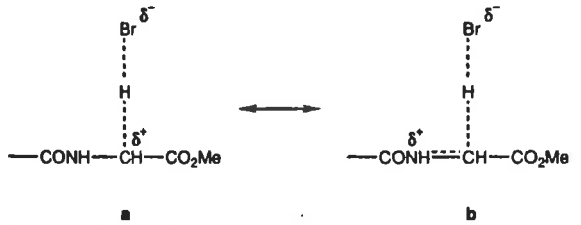


Figure 1. (a) Development of charge in the transition state for hydrogen atom abstraction by bromine atom and (b) delocalization of that charge by an amido substituent.

methylene adjacent to the methoxycarbonyl substituent in **12** would be inconsistent with the greater relative rate of reaction of **12** compared to **11a** and the reaction of **11a** at the methylene substituted with the phthalimido group in preference to reaction adjacent to the methoxycarbonyl substituent.

On this basis, the relative rates of reaction of **9a**, **10a**, **11a**, and **12** reflect the comparative ease of formation of the corresponding radicals **15**–**18**. The faster rate of reaction of **12** compared to **11a** can be attributed to the greater resonance stabilization provided by the benzamido substituent in **18** than by the phthalimido substituent in **17**. From the greater relative rate of formation of **15** compared to **18**, the effect of the methoxycarbonyl group in combination with the benzamido substituent is proactive, whereas the slower relative rate of formation of **16** compared to **17** shows that the effect of the methoxycarbonyl group in combination with the phthalimido substituent is counteractive. A radical formed adjacent to a methoxycarbonyl substituent is resonance stabilized, but formation of such a radical by hydrogen transfer to a bromine atom is often disfavored by a polar effect, involving the inductive interaction between the electron-deficient center of the substituent and that developing in the transition state at the site of hydrogen abstraction (Figure 1a).[13] The proactive nature of the methoxycarbonyl group in combination with the benzamido substituent may be attributed to the ability of the benzamido substituent to delocalize charge developed in the transition state (Figure 1b). The resulting diminution of the polar effect of the methoxycarbonyl substituent leads to the enhanced rate of formation of the resonance-stabilized radical **15**. The charge delocalization provided by the phthalimido substituent is less than that of the benzamido group, to the extent that this effect in the reaction of **10a** is outweighed by steric effects, resulting in the counteractive effect of the methoxycarbonyl group in combination with the phthalimido substituent. The steric effects will arise from interactions of the methoxycarbonyl and phthalimido substituents in **10a** with the hydrogen-abstracting species, and from interactions between the methoxycarbonyl and phthalimido substituents preventing the radical **16** from adopting planar conformations in which there is maximum delocalization of the unpaired spin density (Figure 2). These interactions will be greater than those between the methoxycarbonyl and benzamido substituents in **9a** and **15**.

In our initial report,[12] the contrasting effects of *N*-phthaloyl and *N*-acyl substituents were illustrated with

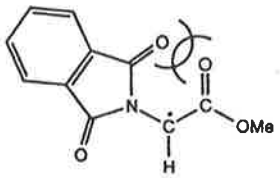
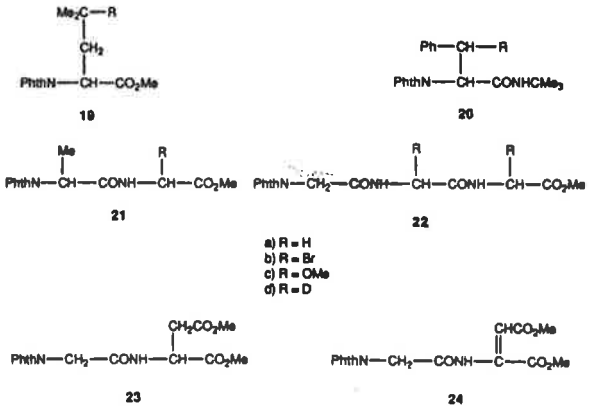


Figure 2. Nonbonding interactions associated with planar conformations of the radical **16**.

the reactions of **5a**–**8a** to give **5b**–**8b**, respectively. The reactions of **19a**–**22a** and **23** with NBS further demonstrate the extent of the counteractive effect of a carboxyl substituent in combination with the phthalimido substituent, to disfavor reaction at the α-position of amino acid derivatives. Treatment of the derivatives of leucine **19a** and phenylalaninamide **20a** with NBS (1 equiv) gave the corresponding bromides **19b** and **20b**, each in high yield. The reaction was repeated with the *S* isomer of **20a**, and the bromide **20b** thus obtained was treated with tributyltin hydride. The reduced product **20a** retained the homochirality of the starting material, as shown by comparison with a racemic sample using an HPLC column with (*S*)-phenylglycine as the chiral stationary phase.



The reaction of the peptide derivative **21a** with NBS (1 equiv) in dichloromethane gave the bromide **21b**, which was characterized by conversion to the methoxyglycine derivative **21c** and the deuteriated peptide derivative **21d**. Mass spectrometric analysis indicated that the deuterium incorporation of 90% in **21d** was regiospecific. The methoxyglycine derivative **21c** was obtained as a 1:1 mixture of diastereomers, as determined by ^{1}H NMR spectroscopy. The tripeptide derivative *N*-phthaloylglycylglycylglycine methyl ester (**22a**) was only sparingly soluble in dichloromethane, and its reaction with NBS had to be conducted in dilute solution. Under those conditions some of the NBS was consumed through reaction with the solvent. Thus, an excess of NBS (4 equiv) was required to produce the dibromide **22b**. Treatment of the crude dibromide **22b** with tributyltin deuteride gave **22d**, in 55% yield based on **22a**, with 90% and 95% deuterium incorporation at the C-terminal and nonterminal glycine residues, respectively. The reaction of *N*-phthaloylglycylaspartic acid dimethyl diester (**23**) with NBS gave an 82% yield of the α,β-dehydroaspartate derivative **24**, which was assumed to have *Z* stereochemistry on the basis of the tendency of dehydro amino acid derivatives to favor this configuration.[14]

In summary, the reactions of **5a**–**11a**, **12**, **19a**–**22a**, and **23** illustrate the contrasting effects of *N*-phthaloyl and

(13) Russell, G. A. *J. Am. Chem. Soc.* **1956**, *78*, 1047. Russell, G. A. *J. Org. Chem.* **1958**, *23*, 1407. Russell, G. A.; Williamson, R. C. *J. Am. Chem. Soc.* **1964**, *86*, 2357. Friedrich, S. S.; Friedrich, E. C.; Andrews, L. J.; Keefer, R. M. *J. Org. Chem.* **1969**, *34*, 900. Russell, G. A. *Free Radicals*; Kochi, J. K., Ed.; Wiley: New York, 1973; Vol. 1, p 275. Poutama, M. L. *Free Radicals*; Kochi, J. K., Ed.; Wiley: New York, 1973; Vol. 2, p 159.

(14) Schmidt, U.; Lieberknecht, A.; Wild, J. *Synthesis* **1988**, 159 and references cited therein.

N-acyl substituents on the regioselectivity of reactions of amino acid and peptide derivatives with NBS. They exemplify procedures for the functionalization of amino acid derivatives and for the regiocontrolled halogenation of peptide derivatives.

Experimental Section

General. Melting points are uncorrected. ^{1}H NMR spectra were recorded as dilute solutions in deuteriochloroform on a Bruker CXP-300 spectrometer. Chemical shifts (δ) are reported in parts per million downfield from tetramethylsilane. Electron-impact mass spectra were recorded on an AEI MS-3010 spectrometer. Microanalyses were performed by the Canadian Microanalytical Service Ltd., New Westminster, British Columbia, Canada. HPLC was performed by using a Waters Model 501 solvent delivery system and a U6K injector with a Waters Model 481 absorbance detector. Preparative chromatography was carried out on a Chromatotron 7924T instrument (Harrison Research, Palo Alto/TC Research, Norwich, CA) by using Merck silica gel 60 PF_{254}, eluting with a gradient of light petroleum/dichloromethane/ethyl acetate. Light petroleum refers to the fraction with bp 55–65 °C. Carbon tetrachloride and dichloromethane were purified by fractional distillation and stored over 4A molecular sieves. NBS was recrystallized from water and dried under reduced pressure before use. A Philips MLU 300-W (220–240-V) ultraviolet lamp was used as the light source in reactions of NBS. Tributyltin deuteride was prepared by reduction of tributyltin chloride with lithium aluminum deuteride.[15] *N*-Benzoylglycine methyl ester (**9a**),[16] *N*-phthaloylglycine methyl ester (**10a**),[17] *N*-phthaloyl-β-alanine methyl ester (**11a**),[18] *N*-benzoyl-β-alanine methyl ester (**12**),[19] *N*-phthaloylleucine methyl ester (**19a**),[20] (*S*)- and (*RS*)-*N*-(*tert*-butyl)-*N*$^\alpha$-phthaloylphenylalaninamide (**20a**), *N*-phthaloylalanylglycine methyl ester (**21a**),[21] *N*-phthaloylglycylglycylglycine methyl ester (**22a**),[21] and *N*-phthaloylglycylaspartic acid dimethyl diester (**23**) were prepared by using standard procedures.

***N*-Benzoyl-α-bromoglycine Methyl Ester (9b).** A mixture of the glycine derivative **9a** (200 mg, 1 mmol) and NBS (180 mg, 1 mmol) in carbon tetrachloride (20 mL) was heated at reflux under nitrogen for 0.25 h, with reaction initiated by irradiation with a 300-W ultraviolet lamp; then the reaction mixture was cooled, filtered, and concentrated under reduced pressure to give crude **9b**;[22] ^{1}H NMR 3.93 (3 H, s), 6.65 (1 H, d, J = 8 Hz), 7.30–7.90 (6 H, m).

***N*-Phthaloyl-α-bromoglycine Methyl Ester (10b).** Treatment of **10a** with NBS as described above for the preparation of **9b**, except that excess NBS (2 equiv) was used and the reaction mixture was heated at reflux for 48 h, gave a mixture of **10a** and **10b** in a ratio of ca. 1:1. Chromatography of the mixture on silica gave **10b** in 27% yield: mp 116–118 °C; ^{1}H NMR 3.88 (3 H, s), 6.68 (1 H, s), 7.80–8.00 (4 H, m); MS m/e 240, 238, 218, 160; MS m/e 237.9508 (M^+ – COOMe). Calcd for $C_9H_5{}^{79}BrNO_2$: 237.9504.

***N*-Phthaloyl-β-bromo-β-alanine Methyl Ester (11b).** Treatment of **11a** with NBS as described above for the preparation of **9b**, except that the reaction mixture was heated at reflux for 2 h, gave **11b** in 84% yield: mp 95–97 °C; ^{1}H NMR 3.64 (1 H, dd, J = 7 and 17 Hz), 3.70 (3 H, s), 3.89 (1 H, dd, J = 8 and 17 Hz), 6.59 (1 H, dd, J = 7 and 8 Hz), 7.20–7.90 (4 H, m); MS m/e 313, 311, 282, 280, 254, 252, 240, 238, 232, 200, 173, 172, 160. Anal. Calcd for $C_{12}H_{10}NO_4$: C, 46.2; H, 3.2; N, 4.5. Found: C, 46.4; H, 3.2; N, 4.6.

Reaction of *N*-Benzoyl-β-alanine Methyl Ester (12) with NBS. Treatment of **12** with NBS as described above for the preparation of **9b**, except that the reaction mixture was heated at reflux for 2 h, gave an oil that contained unreacted starting material and the dehydro-β-alanine derivatives **13** and **14**, in a ratio of ca. 3:2:1, as determined by ^{1}H NMR spectroscopic analysis. The components were separated by chromatography of the mixture on silica, to give **13** and **14** in yields of 32% and 15%, respectively. The stereochemistry of **13** and **14** was assigned on the basis of an X-ray crystal structure determination.[23]

Methyl (*E*)-3-benzamido-2-bromoacrylate (13): mp 98–99 °C; ^{1}H NMR 3.89 (3 H, s), 7.40–7.90 (5 H, m), 8.38 (1 H, br d, J = 12 Hz), 8.60 (1 H, d, J = 12 Hz). Anal. Calcd for $C_{11}H_{10}BrNO_3$: C, 46.5; H, 3.5. Found: C, 46.5; H, 3.5.

Methyl (*Z*)-3-benzamido-2-bromoacrylate (14): mp 122.5–123.5 °C; ^{1}H NMR 3.80 (3 H, s), 7.40–7.90 (5 H, m), 8.20 (1 H, d, J = 12 Hz), 11.30 (1 H, br d, J = 12 Hz). Anal. Calcd for $C_{11}H_{10}BrNO_3$: C, 46.5; H, 3.5. Found: C, 46.5; H, 3.7.

***N*-Phthaloyl-γ-bromoleucine Methyl Ester (19b).** Treatment of **19a** with NBS as described above for the preparation of **9b** gave **19b** in 82% yield: mp 63–64 °C; ^{1}H NMR 1.76 (3 H, s), 1.84 (3 H, s), 2.85 (2 H, m), 3.74 (3 H, s), 5.25 (1 H, dd, J = 4.5 and 8 Hz), 7.75–7.90 (4 H, m); MS m/e 296, 294, 274, 214. Anal. Calcd for $C_{15}H_{16}BrNO_4$: C, 50.8; H, 4.6; N, 4.0. Found: C, 50.9; H, 4.6; N, 4.0.

***N*-*tert*-Butyl-*N*$^\alpha$-phthaloyl-β-bromophenylalaninamide (20b).** Treatment of (*RS*)-**20a** with NBS as described above for the preparation of **9b** gave **20b** as a 1:1 mixture of diastereomers, which were separated by fractional crystallization from 2-propanol/light petroleum. One diastereomer was obtained as needle-shaped crystals in 41% yield: mp 199–200 °C; ^{1}H NMR 1.03 (9 H, s), 5.28 (1 H, d, J = 12 Hz), 5.85 (1 H, br s), 6.25 (1 H, d, J = 12 Hz), 7.30–8.00 (9 H, m); MS m/e 430, 428. Anal. Calcd for $C_{21}H_{21}BrN_2O_3$: C, 58.8; H, 4.9; N, 6.5. Found: C, 58.8; H, 4.9; N, 6.6. The other diastereomer was isolated as pale-yellow granular crystals in 39% yield: mp 213–213.5 °C; ^{1}H NMR 1.43 (9 H, s), 5.32 (1 H, d, J = 11.5 Hz), 6.04 (1 H, d, J = 11.5 Hz), 6.50 (1 H, br s), 7.20–7.80 (9 H, m); MS m/e 430, 428. Anal. Calcd for $C_{21}H_{21}BrN_2O_3$: C, 58.8; H, 4.9; N, 6.5. Found: C, 58.5; H, 5.0; N, 6.8.

Reaction of (*S*)-*N*-*tert*-Butyl-*N*$^\alpha$-phthaloylphenylalaninamide (20a) with NBS and Tributyltin Hydride. Treatment of (*S*)-**20a** with NBS as described above for the reaction of (*RS*)-**20a** gave a 1:1 mixture of the diastereomers of the bromide (**20b**). A sample of this mixture of the bromides (**20b**) (100 mg, 0.23 mmol) was treated with tributyltin hydride (67 mg, 0.23 mmol) in benzene (5 mL), at reflux under nitrogen for 4 h. The product (**20a**) (71 mg, 88%) showed only one component, with a retention time of 21 min, on HPLC analysis using a Regis Pirkle covalent (*S*)-phenylglycine column (25 cm × 4.6 nm), eluting with a gradient of light petroleum/2-propanol (1 mL/min, 5–40% over 40 min). By comparison, under the same conditions (*RS*)-**20a** resolved into two components, with retention times of 21 and 22 min.

***N*-Phthaloylalanyl-α-methoxyglycine Methyl Ester (21c).** The peptide derivative **21a** (0.7 mmol) was treated with NBS (125 mg, 0.7 mmol) in dichloromethane (25 mL), at reflux under nitrogen for 0.5 h, with reaction initiated by irradiation with a 300-W ultraviolet lamp. The reaction mixture was cooled and filtered, and the filtrate was concentrated under reduced pressure to give the crude bromide **21b** (^{1}H NMR 6.56, d, J = 10 Hz). Alternatively, methanol (0.5 mL) was added to the filtrate and the mixture was stirred at room temperature for 2 h; then it was concentrated under reduced pressure. The residual oil was chromatographed on silica and crystallized from dichloromethane/light petroleum to give the methoxyglycine derivative **21c** in 70% yield: mp 134–139 °C; ^{1}H NMR 1.72 (1.5 H, d, J = 7.5 Hz), 1.74 (1.5 H, d, J = 7.5 Hz), 3.45 (1.5 H, s), 3.47 (1.5 H, s), 3.78 (1.5 H, s), 3.79 (1.5 H, s), 5.00 (1 H, m), 5.56 (0.5 H, d, J = 9 Hz), 5.58 (0.5 H, d, J = 9 Hz), 6.93 (0.5 H, br d, J = 9 Hz), 6.98 (0.5 H, br d, J = 9 Hz), 7.70–7.90 (4 H, m); MS m/e 261, 202, 175, 174. Anal. Calcd for $C_{15}H_{16}N_2O_6$: C, 56.3; H, 5.0; N, 8.7. Found: C, 56.4; H, 5.0; N, 8.7.

***N*-Phthaloylalanyl-α-deuterioglycine Methyl Ester (21d).** Tributyltin deuteride (310 mg, 1.07 mmol) was added to a crude,

(15) Kuivila, H. G.; Beumel, O. F. *J. Am. Chem. Soc.* **1961**, *83*, 1246.
(16) Huang, H. T.; Niemann, C. *J. Am. Chem. Soc.* **1952**, *74*, 4634.
(17) Skorna, G.; Ugi, I. *Chem. Ber.* **1979**, *112*, 776.
(18) Clarke, S.; Hider, R. C.; John, D. I. *J. Chem. Soc., Perkin Trans. 1* **1973**, 230.
(19) Garapon, J.; Touet, R.; Sillion, B. Ger. Offen. DE 3,327,285, 1984.
(20) Tascher, E.; Wasielewski, C. *Justus Liebigs Ann. Chem.* **1961**, *640*, 136.
(21) Islam, A. M.; El-Naggar, A. M.; Abd-El-Salam, A. M.; Zaher, M. R. *Egypt. J. Chem.* **1975**, *18*, 35.
(22) Kober, R.; Papadopoulos, K.; Miltz, W.; Enders, D.; Steglich, W.; Reuter, H.; Puff, H. *Tetrahedron* **1985**, *41*, 1693.
(23) Easton, C. J.; Rositano, G.; Tiekink, E. R. T. *Acta Crystallogr.* **1990**, *C46*, 1122.

filtered reaction mixture of the bromide **21b**, prepared from **21a** (0.7 mmol) as described above. The mixture was stirred at room temperature for 2 h; then it was concentrated under reduced pressure. The residual oil was chromatographed on silica and crystallized from dichloromethane/light petroleum to give **21d** in 70% yield: mp 145–146 °C; ^{1}H NMR 1.73 (3 H, d, J = 7.5 Hz), 3.73 (3 H, s), 4.05 (1.1 H, d, J = 5 Hz), 4.98 (1 H, q, J = 7.5 Hz), 6.71 (1 H, br d, J = 5 Hz), 7.70–7.85 (4 H, m); MS m/e 291 (90% $^2H_1$), 232 (90% $^2H_1$), 202 (0% $^2H_1$).

***N*-Phthaloylglycyl-α-deuterioglycyl-α-deuterioglycine Methyl Ester (22d).** Addition of tributyltin deuteride (530 mg, 1.8 mmol) to a crude, filtered reaction mixture of **22b**, prepared from **22a** (200 mg, 0.6 mmol) and NBS (430 mg, 2.4 mmol) in dichloromethane (200 mL), as described above for the preparation of **21b**, gave **22d** (0.11 g, 55%): mp 230–231 °C; ^{1}H NMR 3.69 (3 H, s), 3.86 (1.1 H, d, J = 5 Hz), 3.90 (1.05 H, d, J = 6 Hz), 4.37 (2 H, s), 7.70–7.90 (4 H, m), 8.14 (1 H, br d, J = 6 Hz), 8.51 (1 H, br d, J = 5 Hz); MS m/e 335 (90% $^2H_2$, 5% $^2H_1$), 246 (95% $^2H_1$), 218 (95% $^2H_1$), 188 (0% $^2H_1$).

***N*-Phthaloylglycyl-(*Z*)-α,β-dehydroaspartic Acid Dimethyl Diester (24).** Treatment of **23** (400 mg, 1.15 mmol) with NBS (205 mg, 1.15 mmol), as described above for the preparation of **21b**, gave **24** (330 mg, 82%): mp 175–176 °C; ^{1}H NMR 3.73 (3 H, s), 3.79 (3 H, s), 4.50 (2 H, s), 5.57 (1 H, s), 7.70–7.90 (4 H, m), 10.50 (1 H, br s); MS m/e 346, 345, 314, 287, 188, 161, 160. Anal. Calcd for $C_{16}H_{14}N_2O_7$: C, 55.5; H, 4.1; N, 8.1. Found: C, 55.5; H, 4.0; N, 8.1.

Acknowledgment. This work was supported by a grant from the Australian Research Council.

Registry No. **9a**, 1205-08-9; **9b**, 101649-82-5; **10a**, 23244-58-8; **10b**, 135395-15-2; **11a**, 39739-01-0; **11b**, 135395-26-5; **12**, 89928-06-3; **13**, 129309-14-4; **14**, 129309-13-3; **19a**, 132785-19-4; **19b**, 132785-25-2; (*S*)-**20a**, 135395-13-0; (*RS*)-**20a**, 135501-56-3; (2*S*,3*R*)-**20b**, 135395-16-3; (2*S*,3*S*)-**20b**, 135395-17-4; (±)-(*R**,*R**)-**20b**, 135501-57-4; (±)-(*R**,*S**)-**20b**, 135501-58-5; **21a**, 63267-72-1; **21b** (diastereomer 1), 135395-18-5; **21b** (diastereomer 2), 135395-19-6; **21c** (diastereomer 1), 135395-20-9; **21c** (diastereomer 2), 135395-21-0; **21d** (diastereomer 1), 135395-22-1; **21d** (diastereomer 2), 135395-23-2; **22a**, 63199-92-8; **22b**, 135395-24-3; **22d**, 135395-25-4; **23**, 135395-14-1; **24**, 87358-90-5.

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AN EPR STUDY OF POLAR EFFECTS IN RADICAL REACTIONS OF *N*-ACYLAMINO ACID DERIVATIVES

Key Words: amino acids, radicals, polar effects, EPR spectroscopy

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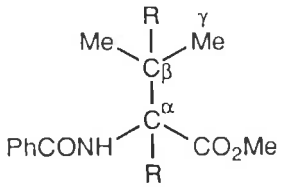
ABSTRACT

Radicals formed by regioselective hydrogen atom transfer from the β- and γ-positions of *N*-acetylvaline (**14**) and the *N*-methyl substituent in *N*-acetylsarcosine (**15**) have been identified by EPR spectroscopy. The detection of these species provides direct evidence of polar effects in radical reactions of amino acid derivatives.

INTRODUCTION

Atom transfer reactions of *N*-acyl-α-amino acid derivatives generally favor formation of α-carbon-centered radicals.[1] For example, the benzoyl peroxide or photochemically initiated reactions of the derivatives of valine (**1a**) and sarcosine (**2a**) with *N*-bromosuccinimide proceed *via* formation of the corresponding radicals (**3**) and (**4**), to give (**1b**) and (**2b**), respectively.[2,3] Radicals such as (**3**) and (**4**) are stabilized by the combined resonance effects of an electron-releasing amido substituent and an electron-withdrawing carboxy group, and they may be classified as captodative,[4] merostabilized,[5] or "push-pull" stabilized[6] radicals.

The reactions of (**1a**) and (**2a**) with sulphuryl chloride are in direct contrast to those with *N*-bromosuccinimide.[2,3] The valine derivative (**1a**) afforded a mixture of the β-chlorovaline derivative (**5**) and the diastereomers of the γ-chlorovaline derivative (**6**), while the sarcosine derivative (**2a**) gave only (**7**). These reactions indicate that (**1a**) and (**2a**) afford the radicals (**8-10**), in preference to (**3**) and (**4**), respectively. To seek direct evidence for this unusual regioselectivity, we undertook an EPR study of the radicals formed by hydrogen atom transfer from *N*-acyl-α-amino acid derivatives.



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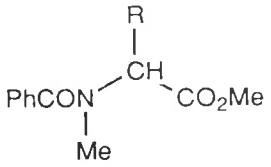
b) R = Br



(2)

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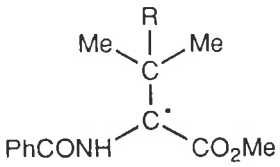

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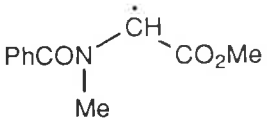

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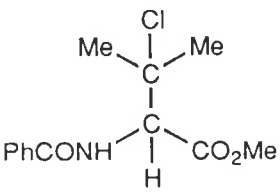

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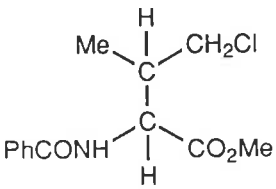

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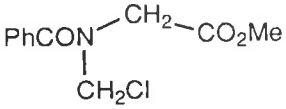

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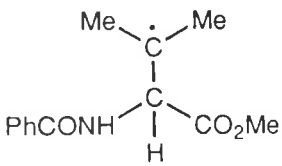

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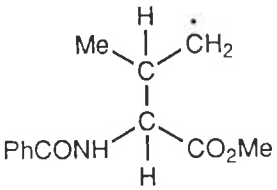

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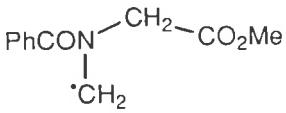
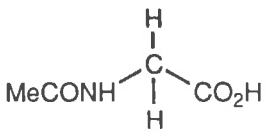


(10)

EXPERIMENTAL

The amino acid derivatives, *N*-acetylglycine (**11**), *N*-acetylalanine (**12**), pyroglutamic acid (**13**), *N*-acetylvaline (**14**) and *N*-acetylsarcosine (**15**), used in this study, were purchased from Sigma Chemical Co. Aqueous solutions of the amino acid derivatives (**11-15**) (20 g/L) and hydrogen peroxide (30 wt %, 2.5 ml/L) were mixed with an aqueous solution of titanous chloride (15 wt %, 10 ml/L) and ethylenediaminetetraacetic acid (EDTA) (0.7 g/L) (system A), at room temperature directly in the cavity of a Varian E9 spectrometer, using a flow system similar to that described by Dixon and Norman.[7] Under these conditions the amino acid derivatives (**11-15**) remain predominantly in the protonated



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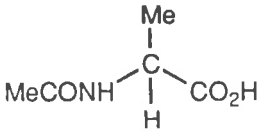

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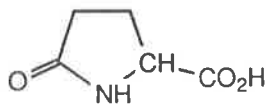

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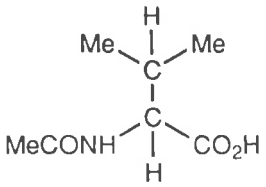

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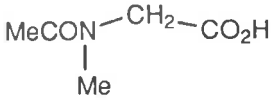


(15)

form. Alternatively, aqueous solutions of the sodium salts of the amino acid derivatives (**11**-**15**) (20 g/L) and hydrogen peroxide (30 wt %, 2.5 ml/L) were mixed with an aqueous solution of ferrous sulphate (1.4 g/L) and EDTA (1.9 g/L) in 0.01 M phosphate buffer at pH 7 (system B).[8] In the absence of EDTA, the reactions of (**11**-**15**) are affected by their complexation with the metal ions.[9]

RESULTS AND DISCUSSION

Spectral parameters for the radicals derived from (**11**-**15**) under these conditions are listed in Table 1. The radicals are formed by hydrogen atom transfer to oxygen centered radicals, derived from hydrogen peroxide. As expected on the basis of previous work,[10,11] *N*-acetylglycine (**11**), *N*-acetylalanine (**12**) and pyroglutamic acid (**13**) gave spectra consistent with formation of the corresponding α-carbon-centered radicals (**16**) (Figure 1), (**17**) and (**18**). In contrast, there was no evidence of formation of the α-carbon-centered radicals (**19a**) and (**19b**) in the reactions of *N*-acetylvaline (**14**). The only radicals evident were (**20a**) and (**21a**) (system A), and (**20b**) and (**21b**) (system B). The ratio of (**20a**) to (**21a**) and (**20b**) to (**21b**) was approximately 2:1, in each case. The radicals (**20a**), (**20b**), (**21a**) and (**21b**) were identified by comparison of their spectral parameters

TABLE 1

EPR Spectral Parameters for Radicals Produced in Reactions of the Amino Acid Derivatives (**11**-**15**) with Titanous Chloride - Hydrogen Peroxide (System A) and Ferrous Sulphate - Hydrogen Peroxide (System B)

Substrate	Reagent System	Radical	aH(α)	aH(β)	aH(β')	aN
			(gauss)			
(**11**)	A	(**16a**)	16.5(d)			2.5(t)
	B	(**16b**)	17.5(d)			3.0(t)
(**12**)	A	(**17a**)		18.0(q)		2.0(t)
	B	(**17b**)		18.0(q)		2.0(t)
(**13**)	A	(**18a**)		23.0(t)		3.0(t)
	B	(**18b**)		23.0(t)		3.0(t)
(**14**)	A	(**20a**)		24.0(s)	4.0(d)	6.0(t)
		(**21a**)	22.0(t)	28.0(d)		
	B	(**20b**)		23.0(s)	NR	NR
		(**21b**)	22.0(t)	28.5(d)		
(**15**)	A	(**22a**)	18.5(t)			NR
	B	(**22b**)	18.5(t)			NR
		(**23**)	17.0(d)			NR

(d) - doublet
(t) - triplet
(q) - quartet
(s) - heptet
NR - other hyperfine splittings were not resolved

The g values for all the radicals (**16**-**23**) were within the range 2.0025-2.0035.

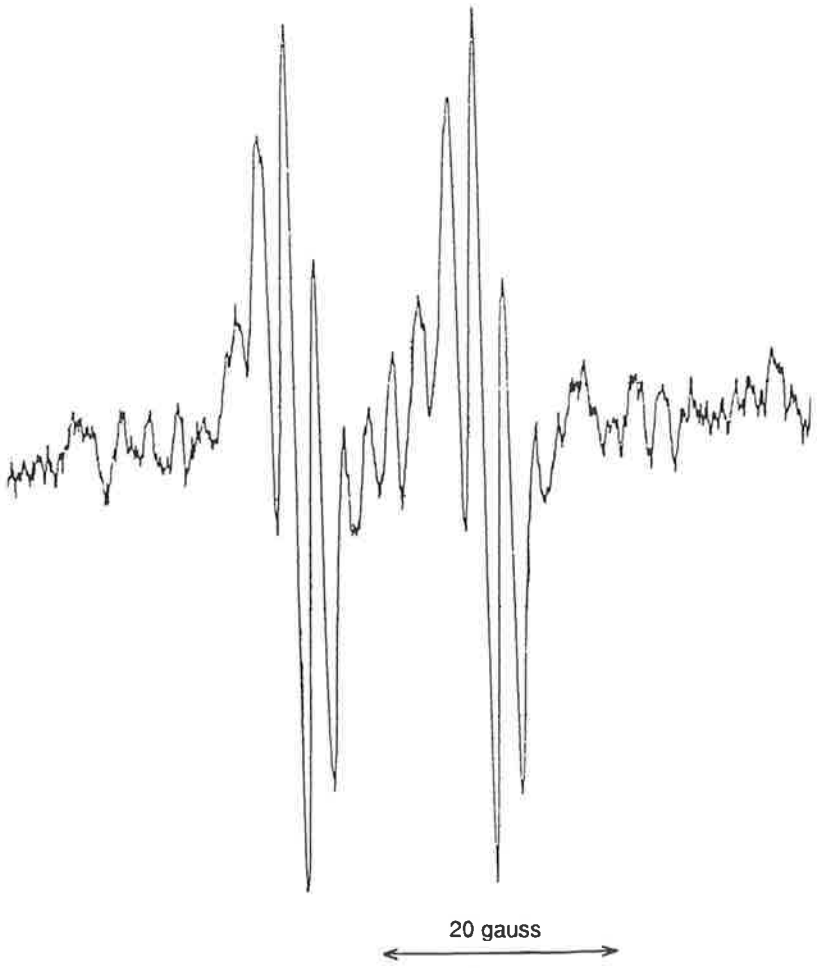


Figure 1. EPR spectrum obtained by treatment of *N*-acetylglycine (**11**) with titanous chloride - hydrogen peroxide.

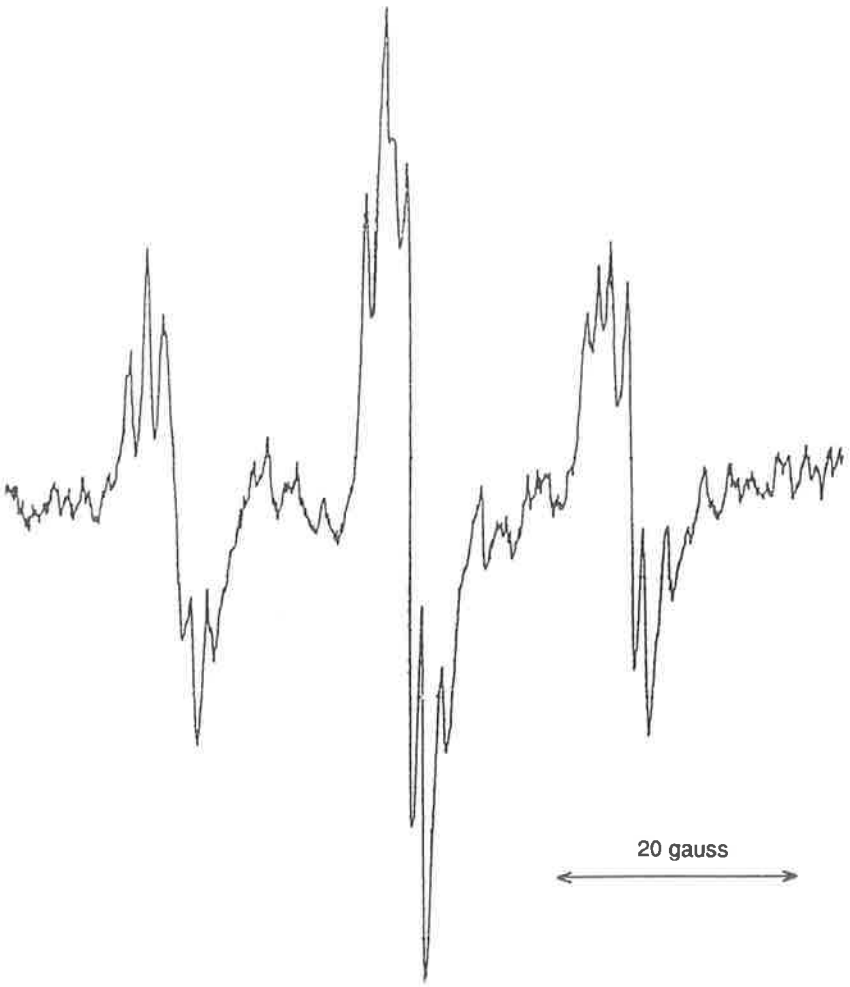
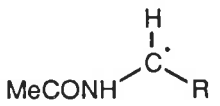


Figure 2. EPR spectrum obtained by treatment of *N*-acetylsarcosine (**15**) with titanous chloride - hydrogen peroxide.

with those formed by hydrogen transfer from the β- and γ-positions of valine.[8,12] When a 1:1 (wt/wt) mixture of *N*-acetylglycine (**11**) and *N*-acetylvaline (**14**) was treated with either titanous chloride - hydrogen peroxide or ferrous sulphate - hydrogen peroxide, the only radicals detected were those derived from (**14**). On treatment with titanous chloride - hydrogen peroxide, *N*-acetylsarcosine (**15**) gave a spectrum consistent with formation of the radical (**22a**) (Figure 2), while both (**22b**) and (**23**) were evident in the spectrum of the reaction of (**15**) with ferrous sulphate - hydrogen peroxide.



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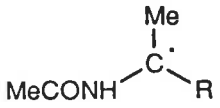

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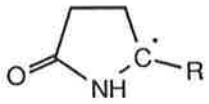

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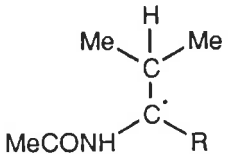

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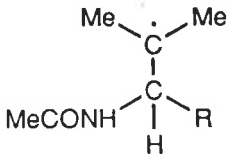

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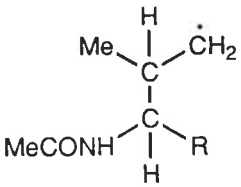

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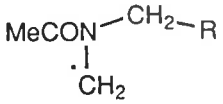

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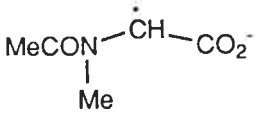

(23)

a) R = CO_2H
b) R = CO_2^-

The regioselectivity observed in these reactions of (**14**) and (**15**) is similar to that of the reactions of (**1a**) and (**2a**) with sulphuryl chloride.[2,3] In each case the outcome of the reaction can be attributed to polar effects. While α-carbon-centered radicals are formed in reactions of *N*-acylamino acid derivatives, for example, in the reactions of (**1a**) and (**2a**) with N-bromosuccinimide to give (**1b**) and (**2b**), respectively, hydrogen atom transfer reactions may afford less stable products if electrophilic radicals are involved in the hydrogen abstraction and if there is little develoment of radical character in the transition state of the reaction. Under these circumstances, the inductive electron-withdrawing effect of the amido and carboxyl groups acts to retard attack at the α-position of *N*-acylamino acid derivatives, thus favoring reaction at the β- and γ-positions in (**1a**) and (**14**), and at the *N*-methyl group in (**2a**) and (**15**). Presumably the deactivating inductive effect of the carboxylate ion is less than that of the free acid, with the result that treatment of the sodium salt of (**15**) with ferrous sulphate - hydrogen peroxide affords both (**22b**) and (**23**).

These reactions provide a clear and direct indication of the importance of polar effects in the atom transfer reactions of *N*-acyl-α-amino acid derivatives.

ACKNOWLEDGEMENT

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REFERENCES

1. Easton CJ. α-Carbon-centered Radicals from Amino Acids and their Derivatives. In: Coxon JM. ed. *Advances in Detailed Reaction Mechanisms, Vol. 1.* Greenwich: JAI Press 1991: 83-126.

2. Bowman NJ., Hay MP., Love SG., Easton CJ. Regioselective Chlorination of Valine Derivatives. J. Chem. Soc., Perkin Trans. I 1988: 259.

3. Easton CJ., Hay MP., Love SG. Regioselective Formation of Amidocarboxy-substituted Free Radicals. J. Chem. Soc., Perkin Trans. I 1988: 265.

4. Viehe HG., Janousek Z., Merenyi R., Stella, L. The Captodative Effect. Acc. Chem. Res. 1985; 18: 148.

5. Baldock RW., Hudson P., Katritzky AR., Soti F. Stable Free Radicals. Part I. A New Principle Governing the Stability of Organic Free Radicals. J. Chem. Soc., Perkin Trans. I 1974: 1422.

6. Balaban, AT. Stable Nitrogen Free Radicals. Rev. Roum. Chim. 1971; 16: 725.

7. Dixon WT., Norman ROC. Electron Spin Resonance Studies of Oxidation. Part I. Alcohols. J. Chem. Soc. 1963: 3119.

8. Taniguchi H., Hasumi H., Hatano H. Electron Spin Resonance Study of Amino Acid Radicals Produced by Fenton's Reagent. Bull. Chem. Soc. Jpn. 1972; 45: 3380.

9. Burgess VA., Easton CJ. Selective γ-Hydrogen Atom Abstraction in Reactions of N-Acetylamino Acids and N-Alkylacetamides with Titanous Ion and Hydrogen Peroxide. Tet. Lett. 1987; 28: 2747.

10. Taniguchi H., Hatano H., Hasegawa H., Maruyama T. Free Radical Intermediates in the Reactions of the Hydroxyl Radical with Amino Acid Derivatives and Related Compounds. J. Phys. Chem. 1970; 74: 3063.

11. Taniguchi H., Kirino Y. An ESR Study of the Acid Dissociation of NH Protons. 2. Cyclic Peptide Radicals and Related Radicals. J. Am. Chem. Soc. 1977; 99: 3625.

12. Armstrong WA., Humphries WG. Amino Acid Radicals Produced Chemically in Aqueous Solutions. Electron Spin Resonance Spectra and Relationship to Radiolysis Products. Canad. J. Chem., 1967; 45: 2589.

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N-Methylation of Carbamate Derivatives of α-Amino Acids

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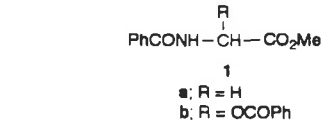
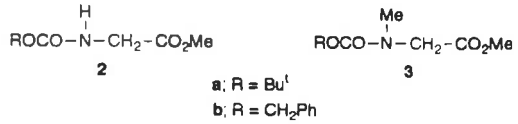
Carbamate derivatives of α-amino acids react by *N*-methylation, without racemization, on treatment with *tert*-butyl perbenzoate in the presence of copper(II) octanoate; the selective reaction of *N*-*tert*-butoxycarbonylglycine methyl ester, in preference to the corresponding alanine and valine derivatives, indicates that the relative reactivity of substrates is determined by the comparative ease of their complexation to the copper.

Copper-catalysed reactions of peresters with organic substrates are often used for introduction of the acyloxy functional group.[1] Accordingly, treatment of *N*-benzoylglycine methyl ester **1a**† with *tert*-butyl perbenzoate, in the presence of copper(II) octanoate, gave the α-benzoyloxyglycine derivative **1b**, in 67% yield. We have now found, however, that the course of reaction depends on the nature of the amino acid *N*-protecting group. With the carbamates **2a** and **2b**, the only products formed were the corresponding sarcosine derivatives **3a** and **3b**. In a typical experiment, treatment of **2a** (0.53 mmol) with *tert*-butyl perbenzoate (4.2 mmol) in the presence of copper(II) octanoate (2 mg) in benzene (40 ml) at reflux under nitrogen for 24 h, gave **3a** in 57% yield, after work-up and chromatography on silica. Under similar conditions, **2b** gave **3b** in 54% yield. Analysis of the crude reaction mixtures by ^{1}H NMR spectroscopy showed the presence of **3a** and **3b** and the corresponding residual starting materials **2a** and **2b**, in the ratio *ca.* 3 : 1 in each case.

The production of **3a** and **3b** may be rationalised as shown in Scheme 1. Electron transfer from copper(I) ion to *tert*-butyl perbenzoate affords copper(II) ion, benzoate and *tert*-butoxyl radical. In turn, electron transfer from the carbamates **2a** and **2b** to copper(II) ion, followed by proton transfer, affords the corresponding carbamate radicals (presumably copper bound rather than discrete species), which react by combination with methyl radical, produced by β-scission of *tert*-butoxyl radical, to give **2b** and **3b**, respectively. The different course of reaction of **2a** and **2b**, compared to **1a**, may be attributed to the propensity of carbamates to react by electron transfer. The selective reaction of a carbamate, in preference to an amide, was clearly demonstrated in the regioselective reaction of the dipeptide derivative **4a** to give **4b**.

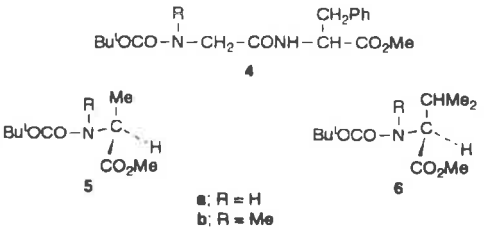


1
a; R = H
b; R = OCOPh



2 **3**
a; R = Bu^t
b; R = CH_2Ph

† The amino acid derivatives **1a,b–6a,b** used in this study, either as substrates or as authentic samples to identify products of reactions, were synthesized using standard procedures, and had spectral and physical properties consistent with those reported previously,[2–6] with the exception of **4a** and **4b** which were completely characterized as new compounds.



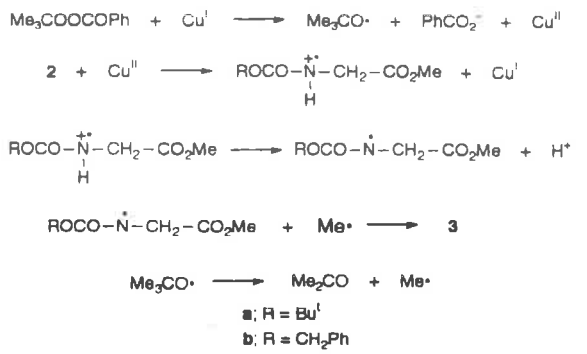
a; R = H
b; R = Me

When the (*S*)-alanine derivative **5a** was treated with *tert*-butyl perbenzoate and copper(II) octanoate, as described above, the yield of the *N*-methylamino acid derivative **5b** was 47%. Analysis of the product by ^{1}H NMR spectroscopy in the presence of tris[heptafluoropropylhydroxymethylene)-(+)-camphorato]europium(III) [Eu(hfc)$_3$],[2] and through comparison with an authentic sample of the corresponding racemate, showed that the reaction occurred without racemization.

The valine derivative **6a** was inert under the reaction conditions used to produce **3a,b**, **4b** and **5b**, from **2a,b**, **4a** and **5a**, respectively. With a mixture of the glycine derivative **2a** and the valine derivative **6a**, only the glycine derivative **2a** reacted, to give **3a**. Similarly, only the glycine derivative **2a** reacted when a mixture of **2a** and the alanine derivative **5a** was treated under the standard reaction conditions. The lack of reaction of the valine derivative **6a**, and the selective reaction of **2a** from mixtures of **2a** and **5a**, and **2a** and **6a**, may be attributed to the relative ease of complexation of these substrates to the copper catalyst. The glycine derivative **2a** binds selectively to the catalyst and, as a consequence, reacts faster than the alanine derivative **5a**. The binding of the valine derivative **6a** to copper is even less efficient, to the extent that no reaction occurs.

Earlier[7] we proposed that the preferential reaction of glycine residues in radical reactions of amino acid derivatives could be attributed to the relative stability and ease of formation of the corresponding α-carbon-centred radicals. That hypothesis does not account for the selectivity observed in the reactions of **2a**, **5a** and **6a**, nor does the rationale proposed above for the selective reaction of **2a** provide a satisfactory explanation for the selective free-radical halogenation of glycine derivatives, on which the earlier hypothesis was based. Instead, the rationale for the selective reaction of **2a** to give **2b**, by preferential binding to the copper catalyst, indicates a different factor which contributes to the selective reaction of glycine derivatives in free-radical reactions of amino acid derivatives.

In summary, the reactions of **2a**, **2b** and **4a–6a**, with *tert*-butyl perbenzoate in the presence of copper(II) octanoate, represent a novel mode of reaction of organic substrates on



a; R = Bu^t
b; R = CH_2Ph

Scheme 1

treatment with peresters in the presence of copper salts, they provide another aspect to account for the selective reaction of glycine residues in free-radical reactions of α-amino acid derivatives, and they illustrate a complementary[3,8] new procedure for the *N*-methylation of α-amino acid derivatives, without racemization. Studies aimed to optimize the synthetic potential of this methodology are continuing in our laboratories.

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References

1 For a review see: D. J. Rawlinson and G. Sosnovsky, *Synthesis*, 1972, 1.
2 H. Kessler and M. Molter, *Angew. Chem., Int. Ed. Engl.*, 1973, **12**, 1011; *J. Am. Chem. Soc.*, 1976, **98**, 5969.
3 R. K. Olsen, *J. Org. Chem.*, 1970, **35**, 1912.
4 H. T. Huang and C. Niemann, *J. Am. Chem. Soc.*, 1952, **74**, 4634.
5 J. E. Baldwin, R. M. Adlington, C. Lowe, I. A. O'Neil, G. L. Sanders, C. J. Schofield and J. B. Sweeney, *J. Chem. Soc., Chem. Commun.*, 1988, 1030.
6 C. Toniolo, G. M. Bonora, F. C. Schilling and F. A. Bovey, *Macromolecules*, 1980, **13**, 1381; C. C. Watson, *Spectrochim. Acta*, 1960, **16**, 1322.
7 C. J. Easton and M. P. Hay, *J. Chem. Soc., Chem. Commun.*, 1986, **55**; V. A. Burgess, C. J. Easton and M. P. Hay, *J. Am. Chem. Soc.*, 1989, **111**, 1047.
8 For examples see: J. R. Coggins and N. L. Benoiton, *Can. J. Chem.*, 1971, **49**, 1968; M. Kawai and U. Nagai, *Chem. Lett.*, 1977, 1397; M. Kawai, N. Fukita, N. Ito, M. Ohya, Y. Butsugan, M. Maruyama and Y. Kudo, *J. Chem. Soc., Chem. Commun.*, 1986, 955; M. Kawai, N. Fukuta, N. Ito, T. Kagami, Y. Butsugan, M. Maruyama and Y. Kudo, *Int. J. Peptide Protein Res.*, 1990, **35**, 452.

α-CARBON-CENTERED RADICALS FROM AMINO ACIDS AND THEIR DERIVATIVES

Christopher J. Easton

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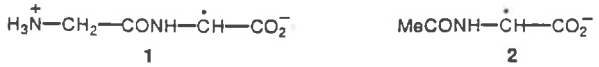
1. INTRODUCTION

Many biosynthetic and biodegradative reactions of proteins, peptides, and other amino acid derivatives involve free-radical processes. For example, there is considerable evidence that free radicals are involved in penicillin and cephalosporin biosynthesis,[1–5] and they have been proposed as intermediates in protein–DNA crosslinking[6–8] and in the yellowing of protein.[9] Radical reactions of amino acids and their derivatives have been studied in order to investigate biochemical systems such as these[5,10,11] and to develop synthetic methods.[12–27] With over 500 amino acids now known, of which circa 240 occur free in nature,[28] these compounds and their derivatives are of interest as synthetic targets.

The free radicals that have been identified in proteins upon irradiation[29,30] may be categorized into three types: aromatic radicals, sulfur radicals, and aliphatic radicals. Aromatic radicals arise from reactions of aromatic side chains in amino acid residues such as phenylalanine and tryptophan.[31] The production of sulfur radicals is thought to involve sensitization of sulfur-containing side chains by aromatic residues.[32–34] The aliphatic radicals produced through the irradiation of proteins are mainly α-carbon-centered radicals (Figure 1). They have been chosen as the focus of this review because they are the radicals that are particular to amino acid derivatives. Other radicals are discussed only in terms of their reactions competing with those of α-carbon-centered radicals. Functional group manipulations such as deaminations and decarboxylations, in which the integrity of the amino acids and their derivatives is not retained, are not discussed. These and other aspects of the radical chemistry of amino acids and their derivatives have been the subject of previous reviews.[29,30]

The main aliphatic radicals produced in proteins upon irradiation have been identified as α-carbon-centered radicals derived from glycine residues.[30] Their electron spin resonance (ESR) spectra show a doublet resonance with hyperfine



1 2

coupling similar to that observed with the radicals **1** and **2**, generated from glycylglycine and *N*-acetylglycine, respectively.[35–42] The spectra of **1** and **2** show that there is extensive conjugation in radicals of this type, with only 70–75% of the unpaired spin density on the α carbon.[30,35,36,42]

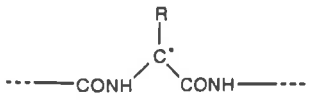


Figure 1. Aliphatic radicals formed by irradiation of proteins.

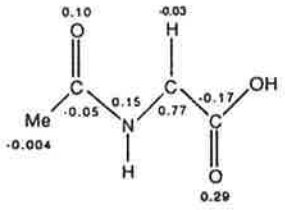
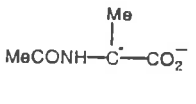


Figure 2. Distribution of the unpaired spin density in the radical produced by hydrogen atom transfer from *N*-acetylglycine.[30]

Molecular orbital calculations have also shown that the unpaired spin density in the radical **2** is distributed over the molecule (Figure 2).[30] The main contribution is from the α center with other contributions from both the carboxyl and amido groups. Radicals of this type belong to the class of captodative radicals. The captodative effect was postulated by Viehe et al.[43,44] as the combined resonance effect of electron-withdrawing (capto) and electron-donating (dative) substitutents on a radical center, leading to enhanced stabilization of the radical. The theoretical basis of this concept was originally formulated by Dewar in 1952.[45] Analogous concepts of "push–pull" radicals and "merostabilization" were independently developed by Balaban[46–48] and by Katritzky et al.,[49,50] respectively. Although the presence or absence of a synergistic radical-stabilizing effect, when a radical is substituted by both electron-donor and electron-acceptor moieties, remains a matter of debate,[51–59] the combined but not necessarily synergistic action of both the substituents results in stabilization of the radical.

Other amido- and carboxyl-substituted radicals analogous to **1** and **2** have been detected by electron spin resonance spectroscopy. The radicals **3** and **4** have been identified in studies of *N*-acetylalanine[37,40,41,60] and pyroglutamic acid,[60,61] respectively. With dipeptides, α-carbon-centered radicals are formed through reaction of C-terminal amino acids.[37,40–42,62] Thus glycylglycine and glycylalanine afford the radicals **1** and **5**, respectively. The selective reaction of C-terminal amino acid residues in dipeptides can be attributed to the stability of the product radicals. For example, the radical **6** is considerably less stable than **1**. The ammonium group strongly destabilizes a radical centered at the adjacent position.[39,41] For this reason α-carbon-centered radicals have been most frequently observed in reactions of amino acid derivatives, rather than with free amino acids. Only the nonprotonated radicals **7a**, **7b**, and **8** have been



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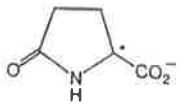

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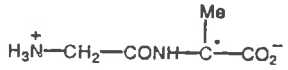

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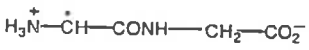

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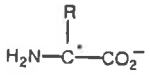

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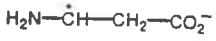


8

a) R = H
b) R = Me

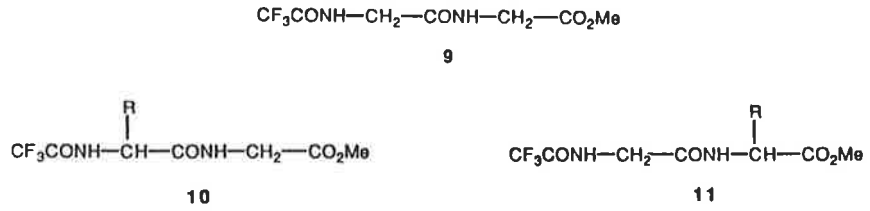
identified in ESR studies of glycine and α- and β-alanine, respectively.[39,63,64] The amino group stabilizes a radical formed at the adjacent position.

The numerous ESR studies have provided valuable qualitative data on the types of radicals produced through reaction of amino acids and their derivatives. The majority of the examples chosen to illustrate this chapter, however, are from studies for which quantitative data are available. Accordingly, the emphasis is on those investigations in which products have been isolated or characterized. Studies in which the conclusions are based solely on the spectroscopic observation of transient species, or on otherwise wholly qualitative data, are included only if they exemplify points raised in other investigations.

2. RADICALS FORMED BY HYDROGEN TRANSFER

Most of the studies in which products have been isolated have involved hydrogen-atom transfer reactions of amino acid derivatives. In a series of papers[65–71] Elad et al. have reported photoalkylation reactions of amino acid derivatives as a tool for the modification of peptides and proteins. In one of many examples,[67] irradiation with ultraviolet light of a mixture of the dipeptide derivative **9** and but-1-ene, in the presence of acetone at room temperature, gave the modified dipeptide derivatives **10a** and **11a**, in yields of 17% and 14%, respectively. With toluene instead of but-1-ene, **10b** and **11b** were formed in yields of 26% and 35%, respectively.[67]

A mechanism involving free-radical intermediates was proposed for the photoalkylation reactions (Scheme 1). Absorption of incident light by acetone



a) R = $CH_2CH_2CH_2Me$
b) R = CH_2Ph
c) R = $CH_2CH(CH_2Me)CH_2CH_2CH_2Me$

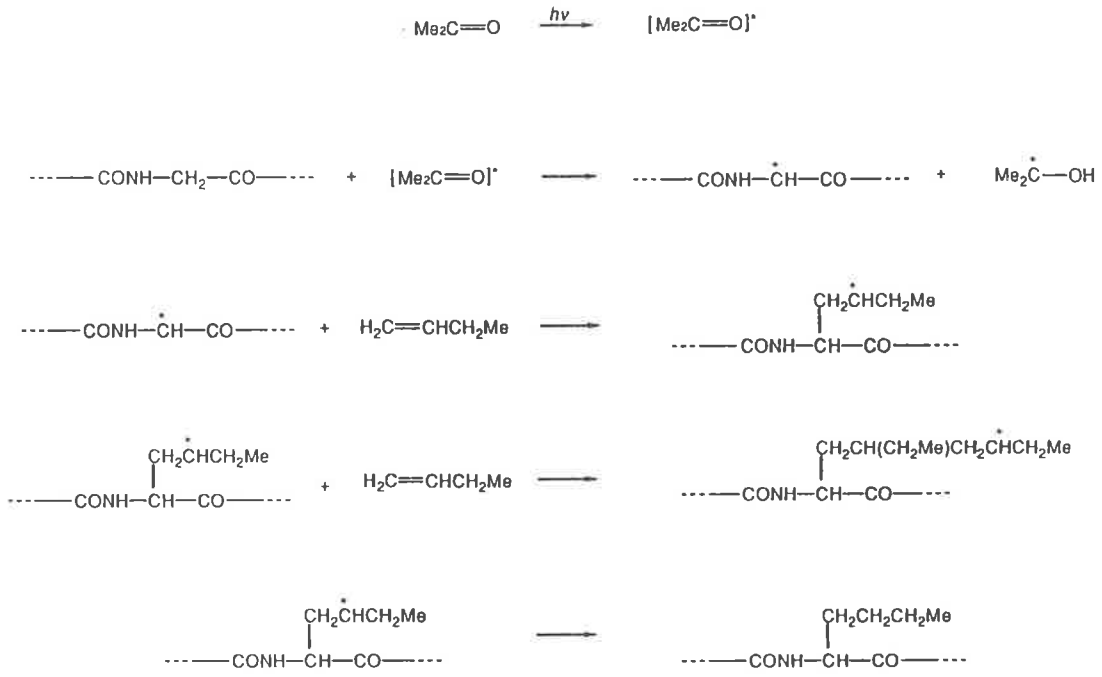
produces the triplet ketone, which abstracts a hydrogen atom from the amino acid derivative to give an α-carbon-centered radical. That radical reacts with but-1-ene by addition. In support of the proposed mechanism, low-molecular-weight telomers were identified in the photoalkylation reactions. For example, evidence was obtained for the production of **10c** and **11c** in the reaction of **9** with but-1-ene. For reactions carried out in the presence of toluene, the α-carbon-centered radical reacts by combination with benzyl radical, formed by hydrogen atom transfer from toluene. Bibenzyl was also formed in reactions when toluene was used, consistent with dimerization of benzyl radical. In the absence of an alkylating agent, dehydrodimers of the amino acid derivatives were formed, presumably as a result of radical coupling.

In a variation of the alkylation procedure,[68,71] the reactions were induced with visible light using a combination of an α-diketone, such as biacetyl or camphorquinone, and a peroxide, such as di-*tert*-butyl peroxide. When used alone α-diketones failed to initiate the photoalkylation reaction, probably because the ability of excited state diketones to abstract hydrogen is rather weak. Presumably, when used in combination, the α-diketone absorbs visible light, then reacts with the peroxide to produce the hydrogen abstracting agent.

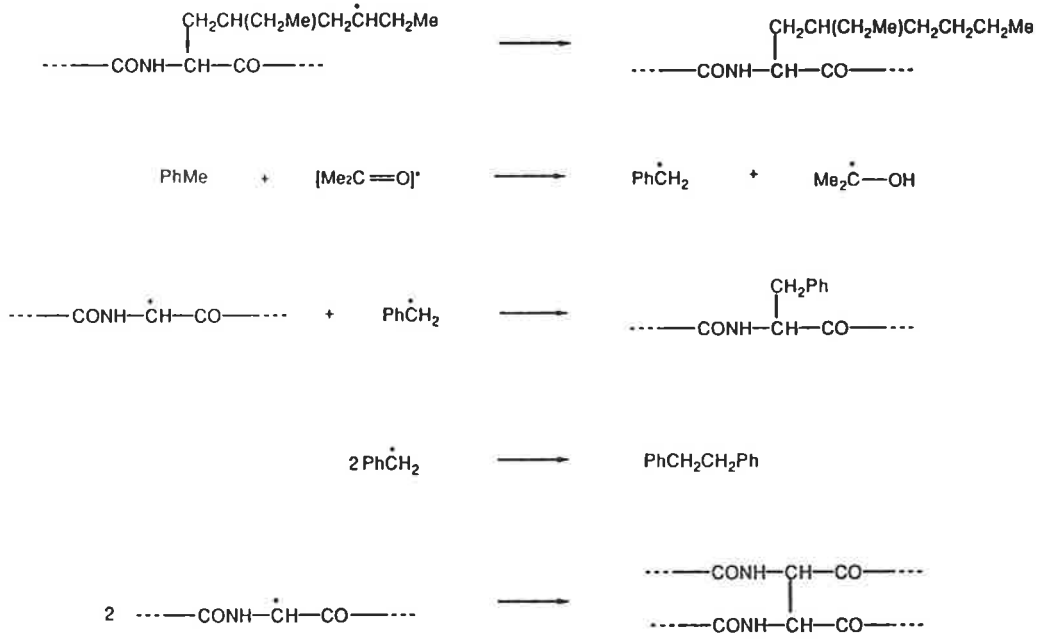
Obata and Niimura[72] used only di-*tert*-butyl peroxide with ultraviolet light as the photoinitiator. They reported the oxidative dimerization of methyl *N*-acetylglycinate **12a** and methyl pyroglutamate **12b** to give the corresponding dehydrodimers **14a** and **14b**, in yields of 51% and 64%, respectively. In a separate study,[21] analogous reactions of *N*-benzoylglycine methyl ester **12c** and *N*-benzoylalanine methyl ester **12d** afforded the corresponding dimers **14c**, in 37% yield, and **14d**, in 20% yield. In these reactions **12c** also afforded **15c** in 34% yield, and **12d** gave **15d** in 10% yield. The production of **14a–d**, **15c**, and **15d** may be rationalized as shown in Scheme 2. Presumably methylation of the amino acid derivatives **12c** and **12d** results from coupling of the corresponding electrophilic α-carbon-centered radicals **13c** and **13d** with nucleophilic methyl radical, produced by β-scission of *tert*-butoxy radical.

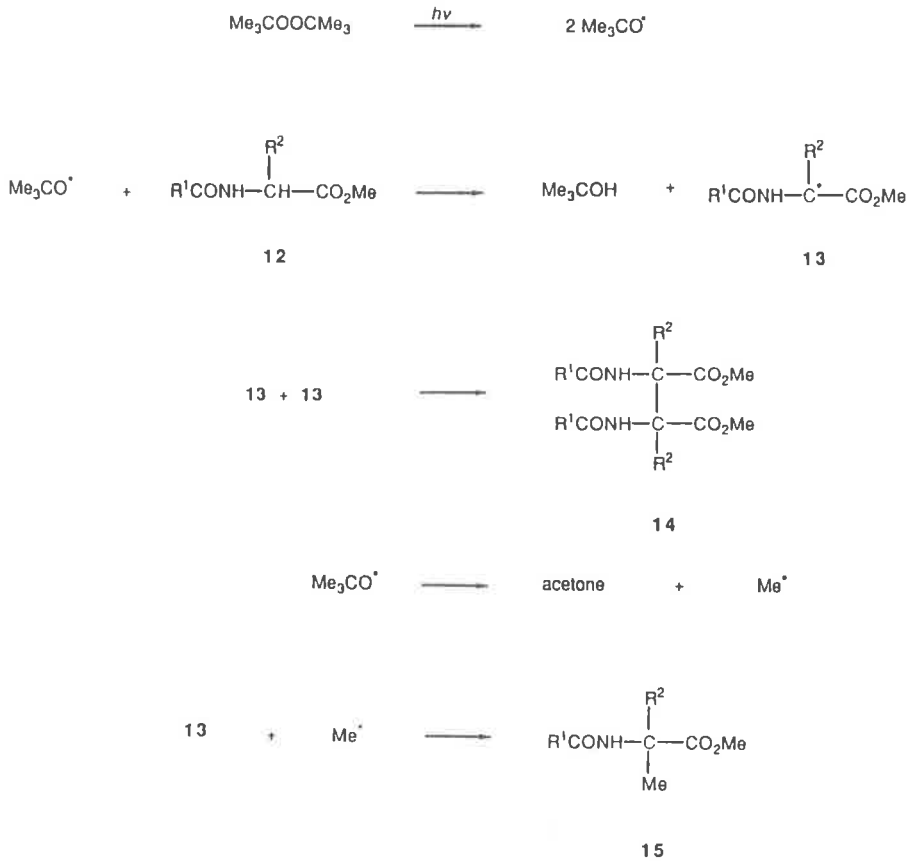
Over the past decade there have been a number of reports on the reaction of glycine derivatives with *N*-bromosuccinimide (NBS) under free-radical reac-

Scheme 1



Scheme 1 (continued)



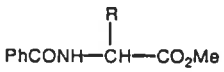


a) R^1 = Me, R^2 = H
b) R^1,R^2 = CH_2CH_2
c) R^1 = Ph, R^2 = H
d) R^1 = Ph, R^2 = Me

Scheme 2

Scheme 3

tion conditions.[73–80] For example, treatment of the glycine derivative **12c** with NBS in carbon tetrachloride, with irradiation to initiate the reaction, gave the α-bromoglycine derivative **16a**.[75] Since the reaction works equally well with

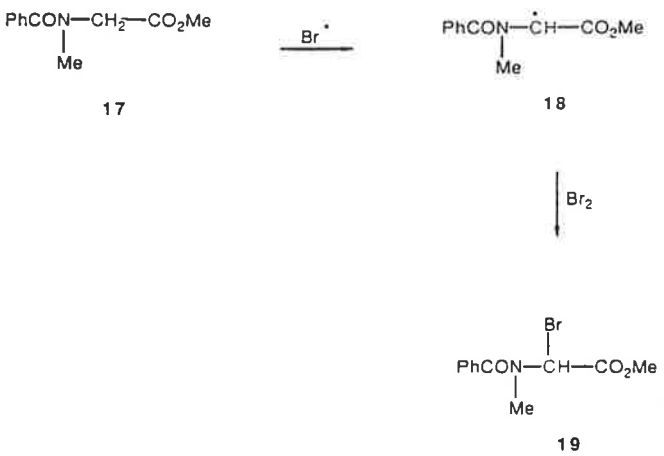


16

a) R = Br
b) R = Cl
c) R = OCOPh

bromine in place of NBS,[74,75] the mechanism most probably involves a bromine atom chain (Scheme 3).

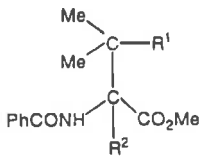
α-Carbon-centered radicals are likely intermediates in a variety of other free-radical substitution reactions of glycine derivatives. Treatment of the glycine derivative **12c** with sulfuryl chloride in carbon tetrachloride, with benzoyl peroxide to initiate the reaction, gave the α-chloroglycine derivative **16b**.[81] Reaction of **12c** with *tert*-butyl perbenzoate in benzene, in the presence of cupric octanoate, gave the benzoate **16c**.[82] Presumably, each of these reac-



Scheme 4

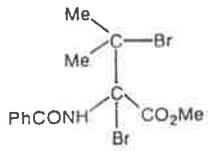
tions involves the glycyl radical **13c** as an intermediate, generated by hydrogen atom transfer from **12c**. Recently, the free-radical carboxylation of glycine derivatives has also been reported.[83]

In reactions of NBS with derivatives of amino acids other than glycine, α-carbon-centered radicals are also implicated as intermediates. The sarcosine derivative **17** gave the bromide **19** on treatment with NBS, presumably via the α-carbon-centered radical **18** (Scheme 4).[84] In other cases the mechanism of reaction has been more difficult to elucidate. The valine derivative **20a** reacted with excess NBS to give the dibromide **21**.[84,85] From the relative rates of reaction of **20a** and the deuteriated analogs **20b** and **20c**, it was established that there is a deuterium isotope effect of 3.7 for cleavage of the α-carbon–hydrogen bond, but no deuterium isotope effect for reaction at the β position. On this basis the



20

a) $R^1 = R^2 = H$
b) $R^1 = D, R^2 = H$
c) $R^1 = H, R^2 = D$

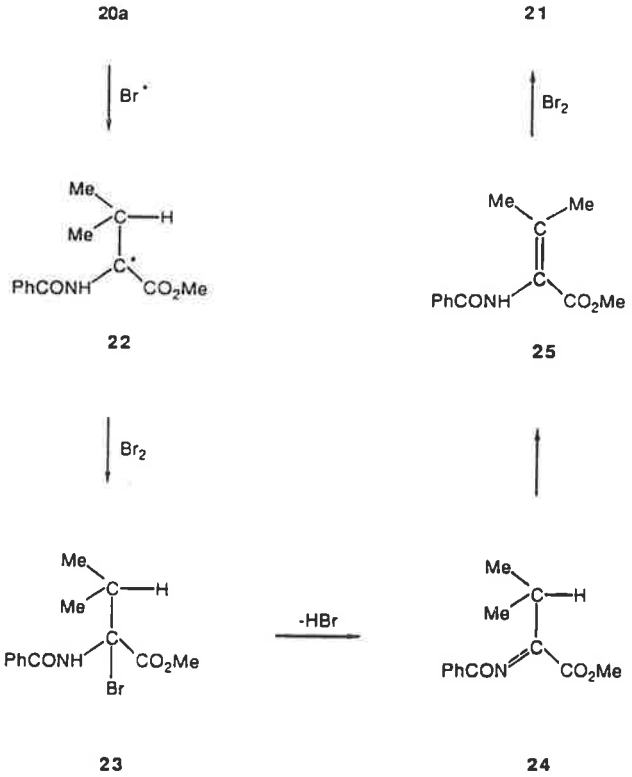


21

mechanism proposed for the reaction is as shown in Scheme 5. Hydrogen atom transfer from **20a** affords the radical **22**, which reacts by bromine atom incorporation to give the bromide **23**. Subsequent elimination of hydrogen bromide from **23** gives the *N*-acylimine **24**, which undergoes tautomerism to give **25**. Addition of bromine to **25** affords the dibromide **21**. The bromine is produced through reaction of hydrogen bromide with NBS. In support of the proposed mechanism, both **24** and **25** were detected in crude reaction mixtures.

Again in the reactions of methyl pyroglutamate **12b** and *N*-benzoylalanine methyl ester **12d** with NBS, there is evidence that reaction occurs via the corresponding α-carbon-centered radicals **13b** and **13d**. In each case a deuterium isotope effect has been measured for α-carbon–hydrogen bond cleavage.[86]

A common feature of the hydrogen-atom transfer reactions of amino acid derivatives is the selectivity for reaction of glycine residues.[30,37,65–71,83] Elad et al.[65–71] observed the selective reaction of glycine residues in the photoalkylation of peptides. This general trend is reflected by the reactions of **26** and **27** with toluene, on irradiation with ultraviolet light in the presence of acetone.[69] The selectivity for alkylation of the glycine residue was 7 : 1 for **26** and 20 : 1 for **27**. With but-1-ene as the alkylating agent, the selectivity was 10 : 1 for **26** and

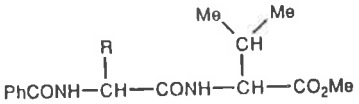


Scheme 5

7 : 1 for **27**.[69] The selective photoalkylation of glycine residues in lysozyme[70] and other polypeptides[68-71] has also been reported.

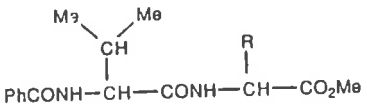
Reactions of dipeptide derivatives with NBS are also selective for glycine residues.[19,85] For example, treatment of **28a** and **29a** with NBS in dichloromethane gave the corresponding bromides **28b** and **29b**. The bromides **28b** and **29b** were characterized by conversion to the corresponding methoxyglycine derivatives **28c** and **29c**, which were isolated in yields of 65% and 73%, based on **28a** and **29a**, respectively.

The preferential reactivity of glycine residues toward hydrogen atom abstrac-



28

a) R = H
b) R = Br
c) R = OMe



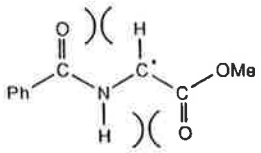
29

tion is contrary to the expectation that tertiary radicals should be formed in preference to secondary ones.[87] Glycine residues afford secondary radicals by α-carbon–hydrogen bond homolysis, whereas analogous reactions of derivatives of other amino acids, such as alanine and valine, produce tertiary radicals. This anomaly was investigated by studying reactions of mixtures of amino acid derivatives with NBS and di-*tert*-butyl peroxide.[85,86] With each reagent the rate of formation of the radical **13c** by hydrogen atom transfer from the glycine derivative **12c** was found to be faster than the rate of reaction of the alanine derivative **12d** to give **13d**, which was in turn faster than the rate of production of the radical **22** by hydrogen transfer from the valine derivative **20a**. To the extent that thermodynamic criteria control the pathways and rates of free-radical reactions, these results indicate that, in direct contrast to expectation, the secondary radical **13c** is marginally more stable than the tertiary radical **13d**, and both **13c** and **13d** are considerably more stable than **22**.

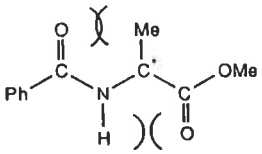
This peculiar stability of the radical **13c** can be attributed to a particularly favorable geometry. Stabilization of the radicals **13c**, **13d**, and **22** will result from overlap of the semioccupied p orbital with the π orbitals of the amido and methoxycarbonyl substituents. There will be maximum overlap of these orbitals in planar conformations of the radicals **13c**, **13d**, and **22**. The radical **13d** will be destabilized compared to **13c** by nonbonding interactions associated with planar conformations of **13d**, and **22** will be even less stable owing to more severe nonbonding interactions (Figure 3). These destabilizing influences outweigh the normal thermodynamic preference for the production of tertiary radicals.

The relative rates of production of the radicals **13d** and **18** in reactions with NBS and di-*tert*-butyl peroxide are very similar. This supports the hypothesis that the rate of hydrogen atom transfer from amino acid derivatives is affected by the extent of nonbonding interactions in the product radicals, since the degree of nonbonding interactions in planar conformations of **13d** and **18** is also very similar (Figure 3).

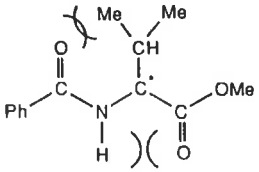
The rates of reaction of **12b** with NBS and di-*tert*-butyl peroxide are faster than the corresponding rates of reaction of the glycine derivative **12c**, consistent with the rationale outlined above. The radical **13b** can adopt planar conformations that are relatively free of nonbonding interactions (Figure 3). Formation of the radical **13b** is favored by the relief of ring strain and by the release of steric interactions between the methoxycarbonyl substituent and the β hydrogens in **12b**. It is possible that formation of the radical **13b** is also favored



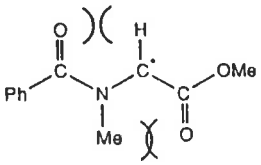
13c



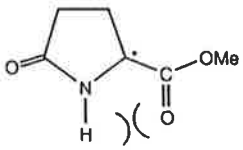
13d



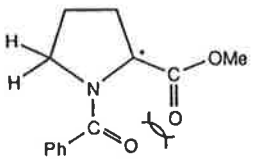
22



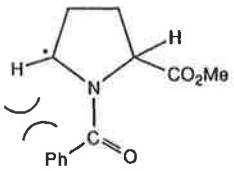
18



13b



30

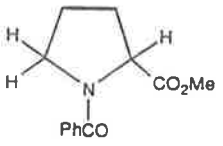


31

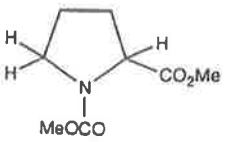
Figure 3. Nonbonding interactions in planar conformations of radicals derived from amino acid derivatives.

entropically by the inflexibility of the ring in **12b**, holding the amido group and the α carbon in the planar orientation as required for stabilization of the product radical **13b**. Thus the radical **13b** is more stable than the glycyl radical **13c** and this is reflected in the relative rates of reaction of **12b** and **12c** with bromine atom and *tert*-butoxy radical.

Based on this hypothesis, reaction of *N*-benzoylproline methyl ester **32** to give the α-carbon-centered radical **30** would be expected to be much slower than the rate of reaction of the glycine derivative **12c** to give **13c**, because the nonbonding interactions are much more severe in **30** than in **13c** (Figure 3). While the rate of reaction of **32** is faster than the rate of reaction of **12c**, the rate of formation of **30** is considerably slower than the rate of formation of **13c**. In



32

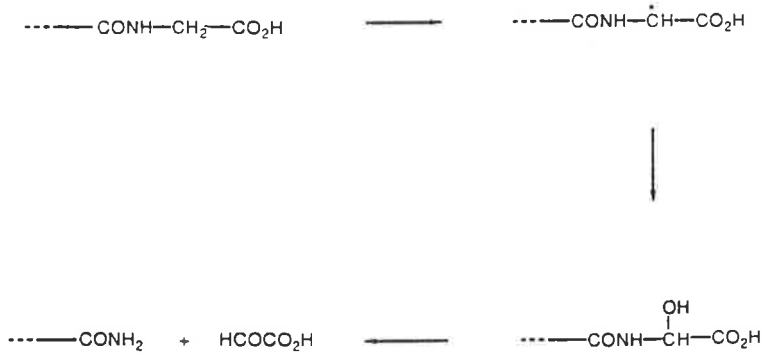


33

fact, steric interactions associated with planar conformations of the radical **30** are so severe that the predominant reaction of **32** is to produce the radical **31**, instead of **30**. Analogous regioselectivity has been observed in an electrochemical reaction of *N*-methoxycarbonylproline methyl ester **33**.[88] Presumably the fact that selective reactions of derivatives of pyroglutamic acid and proline have not been observed in biological systems is due to the relatively rare natural occurrence of these amino acids, compared to that of glycine.

In summary, the relative rates of reaction of **12b**, **12c**, **12d**, **17**, **20a**, and **32** with NBS and di-*tert*-butyl peroxide indicate that the selective reaction of glycine residues in these and other free-radical reactions of amino acid derivatives is due to the stability of the radicals produced by atom transfer reactions. Radicals formed by hydrogen abstraction from *N*-acylglycine derivatives may adopt planar conformations that are relatively free of nonbonding interactions and in which there is maximum delocalization of the unpaired electron. Radicals produced by similar reactions of derivatives of other amino acids such as alanine and valine are relatively unstable because of nonbonding interactions.

The bioactivation of many peptide hormones involves the oxidation of a C-terminal glycine-extended precursor to yield both an α-amidated peptide and glyoxylic acid (Scheme 6).[89,90] The reaction is catalyzed by the enzyme peptidylglycine α-amidating monooxygenase, which requires copper ions, L-ascorbate, and molecular oxygen. The reaction proceeds via an intermediate α-hydroxyglycine derivative,[91] presumably formed from the corresponding glycyl radical. It is interesting to speculate that the substrates of the



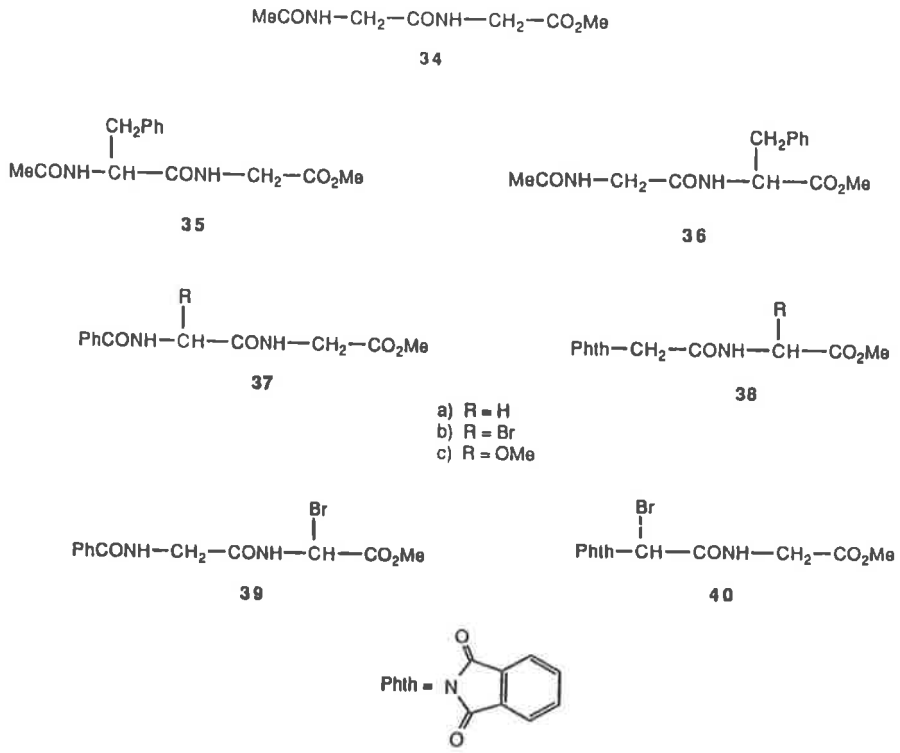
Scheme 6

monooxygenase enzyme are synthesized with glycine at the C terminus because the glycine residue is so easily removed by oxidation.

The regioselectivity of hydrogen transfer reactions of amino acid derivatives is affected by other factors apart from the selectivity for reaction of glycine residues. For example, changes to the amino and carboxyl groups affect the reactivity of an amino acid derivative and the stability of the corresponding α-carbon-centered radical. One example of this phenomenon, mentioned previously, was the selective reaction of C-terminal amino acid residues in dipeptides, as studied by ESR spectroscopy.[37,40–42,62] The ammonium group strongly deactivates the adjacent α position of N-terminal amino acid residues toward hydrogen atom abstraction.

With protected dipeptides reaction has been observed to occur at both the C-terminal and N-terminal residues. The photoalkylation of **34** with toluene, using acetone and ultraviolet light as the photoinitiator, gave **35** and **36** in yields of 27% and 25%, respectively.[67] A similar reaction of **9**, mentioned previously, gave **10b**, in 26% yield, and **11b**, in 35% yield.[67] The reaction of **37a** with NBS in dichloromethane, with irradiation to initiate the reaction, gave only the bromide **37b**, characterized by conversion to the corresponding methoxyglycine derivative **37c**, which was isolated in 62% yield based on **37a**.[23] In direct contrast, the phthaloyl derivative **38a** gave only **38b**, from which **38c** was produced and isolated in 74% yield.[23] Neither **39** nor **40**, nor their derivatives, were detected in the reactions of **37a** and **38a**, respectively.

There is no definitive explanation for the regioselective reaction of the N-terminal amino acid residue of **37a**. It is possible that the selectivity reflects a preferred conformation adopted by **37a** in dichloromethane. It has been reported previously[92,93] that protected dipeptides adopt preferred conformations owing to hydrogen bonding in nonpolar solvents. These conformational effects



are absent in polar solvents, hence the relative lack of regioselectivity in the reactions of **9** and **34**, carried out in the presence of acetone.

The regioselective bromination of the C-terminal amino acid residue in **38a** can be attributed to the effect of the phthaloyl substituent. The α position of an *N*-phthaloyl-substituted amino acid derivative is less reactive than that of an *N*-acylamino acid derivative toward reaction with bromine atom. This may be attributed to the relative stability and ease of formation of the corresponding

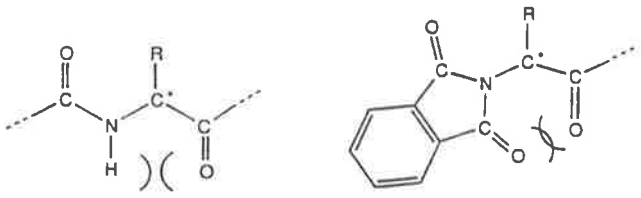
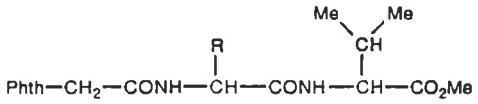


Figure 4. Nonbonding interactions associated with planar conformations of amido- and pthalimido-substituted radicals.

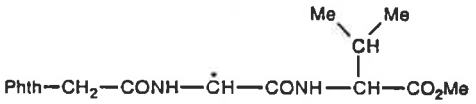
α-carbon-centered radicals. Whereas acylamino-substituted radicals are stabilized by resonance, there is less delocalization of unpaired spin density by a phthalimido substituent. This is the case particularly with α-carbon-centered radicals derived from amino acid derivatives. In planar conformations of the radicals, where there is maximum resonance stabilization, the nonbonding interactions are considerably greater with phthalimido-substituted radicals than with acylamino-substituted radicals (Figure 4). The phthaloyl substituent is also likely to hinder approach of bromine atom to the *N*-terminal amino acid residue in **38a**.

The combined effect of the phthaloyl substituent and the selectivity for reaction of glycine residues is illustrated by the selective formation of the



41

a) R = H
b) R = Br
c) R = OMe

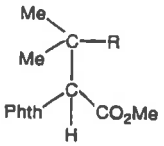


42

radical **42** in the reaction of the tripeptide derivative **41a** with NBS. The reaction afforded **41b**, characterized by conversion to the methoxyglycine derivative **41c**, which was isolated in 73% yield based on **41a**.[23]

The extent of the effect of an *N*-phthaloyl substituent in disfavoring reaction at the α position of an amino acid derivative is illustrated by the reactions of **43a** and **44a** with NBS. The brominated amino acid derivatives **43b** and **44b** were produced in yields of 87% and 83%, respectively, via the corresponding β-carbon-centered radicals **45** and **46**.[23] The regioselectivity observed in these reactions is contrary to that discussed above for reactions of *N*-acylamino acid derivatives with NBS. For example, the reaction of **20a** with bromine atom occurs at the α position to give the intermediate radical **22**.

Reactions of the valine derivative **20a** with sulfuryl chloride gave mixtures of the β-chlorovaline derivative **47** and the diastereomers of the γ-chlorovaline derivative **48**.[10,94] Presumably the peroxide-initiated chlorination of **20a** proceeds by initial hydrogen atom abstraction to give the radicals **49** and **50**, with subsequent chlorine incorporation at the sites of hydrogen abstraction. For



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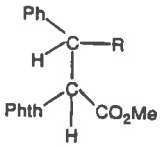
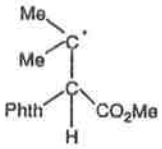


44

a) R = H
b) R = Br



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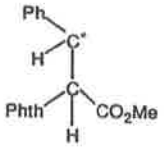

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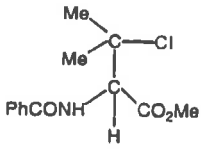

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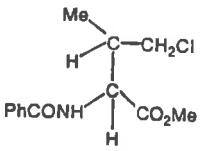

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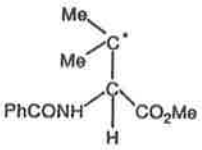

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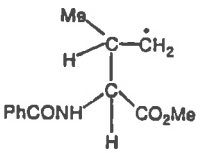


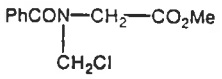
50

reaction in benzene the selectivity for β- to γ-carbon–hydrogen bond homolysis was 9 : 1.

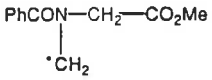
The contrast between the reaction of **20a** with NBS via the α-carbon-centered radical **22** and that with sulfuryl chloride via the β- and γ-centered radicals **49** and **50** may be interpreted in terms of the relative degrees of carbon–hydrogen bond homolysis in the reaction transition states. With little development of radical character in the transition state of the chlorination reaction, the regioselectivity in this case is controlled by the inductive electron-withdrawing effect of the amido and carboxy substituents acting to retard attack at the adjacent α position by electrophilic radicals involved in the hydrogen atom abstraction, thus favoring reaction at the β and γ positions. The reaction with NBS is more sensitive to radical stability effects since there is a greater degree of development of radical character in the transition state. Hydrogen atom transfer from the α position is favored, therefore, because the product radical

22 is stabilized by the combined effect of the resonance electron-donating amido and electron-withdrawing carboxy groups.

Reaction of *N*-benzoylsarcosine methyl ester **17** with sulfuryl chloride gave the chloride **51**.[84] Again this is in contrast to the reaction of **17** with NBS, in which the product was the bromide **19**. This contrast in regioselectivity in the reactions of **17** with NBS and sulfuryl chloride can also be attributed to the degrees of bond homolysis in the transition states of the respective reactions.



51



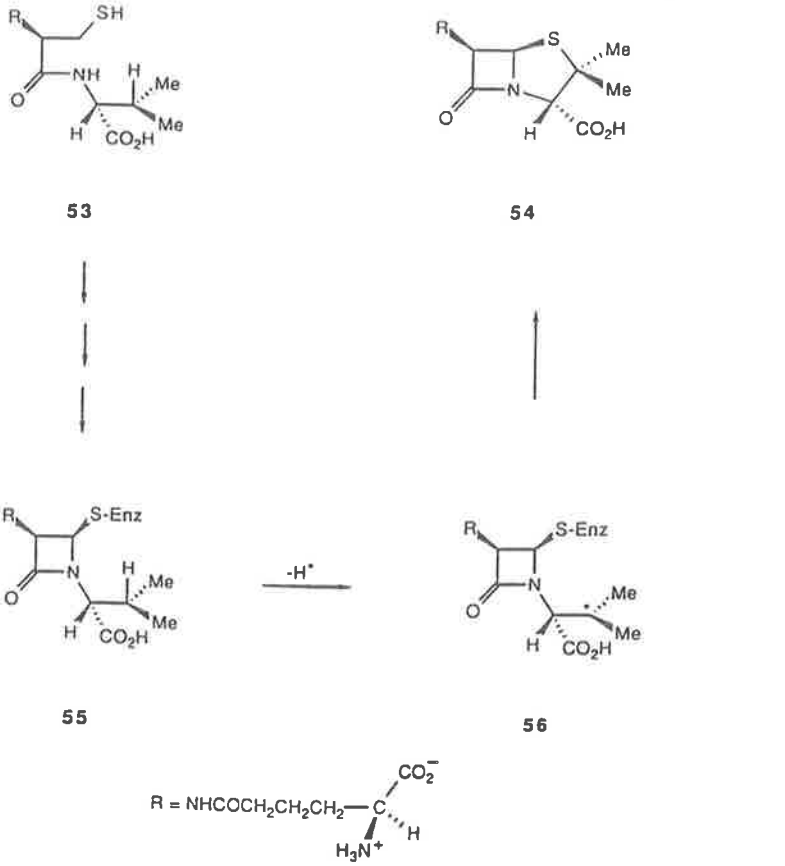
52

The development of appreciable radical character in the transition state of the reaction of **17** with NBS results in reaction via the amidocarboxy-substituted radical **18**, whereas the relative lack of development of radical character in the transition state of the reaction of **17** with sulfuryl chloride is manifest in regioselectivity determined by polar effects and resulting in reaction via the radical **52**.

The valine derivative **20a** reacts with di-*tert*-butyl peroxide to give the radicals **22** and **49**, whereas the sarcosine derivative **17** reacts via the radicals **18** and **52**.[84] With this reagent a balance between polar effects and the stability of the product radicals results in the competing reactions. These studies indicate that amidocarboxy-substituted radicals such as **22** and **18** are considerably more stable than, for example, **49** and **52**, but hydrogen atom transfer reactions may afford the less stable products if electrophilic radicals are involved in the hydrogen atom abstraction, and if there is little development of radical character in the transition state of the reaction.

Polar effects can outweigh the selectivity for reaction of glycine derivatives. Using a mixture of **20a** and **12c**, the reaction of the valine derivative **20a** with sulfuryl chloride, to give the radicals **49** and **50**, was found to be three times faster than reaction of **12c** to give the chloride **16b** via the radical **13c**.[95]

As a result of investigations[1–4] into the biosynthesis of isopenicillin N **54** from Arnstein's tripeptide **53**, a free-radical pathway has been proposed for the formation of the carbon–sulfur bond.[2] It has been suggested that this involves the formation of a radical such as **56**, by hydrogen transfer from the corresponding valine derivative **55**, with subsequent substitution at sulfur to give **54** (Scheme 7). Evidence in support of this proposal has been obtained from reactions of the enzyme isopenicillin N synthetase with modified substrates. For example, δ-(L-α-aminoadipoyl)-L-cysteinyl-D-allylglycine **57** reacted to give three products, **60**, **61**, and **62**.[4] These products are consistent with reaction via the intermediate allylic radical **59**, formed by hydrogen

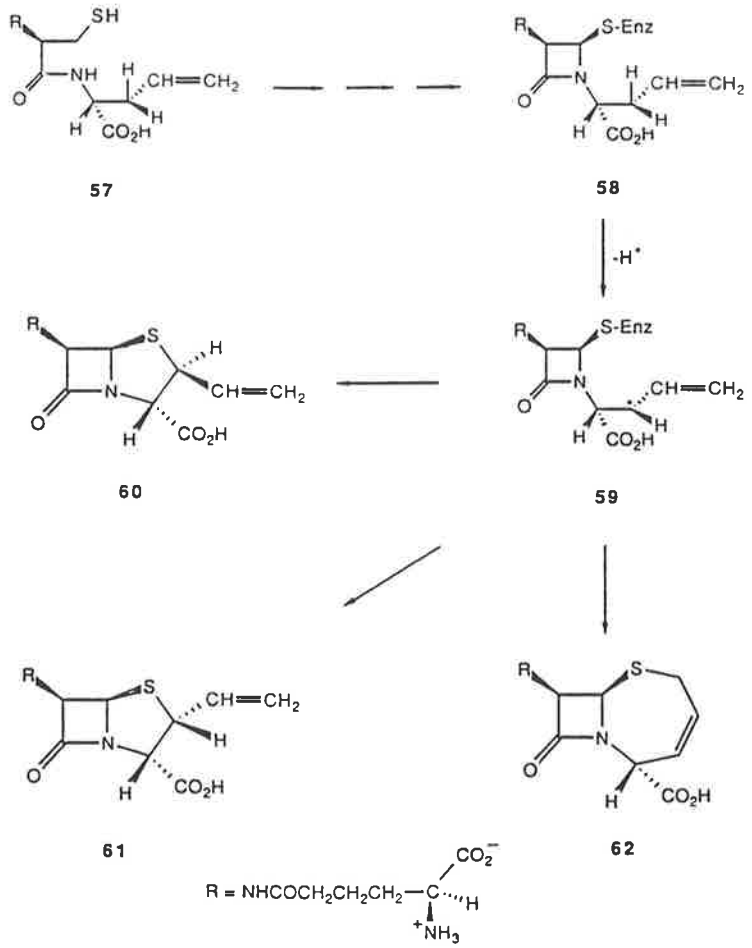


53 54 55 56

Scheme 7

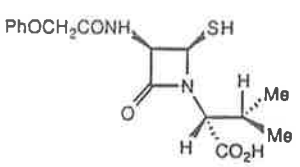
transfer from **58** (Scheme 8). In a model system, treatment of the mercaptoazetidinone **63** with Udenfried's reagent afforded the penicillin derivative **64**.[11] By analogy with the hydrogen transfer reactions of **20a** discussed earlier, the regioselectivity of the reaction of **55** to give **56**, in preference to the α-carbon-centered radical **65**, can be attributed to polar effects. Similar effects may be involved in the regioselective β-hydroxylation of valine and *N*-methylvaline residues in small peptides.[96–99]

In addition to polar and substituent effects, geometrical constraints on the transition state of the hydrogen transfer can affect the regioselectivity of reaction of amino acid derivatives. Photolysis of a solution of *N*-chloro-*N*-acetylnorvaline methyl ester **66a** in benzene afforded a mixture of the chlorides **67**, **68**, and **69**, in the ratio circa 1.4 : 1 : 1.[100] These products are consistent with

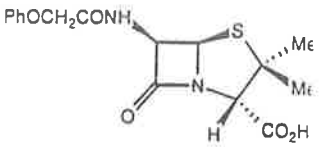


Scheme 8

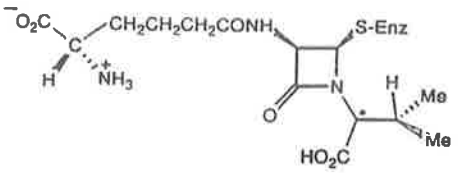
intermolecular hydrogen transfer from **66a** or **66b** to chlorine atom. When 2,4,6-trimethylpyridine was used in the reaction, to react with hydrogen chloride and prevent the intermolecular process,[101] only the chloride **69** was produced. Under these conditions the production of **69** can be attributed to intramolecular 1,5-hydrogen transfer to the amido radical **70** (Scheme 9). The primary radical **71** is formed in preference to the secondary radicals **72** and **73** and the α-carbon-centered radical **74**, owing to the relative ease of formation of the six-membered ring transition state.[101–105] In another example, photolysis of the butyramide **75** in the presence of 2,4,6-trimethylpyridine gave the



63



64



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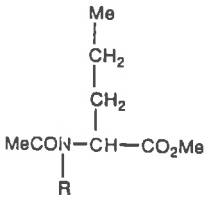
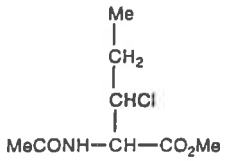


66

a) R = Cl
b) R = H



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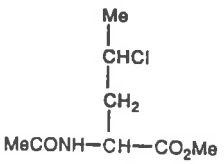
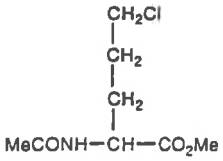


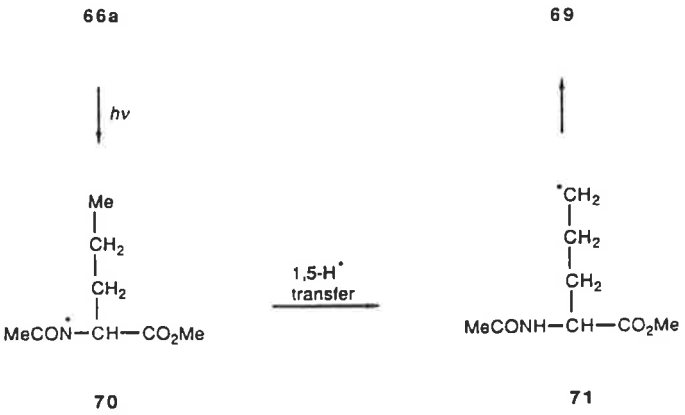
68



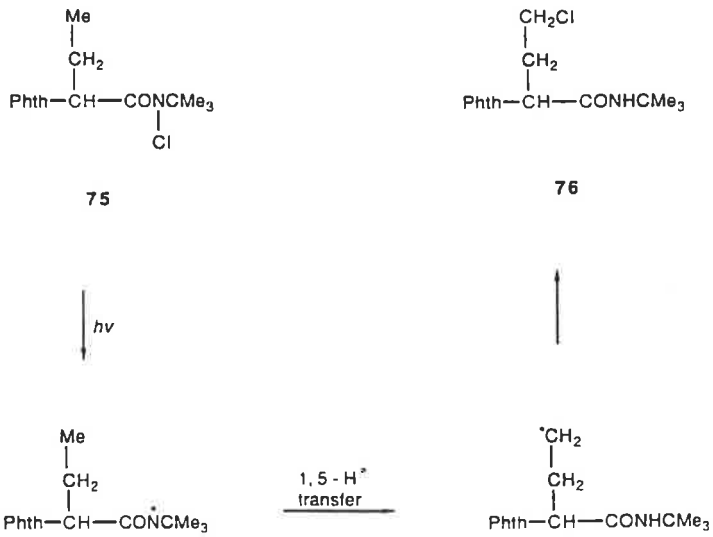
69

chloride **76**, consistent with regioselective intramolecular 1,5-hydrogen atom abstraction by the amido radical **77**, to give **78** (Scheme 10).[106]

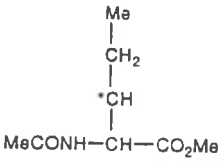
Hydrogen transfer reactions of 2,5-diketopiperazines have attracted particular attention, in synthesis and as models in studies of reactions of proteins. Sperling and Elad[107] reported the photosensitized alkylation of diketopiperazines in reactions with alkenes. Hausler et al.[108] have studied the photochemically induced oxidation of cyclic dipeptides with molecular oxygen. Thus, the anhydrides of prolylproline **79a**, glycylproline **80a** and sarcosylproline **81a**, gave the corresponding hydroperoxides **79b**, **80b**, and **81b**, in yields of 50%, 32%, and 35%, respectively. The products **79b**, **80b**, and **81b** are consistent with an electron transfer mechanism, with reaction proceeding via the corresponding α-carbon-centered radicals **82**, **83**, and **84**. An interesting feature of the reactions of **80a** and **81a** is the selectivity for reaction



Scheme 9



Scheme 10



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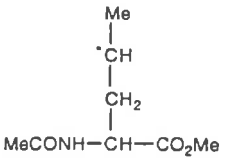

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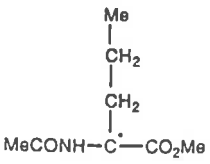

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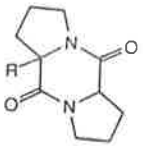

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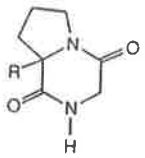

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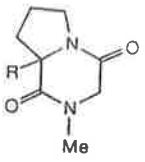
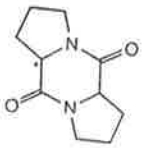


81

a) R = H
b) R = OOH



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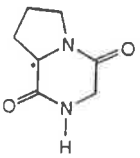

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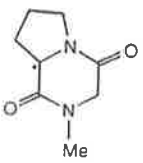

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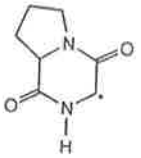

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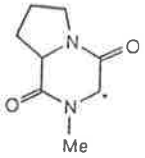

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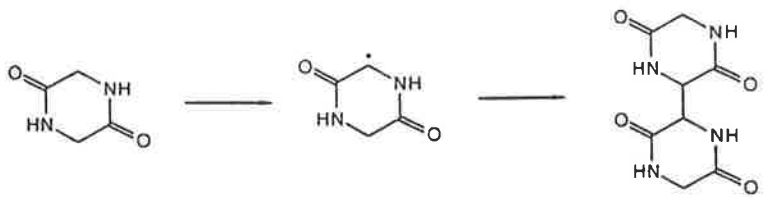


87 88 89

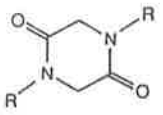
Scheme 11

at the α position of the proline residues. This is in contrast to the reactions of the proline derivative **32** with NBS and di-*tert*-butyl peroxide,[86] discussed previously, in which the radical **30** does not form. Whereas nonbonding interactions inhibit the formation of **30**, the radicals **83** and **84** can adopt planar conformations that are relatively free of such steric effects. Under these circumstances the tertiary amidocarboxy-substituted radicals **83** and **84** are formed in preference to the secondary radicals **85** and **86**.

In the absence of oxygen, irradiation of aqueous solutions of piperazine-2,5-dione **87** gave the dehydrodimer **89**, from coupling of the radical **88** (Scheme 11).[109] The dimer **89** was also formed in thermally and photochemically induced reactions of **87** with di-*tert*-butyl peroxide.[109]

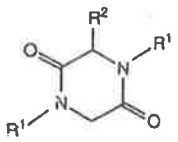
There have been a number of reports of synthetic studies of the reactions of 2,5-diketopiperazines with bromine and NBS, under free-radical reaction conditions.[110–116] In one study,[115,116] irradiation with a 250-W mercury lamp of a mixture of sarcosine anhydride **90a** and NBS (2 equivalents), in dichloromethane at reflux under nitrogen, gave the dibromide **92a**. The dibromide **92a** was characterized by conversion to the dimethoxydiketopiperazine **92d**, which was isolated in 65% yield, based on **90a**. The diketopiperazines **90b** and **90c** gave the corresponding dibromides **92b** and **92c**, characterized by conversion to the derivatives **92e** and **92f**, which were isolated in yields of 60% and 55%, respectively. Treatment of **90a** with 0.85 molar equivalents of NBS gave a mixture of the monobromide **91a**, the dibromide **92a**, and unreacted starting material **90a**, in the ratio circa 15 : 1 : 5. Similar treatment of **90b** and **90c** gave mainly the corresponding monobromides **91b** and **91c**. The monobromides **91a–c** were characterized by conversion to the corresponding monomethoxydiketopiperazines **91d–f**, which were isolated in yields of 54%, 61%, and 41%, respectively.

A reasonable mechanism for the production of **91a–c** and **92a–c** is as shown in Scheme 12. Analysis of reaction mixtures showed that **90a–c** are approximately three times more reactive than the corresponding monobromides **91a–c** toward hydrogen atom transfer. The selective formation of the

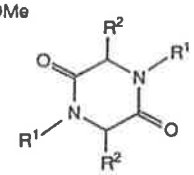


90

a) R = Me
b) R = CH_2Ph
c) R = CH_2Ph-*p*-OMe

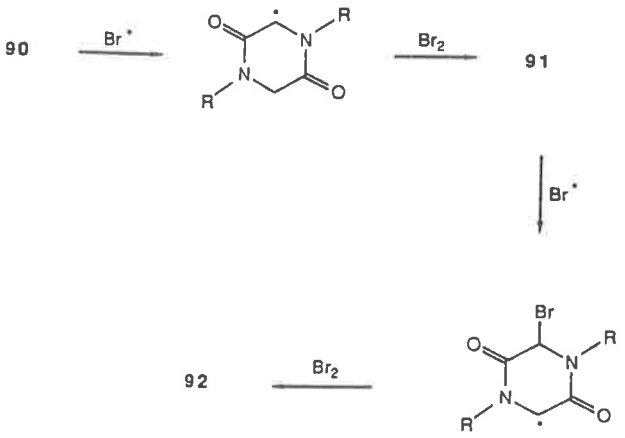


91



92

a) R^1 = Me, R^2 = Br
b) R^1 = CH_2Ph, R^2 = Br
c) R^1 = CH_2Ph-*p*-OMe, R^2 = Br
d) R^1 = Me, R^2 = OMe
e) R^1 = CH_2Ph, R^2 = OMe
f) R^1 = CH_2Ph-*p*-OMe, R^2 = OMe

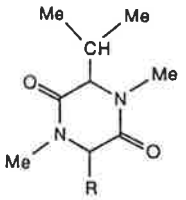


a) R = Me
b) R = CH_2Ph
c) R = CH_2Ph-*p*-OMe

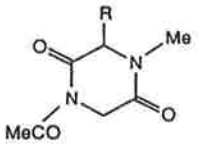
Scheme 12

monobromides **91a–c** is contrary to the report by Williams and Kwast[114] that bromination of diketopiperazines such as **90c** gives exclusively 3,6-dibrominated products.

Bromination of the asymmetric diketopiperazine **93a** gave **93b**.[116] The regioselectivity observed in this reaction is consistent with the selective reaction of glycine residues in peptides,[19,85,86] as discussed previously. Substituent



93



94

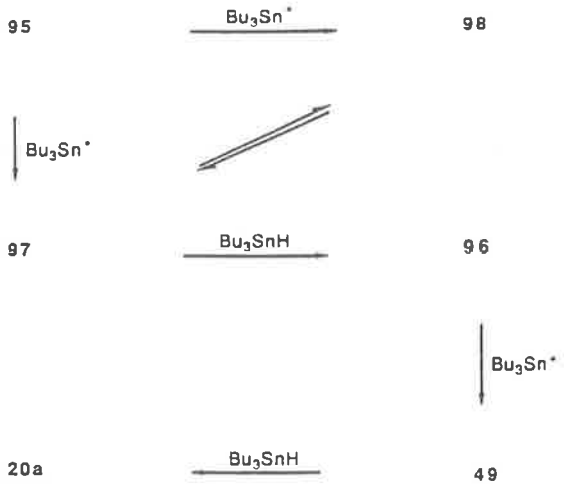
a) R = H
b) R = Br

effects have also been exploited in the regioselective bromination of diketopiperazines. The bromide **94b** was produced on treatment of **94a** with NBS.[116] By analogy with the effect of an *N*-phthaloyl substituent,[23] as discussed earlier, this may be attributed to the deactivating effect of the *N*-acetyl substituent in **94a**.

3. RADICALS FORMED IN REACTIONS WITH STANNANES

A number of studies of α-carbon-centered radicals have involved their generation by halogen transfer to stannyl radicals. Treatment of either of the α-haloglycine derivatives **16a** or **16b** with tributyltin hydride, in benzene or carbon tetrachloride, gave the reduced glycine derivative **12c** (Scheme 13).[81,85] Similarly, the α-bromosarcosine derivative **19** reacted to give **17**.[117] Presumably the selective halogen transfer from **16a**, **16b**, and **19** in the reactions carried out in carbon tetrachloride reflects the stability of the intermediate radicals **13c** and **18**. Less reactive substrates require solvents such as benzene that are inert to reaction with stannyl radicals. Evidence in support of the reaction mechanism was obtained by ESR spectroscopy. Irradiation with a 1000-W high-pressure mercury lamp of mixtures of di-*tert*-butyl peroxide, hexabutylditin, and the

Scheme 13

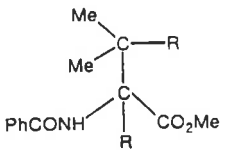


Scheme 14

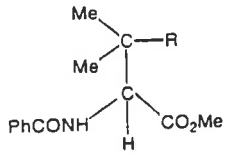
bromides **16a** and **19** in the cavity of an ESR spectrometer gave doublet signals with further complex hyperfine splitting consistent with formation of the corresponding radicals **13c** and **18**.[117] Under these conditions photolysis of the peroxide produces *tert*-butoxy radical, which reacts with hexabutylditin to give tributylstannyl radical. The stannyl radical reacts with **16a** and **19** by bromine atom abstraction to give the radicals **13c** and **18**, respectively.

The α,β-dihalogenated amino acid derivatives **95a** and **95b** reacted with tributyltin hydride to give the corresponding β-halides **96a** and **96b**.[84,85,118] The production of **96a** and **96b** may be rationalized as shown in Scheme 14. Halogen atom transfer from the vicinal dihalides **95a** and **95b** would be expected to give the more stable of the possible corresponding product radicals **97a** and **97b**, and **98a** and **98b**. In the unlikely event that halogen atom abstraction gave as the first formed the less stable of the possible product radicals **97a** and **97b**, and **98a** and **98b**, a facile 1,2-halogen migration to give the thermodynamically more stable radical would be expected.[119] On this basis the formation of the β-halides **96a** and **96b** indicates that the amidocarboxy-substituted radicals **97a** and **97b** are more stable than the corresponding β-carbon-centered radicals **98a** and **98b**, as expected. Furthermore, the formation of only trace amounts of **20a**, the product of subsequent reduction of **96a** and **96b**, in the reactions with tributyltin hydride indicates that the radicals **97a** and **97b** are more stable than **49**.

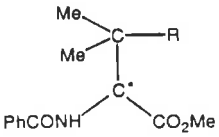
It is interesting to note that while the typical reaction of vicinal dihalides with tributyltin hydride is to give the corresponding alkenes (Scheme 15),[120] there was no evidence of formation of the dehydro amino acid derivative **25** in the



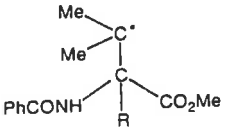
95



96



97



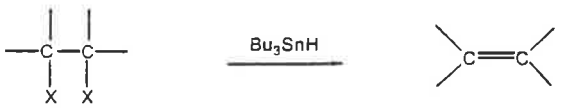
98

a) R = Br
b) R = Cl

reactions of **95a** and **95b**. Presumably the radicals **97a** and **97b** react by hydrogen atom abstraction to give **96a** and **96b**, respectively, rather than by β-scission to give **25**, owing to the destabilizing effect of steric interactions associated with **25** (Figure 5).

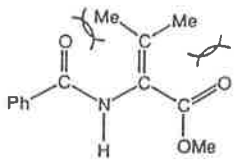
In variations of the reactions of halogenated amino acid derivatives with stannanes, tributyltin deuteride has been used to prepare regiospecifically deuteriated peptides[19,23,85] and other amino acid derivatives.[121] With hexabutylditin the bromoglycine derivative **16a** reacted under irradiation to give a 1 : 1 mixture of the diastereomers of the dimer **14c**, in 67% yield.[21] Formation of **14c** may be attributed to coupling of the glycyl radical **13c** (Scheme 16). Irradiation of a mixture of **16a**, hexabutylditin, and dibenzyldisulfide afforded the amidothioketal **99** as the major product, presumably as a result of **13c** reacting with dibenzyldisulfide by substitution at sulfur (Scheme 16).[82]

There have been two independent reports of the synthesis of the allylglycine derivative **100** by homolytic allylation of the bromide **16a**. Treatment of **16a** with allyltributylstannane gave **100** in 62% yield,[19] while the reaction of **16a**



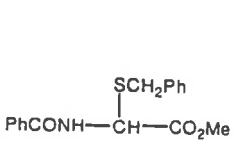
X = Cl, Br

Scheme 15

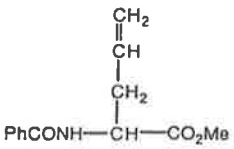


25

Figure 5. Steric interactions in *N*-benzoyl-α,β-dehydrovaline methyl ester.



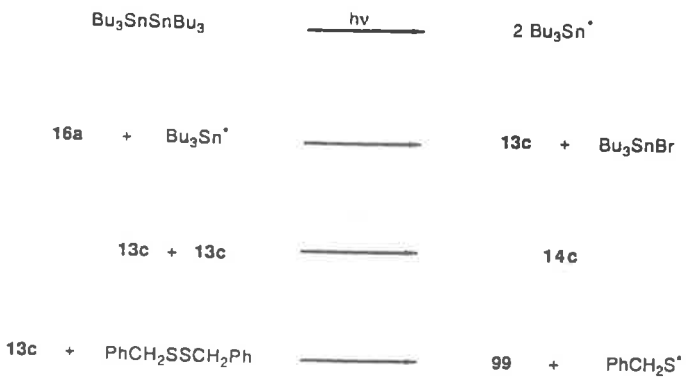
99



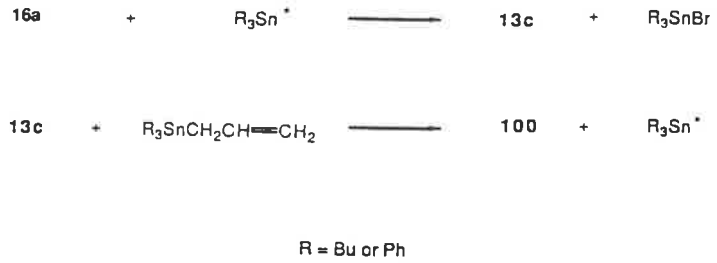
100

with allyltriphenylstannane gave **100** in 65% yield.[20] A probable mechanism for these reactions is as shown in Scheme 17.

Baldwin et al.[20] reported reactions of the 2-substituted allyltributylstannanes **101a–c** to give the corresponding 4-substituted allylglycine derivatives **102a–c**. Allyl transfer reactions of **16a** with 1-, 2-, and 3-alkyl-substituted allyltributylstannanes have also been observed.[24] Treatment of the bromide **16a** with the allylstannanes **103a–d** gave the corresponding glycine derivatives

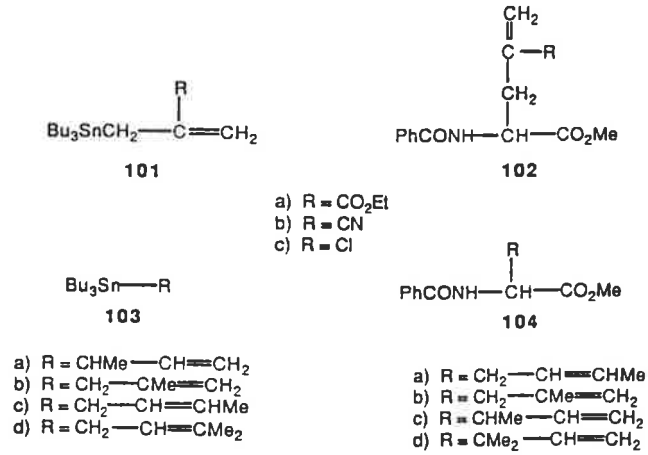


Scheme 16



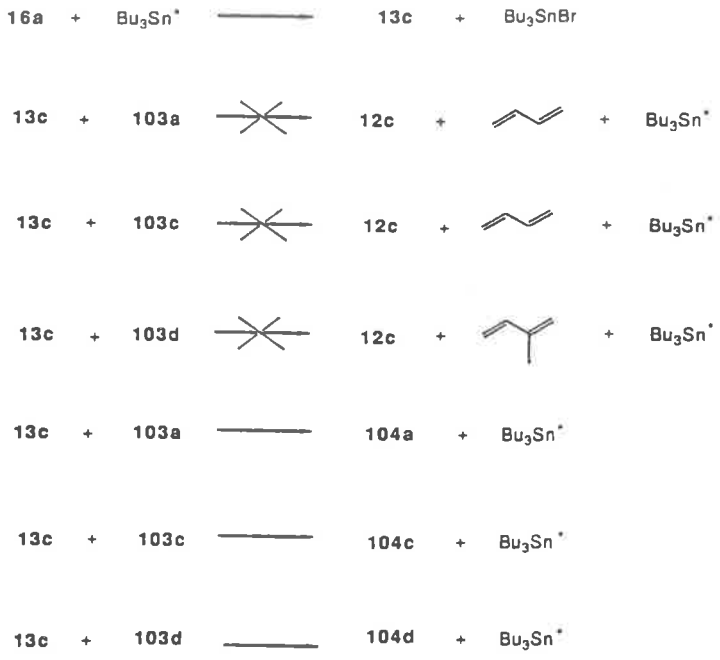
Scheme 17

104a–d. There was no evidence of formation of the reduced glycine derivative **12c** in any of these reactions. The reactions to give **104a**, **104c**, and **104d** are contrary to reports that hydrogen abstraction from 1- and 3-alkyl-substituted allylstannanes occurs in preference to allyl group transfer.[122,123] The radical **13c** reacts by addition to the stannanes **103a**, **103c**, and **103d**, rather than by hydrogen atom abstraction (Scheme 18). Prior to this work, only Pereyre and



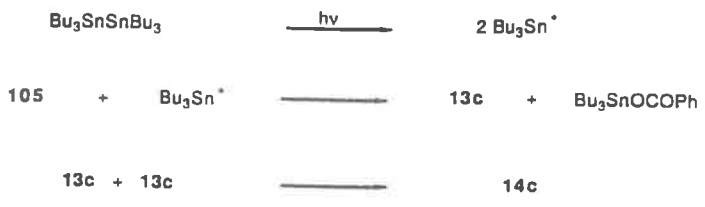
co-workers[124,125] had reported homolytic allyl transfer reactions of tributyl-(3-methylallyl)stannane **103c**. They noted that the allylation reaction is susceptible to polar effects, being faster with substrates which react via intermediate radicals substituted with electron-withdrawing groups. On this basis it seems likely that the allylation reaction is favored with electrophilic radicals such as **13c** and those used by Pereyre and co-workers, whereas nonpolar radicals react by hydrogen atom transfer.

The α-carbon-centered radical **13c** is also implicated as an intermediate in reactions of the benzoate **105** with stannanes. Treatment of **105** with tributyltin

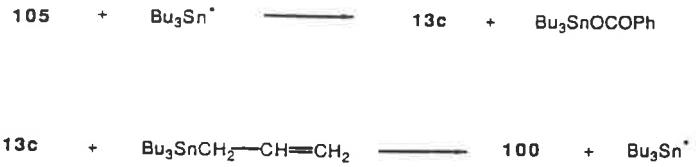


Scheme 18

Scheme 19



Scheme 20



Scheme 21

Scheme 22

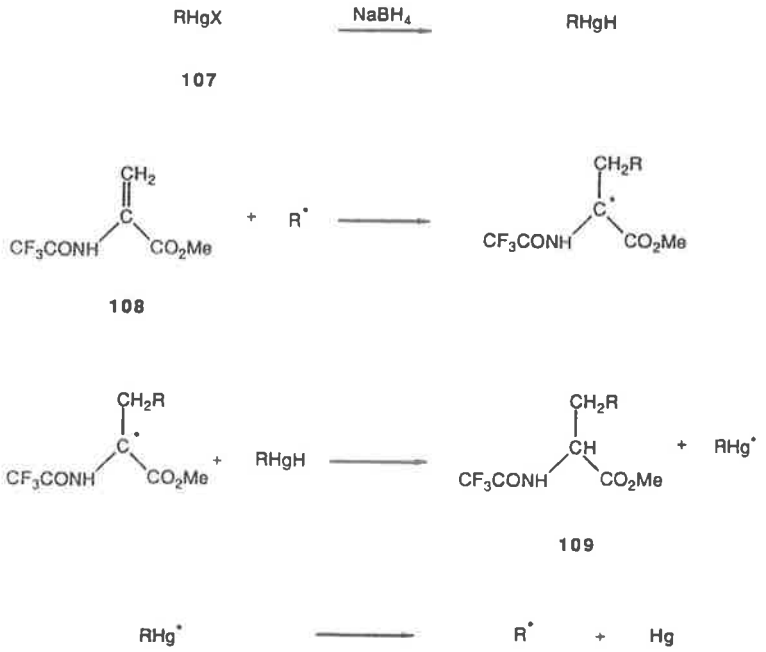
hydride gave the reduced glycine derivative **12c**, in 88% yield.[82] It seems likely that the mechanism of this reaction involves substitution of tributylstannyl radical on the benzoate **105** to give **13c** (Scheme 19). Although it is not a generally used method for the reduction of esters, there has been one other report of the reaction of benzoates with tributyltin hydride.[126] From that work it appears that the efficiency of the reaction depends on the stability of the

radical produced by substitution of the benzoate. Consequently the reaction of **105** via the stable α-carbon-centered radical **13c** is very facile. In related reactions the benzoate **105** reacted with hexabutylditin to give **14c** and with allyltributyltin to give **100**.[82] Formation of the products **14c** and **100** may be attributed to reactions of the radical **13c**, by coupling (Scheme 20) and by allyl group transfer (Scheme 21), respectively.

The peroxide **106** reacted with tributyltin hydride to give **12c**.[82] A reasonable mechanism for this reaction is as shown in Scheme 22. The unusual mode of substitution of the peroxide by carbon–oxygen bond cleavage may be attributed to the stability of the product radical **13c**.

4. RADICALS FORMED BY ADDITION TO DEHYDRO AMINO ACID DERIVATIVES

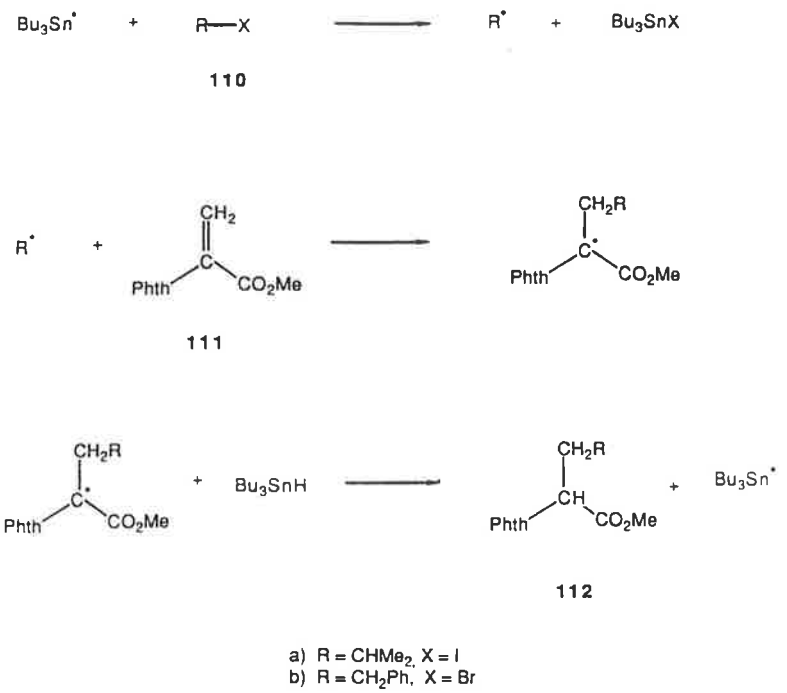
Despite the considerable success that has been achieved with free-radical addition reactions of alkenes,[127] little attention has been given to the addition



a) R = CH_2CHMe_2, X = Br
b) R = c-C_6H_{11}, X = Cl
c) R = CHEtMe, X = Br
d) R = CMe_3, X = Cl
e) R = c-C_3H_5, X = Cl

Scheme 23

reactions of dehydro amino acid derivatives. The reactions of *N*-trifluoroacetyldehydroalanine methyl ester **108** with the alkylmercury halides **107a–e** and sodium borohydride to give **109a–e**, respectively, have been reported.[25] The mechanism proposed for these reactions is as shown in Scheme 23. Recently this work has been extended to reactions of dehydroalanine residues in di- and tripeptides.[27] The vitamin-B_{12}-photoelectrocatalyzed addition of alkyl bromides and carboxylic acids to methyl 2-acetamidoacrylate has also been reported.[26] Crossley and Reid[128] observed the reaction of *N*-phthaloyldehydroalanine methyl ester **111** with radicals generated by the action of tributyltin hydride on alkyl bromides and iodides. For example, heating the halides **110a** and **110b** with mixtures of **111** and tributyltin hydride in benzene at reflux with azobisisobutyronitrile to initiate reaction gave the corresponding adducts **112a** and **112b**, in yields of 79% and 74%, respectively (Scheme 24). These reactions

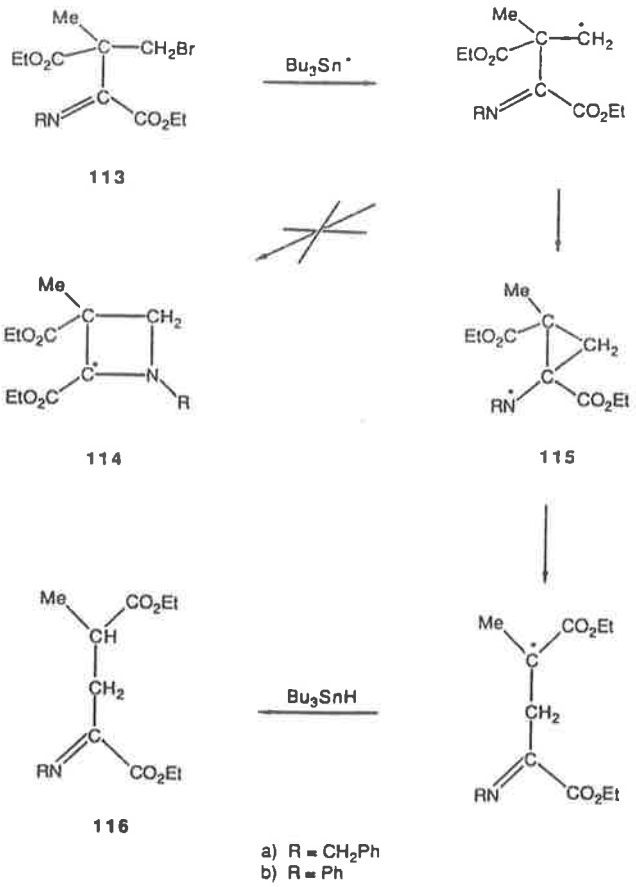


Scheme 24

show that primary, secondary, and tertiary alkyl radicals add to α,β-dehydro amino acid derivatives to give the corresponding α-carbon-centered radicals. The direction of addition of radicals to dehydro amino acid derivatives can be attributed to the stability of the product α-carbon-centered radicals and to steric effects, with addition occurring at the less hindered end of the double bond.

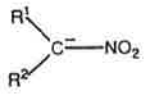
5. RADICALS FORMED IN REACTIONS OF IMINES AND *N*-ACYLIMINES

The intramolecular addition of radicals to imines has been observed in the reactions of **113a** and **113b** with tributyltin hydride to give the corresponding rearranged products **116a** and **116b** (Scheme 25).[129,130] Presumably addition occurs in the *exo* mode to give **115a** and **115b**, in preference to the *endo* mode to give the corresponding α-carbon-centered radicals **114a** and **114b**, due to geometrical constraints on the transition state of the reaction. The reactions of **113a** and **113b** were studied as models of the biochemical interconversion of L-glutamic acid and L-threo-β-methylaspartic acid.[131] In this regard it is interesting to note that **113b** reacted with vitamin B_{12} to give **116b**.[130]

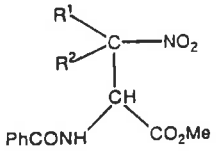


Scheme 25

Electron transfer reactions of *N*-acylimines are thought to be involved in the reactions of **16a** with the deprotonated nitroalkanes **117a–d** (2 equivalents).[22] High yields of the corresponding C-alkylated products **118a–d** were obtained in these reactions. In contrast, alkylation of alkyl nitronates generally takes place overwhelmingly on oxygen.[132–134] Other examples of C-alkylation of alkyl nitronates have been attributed to an electron transfer mechanism.[135,136] Accordingly, it is likely that **16a** reacts with the first mole equivalent of an alkyl nitronate such as **117a** to give the *N*-acylimine **119**. A subsequent electron transfer between **119** and the second mole equivalent of the alkyl nitronate **117a** gives a pair of free radicals that then combine (Scheme 26). It seems probable that the reactions of Grignard reagents with α-haloglycine derivatives[137,138] proceed via an analogous electron transfer mechanism.



117

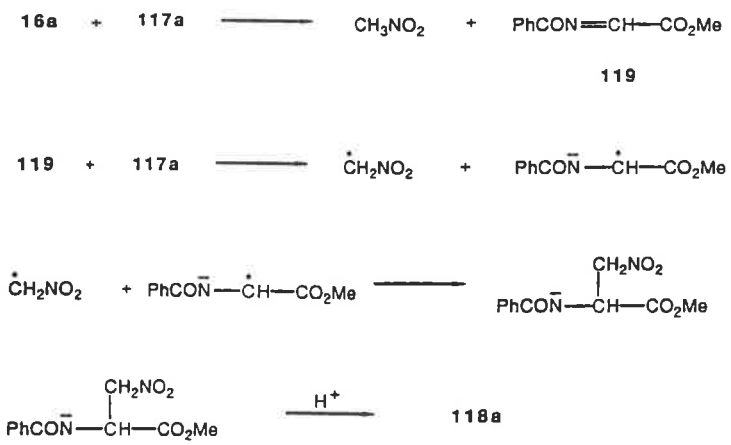


118

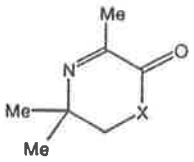
a) $R^1 = R^2 = H$
b) $R^1 = R^2 = Me$
c) $R^1 = Me, R^2 = H$
d) $R^1 = Ph, R^2 = H$

Photochemical reactions of the iminolactone **120a** and the iminolactam **120b** have also been studied.[139–142] Irradiation with a 450-W mercury lamp of **120a** and **120b** in 2-propanol gave the diastereomers of the dimers **122a** and **122b**, respectively, presumably via the corresponding α-carbon-centered radicals **121a** and **121b**. Each of the dimers **122a** and **122b** has an unusually weak carbon–carbon single bond and exists in solution at room temperature in equilibrium with the corresponding radical **121a** or **121b**. The radicals **121a** and **121b** have been identified by ESR spectroscopy and in spin trapping experiments.

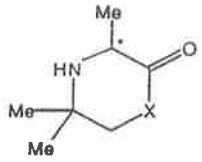
The enthalpy of dissociation of the dimers **122a** and **122b** is solvent dependent.[139,142] For **122a** the enthalpy of dissociation is 22 kcal/mol in chloroform and 11 kcal/mol in ethanol.[139] The solvent effect has been rationalized in terms of the greater solvation in polar solvents of the polar radicals **121a** and **121b**



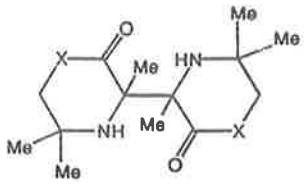
Scheme 26



120



121



122

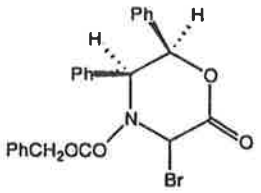
a) X = O
b) X = NH

compared to the corresponding dimers **122a** and **122b**. The dissociation of **122a** occurs more easily than that of **122b**. This has been attributed to the greater stability of the radical **121a** compared to **121b**, because the carboxyamido substituent in **121b** is less effective than the carboxyl group in **121a** as a resonance electron acceptor.[141]

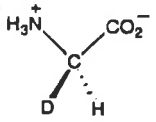
The radicals **121a** and **121b** have been utilized in a number of studies as one-electron reducing agents.[141,143–149] In these processes the radicals **121a** and **121b** are oxidized to the iminolactone **120a** and the iminolactam **120b**, respectively. The reactions are thought to involve electron transfer from the radicals **121a** and **121b**, followed by proton transfer.

6. REACTIONS WITH ASYMMETRIC INDUCTION

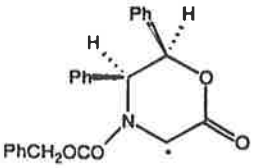
There have been a number of reports of asymmetric induction in reactions of α-carbon-centered radicals derived from amino acid derivatives. Williams et al.[77] reported that the reduction of (–)-5(*S*),6(*R*)-**123** with tributyltin deuteride, followed by hydrogenolysis, gave (*R*)-**124** in 60% enantiomeric excess. Presumably the intermediate radical **125** adopts a conformation in which the phenyl substituents block one side to a lesser extent than the carbobenzoxy group blocks the opposite side. In this conformation tributyltin deuteride delivers the deuterium *cis* to the phenyl substituents. It should be noted that the asymmetric induction observed in the preparation of the bromide **123**[76] and other halogenated glycine derivatives[75,78,80,138] does not necessarily reflect the stereoselectivity of transfer of bromine atom to the corresponding intermediate radicals. It is more likely that the ratio of products reflects the relative stability



123



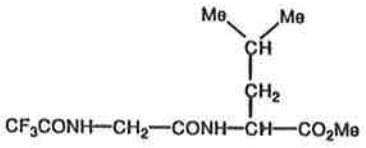
124



125

of the diastereomers because equilibration of α-halogenated glycine derivatives occurs readily.

The conversion of a glycine residue into a residue of an α-substituted amino acid generates a new chiral center. In the photoalkylation of peptides this occurs with a low degree of asymmetric induction.[66,67,69,71] For example, the



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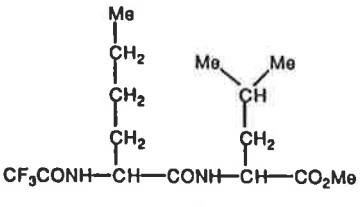
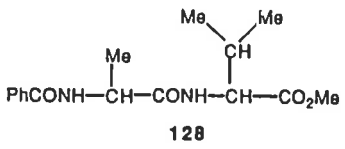
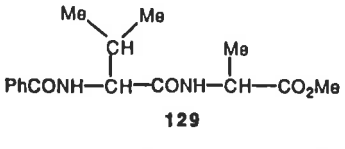


127



128



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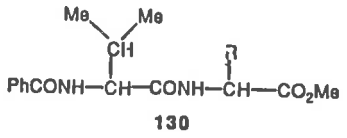
photoalkylation of (*S*)-*N*-trifluoroacetylglycylleucine methyl ester **126** with but-1-ene gave the (*S*),(*S*) and (*S*),(*R*) diastereomers of **127** in the ratio circa 6 : 4.[69] A similar degree of asymmetric induction was observed in the reactions of **28a** and **29a** with di-*tert*-butyl peroxide to give the diastereomers of **128** and **129**, respectively.[95]

A more significant degree of asymmetric induction has been observed in reactions of the brominated dipeptide **29b**, prepared from (*R*,*S*)-**29a**. Treatment of **29b** with allyltributyltin gave the (*R*,*S*),(*S*,*R*) and (*R*,*R*),(*S*,*S*) diastereomers of **130a** in the ratio circa 3 : 1.[19] The mixture of diastereomers of **130a** was characterized by hydrogenation to give **130b**, which compared with an authen-

tic sample. Reaction of **29a** with the anion of 2-nitropropane gave the (*R*,*S*),(*S*,*R*) and (*R*,*R*),(*S*,*S*) diastereomers of **130c** in the ratio circa 3 : 1, characterized by reduction with tributyltin hydride to give **130d**.[95] Although there is no definitive explanation for the asymmetric induction observed in



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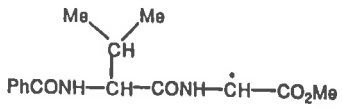
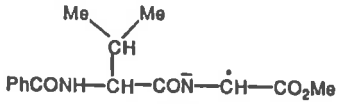
a) R = $CH_2CH{=}CH_2$
b) R = CH_2CH_2Me
c) R = $C(NO_2)Me_2$
d) R = $CHMe_2$



131



132

these reactions, it is possible that the stereoselectivity reflects preferred conformations of the radical **131** and the radical anion **132**, intermediates in the reactions of **29a** with allyltributyltin and deprotonated 2-nitropropane, respectively. Obviously the induction observed in these reactions warrants further investigation.

7. CONCLUSION

The foregoing survey illustrates factors that affect the formation and reaction of α-carbon-centered radicals derived from amino acids and their derivatives. The reactions include atom transfer, electron transfer, radical coupling, and radical addition processes. The reactions are affected markedly by the particular stability of the α-carbon-centered radicals, but substituent effects, polar effects, and geometrical constraints on reaction transition states also affect the reactions of these species. Each of these factors must be considered in order to understand the reactions of α-carbon-centered radicals. Although these radicals require further investigation in order that we can understand their reactions more completely, the results already obtained indicate the types of reactions that can be expected in biochemical systems, and illustrate the potential of this work for exploitation in the synthesis of amino acids and their derivatives.

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REFERENCES

1. Baldwin, J. E.; Abraham, E. P. *Nat. Prod. Rep.* **1988**, *5*, 129–145.
2. Baldwin, J. E.; Wan, T. S. *J. Chem. Soc., Chem. Commun.* **1979**, 249–250.
3. Baldwin, J. E.; Adlington, R. M.; Domayne-Hayman, B. P.; Knight, G.; Ting, H.-H. *J. Chem. Soc., Chem. Commun.* **1987**, 1661–1663.
4. Baldwin, J. E.; Adlington, R. M.; Derome, A. E.; Ting, H.-H.; Turner, N. J. *J. Chem. Soc., Chem. Commun.* **1984**, 1211–1214.
5. Baldwin, J. E.; Adlington, R. M.; Kang, T. W.; Lee, E.; Schofield, C. J. *J. Chem. Soc., Chem. Commun.* **1987**, 104–106.
6. Smith, K. C. *Photochem. Photobiol.* **1964**, *3*, 415–427.
7. Toth, B.; Dose, K. *Rad. Environ. Biophys.* **1976**, *13*, 105–113.
8. Dietz, T. M.; von Trebra, R. J.; Swanson, B. J.; Koch. T. D. *J. Am. Chem. Soc.* **1987**, *109*, 1793–1797.
9. Collins, M. A.; Grant, R. A. *Photochem. Photobiol.* **1969**, *9*, 369–375.
10. Easton, C. J.; Bowman, N. J. *J. Chem. Soc., Chem. Commun.* **1983**, 1193–1194.
11. Baldwin, J. E.; Adlington, R. M.; Bohlmann, R. *J. Chem. Soc., Chem. Commun.* **1985**, 357–359.
12. Barton, D. H. R.; Hervé, Y.; Potier, P.; Thierry, J. *Tetrahedron* **1987**, *43*, 4297–4308.
13. Barton, D. H. R.; Hervé, Y.; Potier, P.; Thierry, J. *Tetrahedron* **1988**, *44*, 5479–5486.
14. Barton, D. H. R.; Crich, D.; Hervé, Y.; Potier, P.; Thierry, J. *Tetrahedron* **1985**, *41*, 4347–4357.
15. Barton, D. H. R.; Bridon, D.; Hervé, Y.; Potier, P.; Thierry, J.; Zard, S. Z. *Tetrahedron* **1986**, *42*, 4983–4990.
16. Barton, D. H. R.; Guilhem, J.; Hervé, Y.; Potier, P.; Thierry, J. *Tetrahedron Lett.* **1987**, *28*, 1413–1416.
17. Baldwin, J. E.; Adlington, R. M.; Basak, A. *J. Chem. Soc., Chem. Commun.* **1984**, 1284–1285.
18. Baldwin, J. E.; Li, C.-S. *J. Chem. Soc., Chem. Commun.* **1987**, 166–168.
19. Easton, C. J.; Scharfbillig, I. M.; Tan, E. W. *Tetrahedron Lett.* **1988**, *29*, 1565–1568.
20. Baldwin, J. E.; Adlington, R. M.; Lowe, C.; O'Neil, I. A.; Sanders, G. L.; Schofield, C. J.; Sweeney, J. B. *J. Chem. Soc., Chem. Commun.* **1988**, 1030–1031.
21. Burgess, V. A.; Easton, C. J.; Hay, M. P.; Steel, P. J. *Aust. J. Chem.* **1988**, *41*, 701–710.
22. Burgess, V. A.; Easton, C. J. *Aust. J. Chem.* **1988**, *41*, 1063–1070.
23. Easton, C. J.; Tan, E. W.; Hay, M. P. *J. Chem. Soc., Chem. Commun.* **1989**, 385–386.
24. Easton, C. J.; Scharfbillig, I. M. *J. Org. Chem.* **1990**, *55*, 384–386.
25. Crich, D.; Davies, J. W.; Negrón, G.; Quintero, L.; *J. Chem. Res. (S)* **1988**, 140–141.
26. Orlinski, R.; Stankiewicz, T. *Tetrahedron Lett.* **1988**, *29*, 1601–1602.
27. Crich, D.; Davies, J. W. *Tetrahedron* **1989**, *45*, 5641–5654.
28. Wagner, I.; Musso, H. *Angew. Chem., Int. Ed. Eng.* **1983**, *22*, 816–828.
29. Schaich, K. M. *CRC Crit. Rev. Food Sci. Nutr.* **1980**, *13*, 131–159.
30. Henriksen, T.; Melø, T. B.; Saxebøl, G. In *Free Radicals in Biology*; Pryor, W. A., Ed.; Academic Press: New York, 1976; Vol. 2, pp 213–256.
31. Santus, R.; Grossweiner, L. I. *Photochem. Photobiol.* **1972**, *15*, 101–105.
32. Grossweiner, L. I.; Usui, Y. *Photochem. Photobiol.* **1970**, *11*, 53–56.

33. Androes, G. M.; Gloria, H. R.; Reinisch, R. F. *Photochem. Photobiol.* **1972**, *15*, 375–393.
34. Cowgill, R. W. *Biochim. Biophys. Acta* **1967**, *140*, 37–44.
35. Miyagawa, I.; Kurita, Y.; Gordy, W. *J. Chem. Phys.* **1960**, *33*, 1599–1603.
36. Katayama, M.; Gordy, W. *J. Chem. Phys.* **1961**, *35*, 117–122.
37. Rosenthal, I.; Poupko, R.; Elad, D. *J. Phys. Chem.* **1973**, *77*, 1944–1948.
38. Paul, H.; Fischer, H. *Ber. Bunsenges. Phys. Chem.* **1969**, *73*, 972–980.
39. Neta, P.; Fessenden, R. W. *J. Phys. Chem.* **1971**, *75*, 738–748.
40. Kirino, Y.; Taniguchi, H. *J. Am. Chem. Soc.* **1976**, *98*, 5089–5096.
41. Taniguchi, H.; Hatano, H.; Hasegawa, H.; Maruyama, T. *J. Phys. Chem.* **1970**, *74*, 3063–3065.
42. Livingston, R.; Doherty, D. G.; Zeldes, H. *J. Am. Chem. Soc.* **1975**, *97*, 3198–3204.
43. Viehe, H. G.; Merényi, R.; Stella, L.; Janousek, Z. *Angew. Chem., Int. Ed. Eng.* **1979**, *18*, 917–932.
44. Viehe, H. G.; Janousek, Z.; Merényi, R.; Stella, L. *Acc. Chem. Res.* **1985**, *18*, 148–154.
45. Dewar, M. J. S. *J. Am. Chem. Soc.* **1952**, *74*, 3353–3354.
46. Balaban, A. T. Rev. Roum. Chim. **1971**, *16*, 725–737.
47. Negoita, N.; Baican, R.; Balaban, A. T. *Tetrahedron Lett.* **1973**, 1877–1878.
48. Balaban, A. T.; Istratoiu, R. *Tetrahedron Lett.* **1973**, 1879–1880.
49. Baldock, R. W.; Hudson, P.; Katritzky, A. R.; Soti, F. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1422–1427.
50. Katritzky, A. R.; Soti, F. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1427–1432.
51. Katritzky, A. R.; Zerner, M. C.; Karelson, M. M. *J. Am. Chem. Soc.* **1986**, *108*, 7213–7214.
52. Pasto, D. J.; Krasnansky, R.; Zercher, C. *J. Org. Chem.* **1987**, *52*, 3062–3072.
53. Pasto, D. J. *J. Am. Chem. Soc.* **1988**, *110*, 8164–8175.
54. Korth, H.-G.; Lommes, P.; Sustmann, R. *J. Am. Chem. Soc.* **1984**, *106*, 663–668.
55. Korth, H.-G.; Lommes, P.; Sicking, W.; Sustmann, R. *Chem. Ber.* **1985**, *118*, 4627–4631.
56. MacInnes, I.; Walton, J. C.; Nonhebel, D. C. *J. Chem. Soc., Chem. Commun.* **1985**, 712–713.
57. Louw, R.; Bunk, J. *Recl.: J. R. Neth. Chem. Soc.* **1983**, *102*, 119–120.
58. Barbe, W.; Beckhaus, H.-D.; Rückhart, C. *Chem. Ber.* **1983**, *116*, 3216–3234.
59. Birkhofer, H.; Hádrich, J.; Beckhaus, H.-D.; Rückart, C. *Angew. Chem., Int. Ed. Eng.* **1987**, *26*, 573–575.
60. Burgess, V. A.; Easton, C. J. *Tetrahedron Lett.* **1987**, *28*, 2747–2750.
61. Taniguchi, H.; Kirino, Y. *J. Am. Chem. Soc.* **1977**, *99*, 3625–3631.
62. Moriya, F.; Makino, K.; Suzuki, N.; Rokushika, S.; Hatano, H. *J. Am. Chem. Soc.* **1982**, *104*, 830–836.
63. Poupko, R.; Silver, B. L.; Lowenstein, A. *J. Chem. Soc., Chem. Commun.* **1968**, 453.
64. Smith, P.; Fox, W. M.; McGinty, D. J.; Stevens, R. D. *Can. J. Chem.* **1970**, *48*, 480–491.
65. Elad, D.; Sperling, J. *J. Chem. Soc., Chem. Commun.* **1968**, 655–656.
66. Elad, D.; Sperling, J. *J. Chem. Soc., Chem. Commun.* **1969**, 234.
67. Elad, D.; Sperling, J. *J. Chem. Soc. C* **1969**, 1579–1585.
68. Elad, D.; Schwarzberg, M.; Sperling, J. *J. Chem. Soc., Chem. Commun.* **1970**, 617–618.
69. Sperling, J.; Elad, D. *J. Am. Chem. Soc.* **1971**, *93*, 967–971.
70. Sperling, J.; Elad, D. *J. Am. Chem. Soc.* **1971**, *93*, 3839–3840.
71. Schwarzberg, M.; Sperling, J.; Elad, D. *J. Am. Chem. Soc.* **1973**, *95*, 6418–6426.
72. Obata, N.; Niimura, K. *J. Chem. Soc., Chem. Commun.* **1977**, 238–239.
73. Lidert, Z.; Gronowitz, S. *Synthesis* **1980**, 322–324.
74. Kober, R.; Steglich, W. *Liebigs Ann. Chem.* **1983**, 599–609.
75. Kober, R.; Papadopoulos, K.; Miltz, W.; Enders, D.; Steglich, W.; Reuter, H.; Puff, H. *Tetrahedron* **1985**, *41*, 1693–1701.
76. Sinclair, P. J.; Zhai, D.; Reibenspeis, J.; Williams, R. M. *J. Am. Chem. Soc.* **1986**, *108*, 1103–1104.

77. Williams, R. M.; Zhai, D.; Sinclair, P. J.; *J. Org. Chem.* **1986**, *51*, 5021–5022.
78. Zimmermann, J.; Seebach, D. *Helv. Chim. Acta* **1987**, *70*, 1104–1114.
79. Williams, R. M.; Sinclair, P. J.; Zhai, D.; Chen, D. *J. Am. Chem. Soc.* **1988**, *110*, 1547–1557.
80. Ermert, P.; Meyer, J.; Stucki, C.; Schneebeli, J.; Obrecht, J.-P. *Tetrahedron Lett.* **1988**, *29*, 1265–1268.
81. Bowman, N. J.; Easton, C. J., University of Canterbury, New Zealand, unpublished observations.
82. Easton, C. J.; Peters, S. C., University of Adelaide, Australia, unpublished observations.
83. Wheelan, P.; Kirsch, W. M.; Koch, T. D. *J. Org. Chem.* **1989**, *54*, 4360–4364.
84. Easton, C. J.; Hay, M. P.; Love, S. G. *J. Chem. Soc., Perkin Trans. 2* **1988**, 265–268.
85. Easton, C. J.; Hay, M. P. *J. Chem. Soc., Chem. Commun.* **1986**, 55–57.
86. Burgess, V. A.; Easton, C. J.; Hay, M. P. *J. Am. Chem. Soc.* **1989**, *111*, 1047–1052.
87. Russell, G. A. In *Free Radicals*; Kochi, J. K., Ed.; Wiley: New York, 1973; Vol. 1, pp 275–331.
88. Shono, T.; Matsumura, Y.; Tsubata, K.; Sugihara, Y.; Yamane, S.; Kanazawa, T.; Aoki, T. *J. Am. Chem. Soc.* **1982**, *104*, 6697–6703.
89. Bradbury, A. F.; Finnie, M. D. A.; Smyth, D. G. *Nature* **1982**, *298*, 686–688.
90. Eipper, B. A.; Mains, R. E.; Glembotski, C. C. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 5144–5148.
91. Young, S. D.; Tamburini, P. P. *J. Am. Chem. Soc.* **1989**, *111*, 1933–1934.
92. Davies, J. S.; Thomas, R. J.; Williams, M. K. *J. Chem. Soc., Chem. Commun.* **1975**, 76–77.
93. Davies, J. S.; Thomas, R. J. *J. Chem. Soc., Perkin Trans. 1* **1981**, 1639–1646.
94. Bowman, N. J.; Hay, M. P.; Love, S. G.; Easton, C. J. *J. Chem. Soc., Perkin Trans. 1* **1988**, 259–264.
95. Burgess, V. A., Ph.D. Dissertation, University of Adelaide, Adelaide, Australia, 1989.
96. Liesch, J. M.; Millington, D. S.; Pandey, R. C.; Rinehart, K. L. *J. Am. Chem. Soc.* **1976**, *98*, 8237–8249.
97. Ito, Y.; Ohashi, Y.; Kawabe, S.; Abe, H.; Okuda, T. *J. Antibiot.* **1972**, *25*, 360–361.
98. Konishi, M.; Ohkuma, H.; Sakai, F.; Tsuno, T.; Koshiyama, H.; Naito, T.; Kawaguchi, H. *J. Am. Chem. Soc.* **1981**, *103*, 1241–1243.
99. Arnold, E.; Clardy, J. *J. Am. Chem. Soc.* **1981**, *103*, 1243–1244.
100. Clark, B. M., M.Sc. Dissertation, University of Canterbury, Christchurch, New Zealand, 1987.
101. Johnson, R. A.; Greene, F. D. *J. Org. Chem.* **1975**, *40*, 2186–2192.
102. Barton, D. H. R.; Beckwith, A. L. J.; Goosen, A. *J. Chem. Soc.* **1965**, 181–190.
103. Beckwith, A. L. J.; Goodrich, J. E. *Aust. J. Chem.* **1965**, *18*, 747–757.
104. Neale, R. S.; Marcus, N. L.; Schepers, R. G. *J. Am. Chem. Soc.* **1966**, *88*, 3051–3058.
105. Chow, Y. L.; Joseph, T. C. *J. Chem. Soc., Chem. Commun.* **1969**, 490–491.
106. Clark, B. M.; Easton, C. J., unpublished observations.
107. Sperling, J.; Elad, D. *Isr. J. Chem.* **1967**, *5*, 40p.
108. Hausler, J.; Jahn, R.; Schmidt, U. *Chem. Ber.* **1978**, *111*, 361–368.
109. Coyle, J. D.; Hill, R. R.; Randall, D.; *Photochem. Photobiol.* **1984**, *40*, 153–159.
110. Trown, P. W. *Biochem. Biophys. Res. Commun.* **1968**, *33*, 402–407.
111. Yoshimura, J.; Nakamura, H.; Matsunari, K. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 605–609.
112. Nakatsuka, S.; Yamada, K.; Yoshida, K.; Asano, O.; Murakami, Y.; Goto, T. *Tetrahedron Lett.* **1983**, *24*, 5627–5630.
113. Shimazaki, N.; Shima, I.; Hemmi, K.; Tsurumi, Y.; Hashimoto, M. *Chem. Pharm. Bull.* **1987**, *35*, 3527–3530.
114. Williams, R. M.; Kwast, A. *J. Org. Chem.* **1988**, *53*, 5785–5787.
115. Badran, T. W.; Easton, C. J. *Aust. J. Chem.* **1990**, *43*, 1455–1459.
116. Badran, T. W.; Easton, C. J., University of Adelaide, Australia, unpublished observations.
117. Easton, C. J.; Scharfbillig, I. M., University of Adelaide, Australia, unpublished observations.

118. Easton, C. J.; Hay, M. P. *J. Chem. Soc., Chem. Commun.* **1985**, 425–427.
119. Beckwith, A. L. J.; Ingold, K. U. In *Rearrangements in Ground and Excited States*; de Mayo, P., Ed.; Academic Press: New York, **1980**; Vol. 1, pp 161–310.
120. Kuivila, H. G. *Acc. Chem. Res.* **1968**, *1*, 299–305.
121. Easton, C. J.; Findlay, N. G. *J. Lab. Comp. Radiopharm.* **1984**, *22*, 667–672.
122. Keck, G. E.; Yates, J. B. *J. Organomet. Chem.* **1983**, *248*, C21–C25.
123. Keck, G. E.; Enholm, E. J.; Yates, J. B.; Wiley, M. R. *Tetrahedron* **1985**, *41*, 4079–4094.
124. Grignon, J.; Pereyre, M. *J. Organomet. Chem.* **1973**, *61*, C33–C35.
125. Servens, C.; Pereyre, M. *J. Organomet. Chem.* **1971**, *26*, C4–C6.
126. Khoo, L. E.; Lee, H. H. *Tetrahedron Lett.* **1968**, 4351–4354.
127. Giese, B. In *Radicals In Organic Synthesis: Formation of Carbon–Carbon Bonds*; Pergamon Press: New York, 1986.
128. Crossley, M. J.; Reid, R. C., University of Sydney, Australia, personal communication.
129. Dowd, P.; Choi, S.-C.; Duah, F.; Kaufman, C. *Tetrahedron* **1988**, *44*, 2137–2148.
130. Choi, S.-C.; Dowd, P. *J. Am. Chem. Soc.* **1989**, *111*, 2313–2314.
131. Barker, H. A.; Weissbach, H.; Smyth, R. D. *Proc. Natl. Acad. Sci. U.S.A.* **1958**, *44*, 1093–1097.
132. Seebach, D.; Colvin, E. W.; Lehr, F.; Weller, T. *Chimia* **1979**, *33*, 1–18.
133. Seebach, D.; Lehr, F. *Angew. Chem., Int. Ed. Eng.* **1976**, *15*, 505–506.
134. Seebach, D.; Henning, R.; Lehr, F.; Gonnermann, J. *Tetrahedron Lett.* **1977**, *18*, 1161–1164.
135. Kerber, R. C.; Urry, G. W.; Kornblum, N. *J. Am. Chem. Soc.* **1964**, *86*, 3904–3905.
136. Kornblum, N.; Boyd, S. D.; Ono, N. *J. Am. Chem. Soc.* **1974**, *96*, 2580–2587.
137. Castelhano, A. L.; Horne, S.; Billedeau, R.; Krantz, A. *Tetrahedron Lett.* **1986**, *27*, 2435–2438.
138. Munster, P.; Steglich, W. *Synthesis* **1987**, 223–225.
139. Koch, T. H.; Olesen, J. A.; DeNiro, J. *J. Am. Chem. Soc.* **1975**, *97*, 7285–7288.
140. Koch, T. H.; Olesen, J. A.; DeNiro, J. *J. Org. Chem.* **1975**, *40*, 14–19.
141. Kleyer, D. L.; Haltiwanger, R. C.; Koch, T. H. *J. Org. Chem.* **1983**, *48*, 147–152.
142. Olson, J. B.; Koch, T. H. *J. Am. Chem. Soc.* **1986**, *108*, 756–761.
143. Bennett, R. W.; Wharry, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1980**, *102*, 2345–2349.
144. Burns, J. M.; Wharry, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1981**, *103*, 849–856.
145. Kleyer, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1983**, *105*, 5154–5155.
146. Kleyer, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1983**, *105*, 5911–5912.
147. Kleyer, D. L.; Gaudiano, G.; Koch, T. H. *J. Am. Chem. Soc.* **1984**, *106*, 1105–1109.
148. Kleyer, D. L.; Koch, T. H. *J. Am. Chem. Soc.* **1984**, *106*, 2380–2387.
149. Bird, D. M.; Boldt, M.; Koch, T. H. *J. Am. Chem. Soc.* **1987**, *109*, 4046–4053.

Zeitschrift für Kristallographie 198, 142–144 (1992)

Crystal structure of (Z)-1-methoxycarbonyl-2-phenyl-1-phthalimidocyclopropane, $C_{19}H_{15}NO_4$

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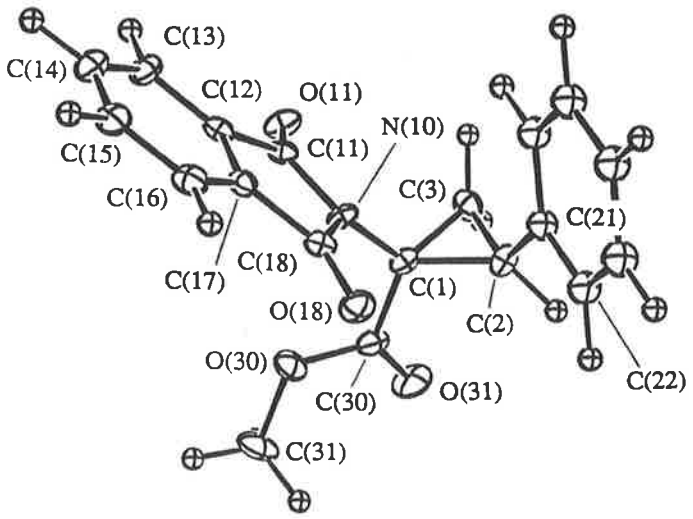

Source of material: see ref. 1.
The title compound is the major diastereomer formed by base-induced cyclization of the corresponding γ-bromohomophenylalanine derivative. The X-ray structure shows that this isomer has the Z-configuration.

Monoclinic, $P12_1/n1$ (no 14), $a = 10.122(2)$, $b = 11.799(2)$, $c = 14.569(2)$ Å, $\beta = 109.69(2)^\circ$, $V = 1638.2$ Å^3, $Z = 4$, $R = 0.061$.

Table 1. Parameters used for the X-ray data collection

Diffractometer type:	Enraf-Nonius CAD4	Number of unique reflections:	2879
Wave length:	Mo K_α radiation (0.7107 Å)	Criterion for unobserved reflections:	$I_O < 2.5\sigma(I_O)$
Crystal characteristics:	colourless, size $0.06 \times 0.16 \times 0.42$ mm	Number of refined parameters:	221
Temperature of measurement:	293 K	Scan mode:	$\omega/2\Theta$
$2\theta_{max}$:	50°	μ:	0.55 cm^{-1}
		Structure solution program used:	SHELX

Table 2. Final atomic coordinates and displacement parameters (in $Å^2$)

Atom	x	y	z	U_{iso}/U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O(11)	0.1456(6)	0.6226(5)	0.9968(4)	0.067(4)	0.054(4)	0.053(4)	−0.014(3)	−0.002(3)	0.013(3)
O(18)	0.0873(6)	0.9124(5)	0.7807(4)	0.073(4)	0.048(4)	0.072(4)	0.003(3)	0.022(3)	0.019(3)
O(30)	0.1835(7)	0.9346(5)	1.0190(4)	0.087(5)	0.066(4)	0.050(4)	0.005(4)	0.031(4)	−0.013(3)
O(31)	0.4182(7)	0.9321(5)	1.0754(5)	0.097(5)	0.101(5)	0.073(5)	−0.049(4)	0.017(4)	−0.040(4)
N(10)	0.1570(6)	0.7687(5)	0.8946(5)	0.049(4)	0.031(3)	0.041(4)	−0.010(3)	0.013(4)	0.008(3)
C(1)	0.2958(8)	0.8061(7)	0.9491(6)	0.044(5)	0.050(6)	0.054(6)	−0.010(5)	0.012(5)	−0.007(5)
C(2)	0.4056(9)	0.8088(8)	0.8973(7)	0.045(5)	0.063(6)	0.057(6)	−0.006(5)	0.009(5)	−0.005(6)
H(2)	0.462(8)	0.872(7)	0.923(6)	0.088(8)					
C(3)	0.4120(9)	0.7199(9)	0.9700(7)	0.046(6)	0.065(6)	0.062(7)	0.006(5)	0.005(5)	−0.007(6)
H(31)	0.380(8)	0.636(7)	0.944(6)	0.088(8)					
H(32)	0.482(9)	0.732(7)	1.031(7)	0.088(8)					
C(11)	0.0910(8)	0.6814(6)	0.9271(6)	0.050(5)	0.035(5)	0.038(5)	0.010(4)	0.006(4)	−0.001(5)
C(12)	−0.0576(8)	0.6782(6)	0.8604(5)	0.049(5)	0.035(4)	0.033(5)	0.011(4)	0.011(4)	−0.009(4)
C(13)	−0.169(1)	0.6098(8)	0.8600(6)	0.057(6)	0.060(6)	0.050(6)	−0.019(5)	0.012(5)	−0.010(5)
H(13)	−0.147(8)	0.552(7)	0.909(6)	0.088(8)					
C(14)	−0.298(1)	0.6329(9)	0.7897(9)	0.057(7)	0.065(7)	0.083(8)	−0.018(5)	0.019(6)	−0.009(7)
H(14)	−0.364(9)	0.589(7)	0.794(6)	0.088(8)					
C(15)	−0.313(1)	0.7182(9)	0.7230(8)	0.041(6)	0.073(7)	0.073(8)	−0.009(6)	−0.000(5)	−0.024(6)
H(15)	−0.404(9)	0.726(7)	0.688(6)	0.088(8)					
C(16)	−0.204(1)	0.7860(7)	0.7224(7)	0.059(6)	0.050(6)	0.056(6)	0.003(5)	0.013(5)	−0.002(5)
H(16)	−0.206(9)	0.843(7)	0.671(6)	0.088(8)					
C(17)	−0.0755(8)	0.7646(6)	0.7921(6)	0.037(5)	0.042(5)	0.046(5)	0.006(4)	0.009(4)	−0.012(5)
C(18)	0.0609(8)	0.8280(7)	0.8164(6)	0.054(5)	0.037(5)	0.042(5)	0.006(5)	0.016(4)	−0.003(5)
C(22)	0.3804(6)	0.8824(4)	0.7335(4)	0.060(2)					
C(23)	0.3409(6)	0.8704(4)	0.6325(4)	0.071(3)					
C(24)	0.2888(6)	0.7670(4)	0.5888(4)	0.071(3)					
C(25)	0.2762(6)	0.6756(4)	0.6461(4)	0.063(3)					
C(26)	0.3157(6)	0.6876(4)	0.7470(4)	0.057(2)					
C(21)	0.3678(6)	0.7910(4)	0.7907(4)	0.051(2)					
H(22)	0.437(7)	0.962(6)	0.783(5)	0.088(8)					
H(23)	0.330(7)	0.942(6)	0.599(5)	0.088(8)					
H(24)	0.259(7)	0.758(6)	0.525(6)	0.088(8)					
H(25)	0.234(7)	0.592(6)	0.611(5)	0.088(8)					
H(26)	0.313(7)	0.615(7)	0.794(5)	0.088(8)					
C(30)	0.310(1)	0.8965(7)	1.0219(6)	0.082(7)	0.046(6)	0.050(6)	−0.017(5)	0.027(6)	−0.007(5)
C(31)	0.187(2)	1.029(1)	1.084(1)	0.18(2)	0.059(8)	0.08(1)	0.02(1)	0.07(1)	−0.009(7)
H(311)	0.12(1)	1.04(1)	1.086(9)	0.088(8)					
H(312)	0.204(8)	1.105(7)	1.049(6)	0.088(8)					
H(313)	0.237(9)	1.001(8)	1.139(7)	0.088(8)					

Further details of the structure determination (e.g. structure factors) have been deposited within the relevant database and can be accessed as Collection No. 320242 or ordered from the Fachinformationszentrum Karlsruhe, D-7514 Eggenstein-Leopoldshafen.

References:

1. Ward, C. M.: Synthesis of cyclopropylamino acid derivatives. B.Sc.(Hons) Dissertation, 1990.

Zeitschrift für Kristallographie 198, 145–147 (1992)

Crystal structure of meso-bis(1,4-dimethyl-piperazine-2,5-dione-3-yl), $C_{12}H_{18}N_4O_4$

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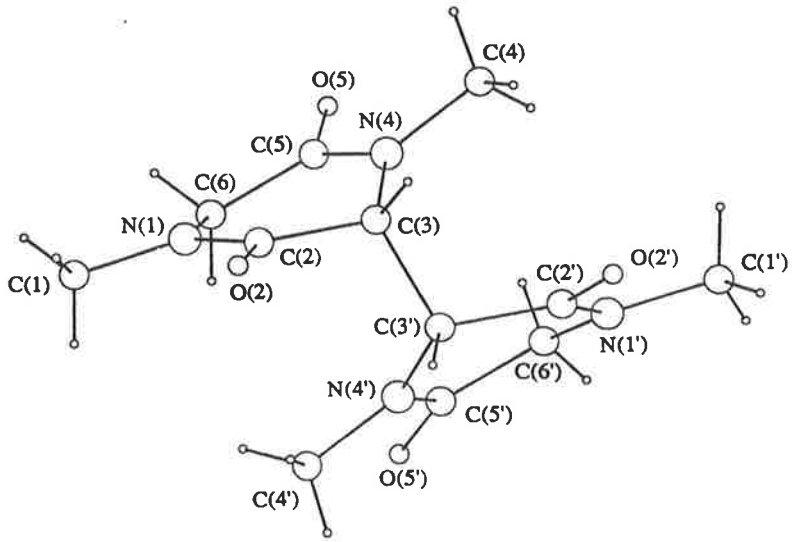

Source of material: Produced by photolysis of a mixture of sarcosine anhydride and di-tert-butyl peroxide in benzene.
The elongation of the C(3)-C(3′) bond distance (1.566(2) Å) is thought to arise from the stability of the radicals formed by the dissociation of that bond. Corresponding distances in the photoreductive dimers of 1,2,5,6-tetrahydro-3,5,5-trimethyl-2-pyrazinone and 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one are 1.581(5) Å (see ref. 2) and 1.591(4) Å (see ref. 1), respectively.

Monoclinic, $P12_1/c1$ (no 14), $a = 14.965(1)$, $b = 8.175(2)$, $c = 10.812(2)$ Å, $\beta = 91.82(1)^\circ$, $V = 1322.1$ Å^3, $Z = 4$, $R = 0.040$.

Table 1. Parameters used for the X-ray data collection

Diffractometer type:	Enraf-Nonius CAD4	Number of unique reflections:	2876
Wave length:	Mo K_α radiation (0.7107 Å)	Criterion for unobserved reflections:	$I_o < 2.5\sigma(I_o)$
Crystal characteristics:	colourless crystal, size $0.10 \times 0.40 \times 0.55$ mm	Number of refined parameters:	253
Temperature of measurement:	293 K	Scan mode:	$\omega/2\Theta$
		μ:	0.68 cm^{-1}
$2\theta_{max}$:	50°	Structure solution program used:	SHELX

Table 2. Final atomic coordinates and displacement parameters (in $Å^2$)

Atom	x	y	z	U_{iso}/U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O(2)	0.8918(1)	−0.0758(2)	−0.1636(1)	0.0586(9)	0.0458(8)	0.0327(8)	0.0104(7)	0.0104(6)	−0.0054(6)
O(5)	0.8512(1)	0.0945(2)	0.3047(1)	0.071(1)	0.061(1)	0.0277(8)	0.0039(8)	−0.0060(7)	−0.0058(7)
N(1)	0.9151(1)	−0.1369(2)	0.0385(2)	0.0386(8)	0.0310(8)	0.0391(9)	0.0071(7)	−0.0020(7)	−0.0014(7)
N(4)	0.8134(1)	0.1328(2)	0.1033(1)	0.0417(8)	0.0268(8)	0.0264(7)	0.0020(6)	−0.0005(6)	−0.0025(6)
C(1)	0.9755(2)	−0.2719(3)	0.0119(3)	0.048(1)	0.044(1)	0.073(2)	0.017(1)	−0.007(1)	−0.007(1)
C(2)	0.8752(1)	−0.0547(2)	−0.0548(2)	0.0348(9)	0.0274(9)	0.0319(9)	−0.0007(7)	0.0013(7)	−0.0012(7)
C(3)	0.8032(1)	0.0674(2)	−0.0215(2)	0.0358(9)	0.0266(9)	0.0223(8)	0.0017(7)	0.0028(6)	0.0016(7)
C(4)	0.7846(2)	0.3006(3)	0.1266(2)	0.057(1)	0.032(1)	0.045(1)	0.003(1)	−0.003(1)	−0.0083(9)
C(5)	0.8502(1)	0.0462(3)	0.1973(2)	0.0401(9)	0.041(1)	0.0261(9)	−0.0050(8)	−0.0015(7)	0.0005(8)
C(6)	0.8933(2)	−0.1140(3)	0.1667(2)	0.057(1)	0.042(1)	0.034(1)	0.009(1)	−0.0067(9)	0.0068(9)
O(2')	0.6302(1)	0.2227(2)	−0.1193(1)	0.062(1)	0.056(1)	0.0454(9)	0.0226(8)	0.0044(7)	0.0190(8)
O(5')	0.6409(1)	−0.2733(2)	0.1975(2)	0.074(1)	0.055(1)	0.051(1)	−0.0074(8)	0.0062(8)	0.0230(8)
N(1')	0.5903(1)	0.1211(2)	0.0647(2)	0.0395(9)	0.0407(9)	0.0358(9)	0.0041(7)	0.0046(6)	−0.0008(7)
N(4')	0.6929(1)	−0.1569(2)	0.0252(2)	0.0411(8)	0.0286(8)	0.0350(8)	−0.0004(6)	0.0008(6)	0.0019(6)
C(1')	0.5294(2)	0.2567(4)	0.0869(3)	0.045(1)	0.062(2)	0.061(2)	0.014(1)	0.008(1)	−0.012(1)
C(2')	0.6384(1)	0.1199(2)	−0.0369(2)	0.036(1)	0.038(1)	0.0311(9)	0.0035(8)	−0.0027(7)	0.0016(8)
C(3')	0.7092(1)	−0.0115(2)	−0.0485(2)	0.0369(9)	0.0303(9)	0.0221(8)	0.0014(7)	−0.0008(6)	0.0008(7)
C(4')	0.7249(2)	−0.3157(3)	−0.0178(3)	0.057(1)	0.027(1)	0.062(2)	−0.002(1)	−0.001(1)	0.001(1)
C(5')	0.6483(1)	−0.1521(3)	0.1315(2)	0.042(1)	0.044(1)	0.034(1)	−0.0086(9)	−0.0023(8)	0.0087(8)
C(6')	0.6056(2)	0.0058(3)	0.1663(2)	0.055(1)	0.054(1)	0.032(1)	−0.002(1)	0.0108(9)	0.003(1)
H(1A)	1.019(2)	−0.281(4)	0.075(3)	0.072(9)					
H(1B)	1.005(2)	−0.247(4)	−0.067(3)	0.08(1)					
H(1C)	0.943(3)	−0.355(5)	−0.017(3)	0.09(1)					
H(3A)	0.808(1)	0.160(2)	−0.079(2)	0.024(5)					
H(4A)	0.832(2)	0.359(3)	0.174(2)	0.048(6)					
H(4B)	0.771(2)	0.345(4)	0.043(3)	0.081(9)					
H(4C)	0.730(2)	0.295(4)	0.183(3)	0.09(1)					
H(6A)	0.947(2)	−0.120(4)	0.212(3)	0.065(8)					
H(6B)	0.856(2)	−0.207(4)	0.196(3)	0.062(7)					
H(1A')	0.561(3)	0.343(5)	0.122(4)	0.10(1)					
H(1B')	0.506(2)	0.295(4)	0.000(3)	0.072(8)					
H(1C')	0.485(3)	0.216(5)	0.131(4)	0.11(1)					
H(3A')	0.706(1)	−0.046(2)	−0.135(2)	0.024(4)					
H(4A')	0.768(2)	−0.364(4)	0.047(3)	0.080(9)					
H(4B')	0.750(2)	−0.303(4)	−0.097(3)	0.067(8)					
H(4C')	0.679(2)	−0.382(4)	−0.031(3)	0.071(9)					
H(6A')	0.640(2)	0.047(4)	0.237(3)	0.068(8)					
H(6B')	0.554(2)	−0.016(3)	0.199(2)	0.057(7)					

Further details of the structure determination (e.g. structure factors) have been deposited within the relevant database and can be accessed as Collection No. 320243 or ordered from the Fachinformationszentrum Karlsruhe, D-7514 Eggenstein-Leopoldshafen.

References:

1. Haltiwanger, R. C., Koch, T. H., Olesen, J. A., Kim, C. S., Kim, N. K.: Crystal and molecular structure of the photoreductive dimer of 5,6-dihydro-3,5,5-trimethyl-1,4-oxazin-2-one. J. Am. Chem. Soc. **99** (1977) 6327–6331.
2. Kleyer, D. L., Haltiwanger, R. C., Koch, T. H.: Synthesis and characterization of the 3,5,5-trimethyl-2-oxopiperazin-3-yl radical. An amino carboxamido type merostabilized free radical. J. Org. Chem. **48** (1983) 147–152.

Zeitschrift für Kristallographie 198, 155–157 (1992)

Crystal structure of methyl-2-phthalimido-3-methylbut-2-enoate, $C_{14}H_{13}N_1O_4$

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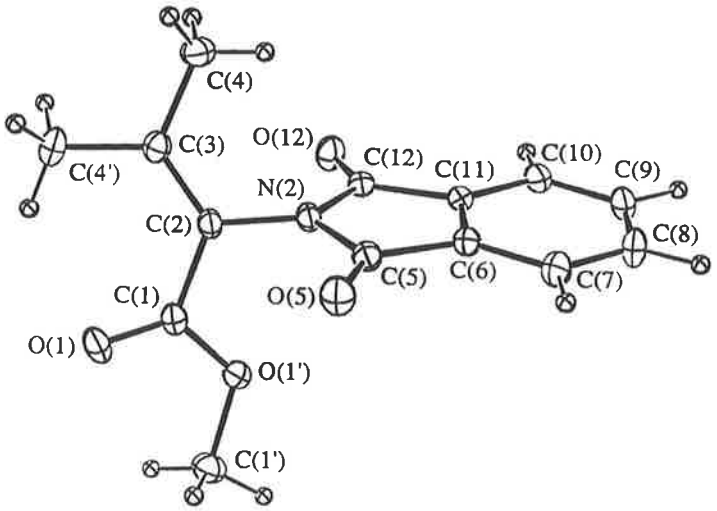

Source of material: Treatment of the parent valine derivative with N-bromosuccinimide and subsequently with sodium hydride (see ref. 2).
The compound was investigated as a part of a study of conjugation in dehydroamino acid derivatives (see ref. 1). The C(1)-C(2) and C(2)-C(3) separations of 1.481(4) and 1.343(4) Å respectively, coupled with the torsion angle of $-25.1°$ for C(3)C(2)C(1)O(1) and the relative disposition of the substituents indicate that there is no extended conjugation in this molecule.

Triclinic, $P\bar{1}$ (no 2), $a = 9.444(4)$, $b = 9.715(1)$, $c = 7.732(2)$ Å, $\alpha = 102.09(2)°$, $\beta = 100.19(3)°$, $\gamma = 75.98(3)°$, V = 667.0 Å^3, Z = 2, R = 0.038.

Table 1. Parameters used for the X-ray data collection

Diffractometer type:	Enraf-Nonius CAD4	Number of unique reflections:	1744
Wave length:	Mo K_α radiation (0.7107 Å)	Criterion for unobserved reflections:	$I_O < 2.5\sigma(I_O)$
Crystal characteristics:	colourless crystal, size $0.05 \times 0.15 \times 0.40$ mm	Number of refined parameters:	224
Temperature of measurement:	293 K	Scan mode:	$\omega/2\Theta$
		μ:	0.58 cm^{-1}
$2\Theta_{max}$:	45°	Structure solution program used:	SHELX

Table 2. Final atomic coordinates and displacement parameters (in $Å^2$)

Atom	x	y	z	U_{iso}/U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O(1)	0.2902(2)	−0.1096(2)	−0.4315(3)	0.089(2)	0.039(1)	0.108(2)	−0.014(1)	−0.008(1)	−0.003(1)
O(1')	0.4322(2)	0.0383(2)	−0.2737(2)	0.054(1)	0.048(1)	0.065(1)	−0.0096(9)	−0.0014(8)	0.0092(8)
O(5)	0.1602(2)	0.2018(2)	−0.0472(2)	0.099(2)	0.066(1)	0.060(1)	−0.033(1)	0.019(1)	0.0157(9)
O(12)	0.3093(2)	0.3932(2)	−0.4572(2)	0.099(2)	0.066(1)	0.049(1)	−0.033(1)	0.0100(9)	0.0096(8)
N(2)	0.2217(2)	0.2706(2)	−0.2873(2)	0.061(1)	0.036(1)	0.040(1)	−0.013(1)	0.0040(9)	0.0039(8)
C(1)	0.3098(3)	0.0106(3)	−0.3782(3)	0.060(2)	0.045(2)	0.051(1)	−0.013(1)	0.009(1)	−0.001(1)
C(1')	0.5394(4)	−0.0863(4)	−0.2263(5)	0.065(2)	0.061(2)	0.081(2)	0.000(2)	0.009(2)	0.016(2)
H(11)	0.493(4)	−0.136(4)	−0.163(5)	0.10(1)					
H(12)	0.615(5)	−0.043(4)	−0.142(5)	0.12(1)					
H(13)	0.559(4)	−0.157(4)	−0.332(6)	0.11(1)					
C(2)	0.2045(3)	0.1443(2)	−0.4159(3)	0.057(2)	0.039(1)	0.043(1)	−0.015(1)	0.006(1)	0.003(1)
C(3)	0.1008(3)	0.1528(3)	−0.5590(3)	0.054(2)	0.054(2)	0.051(1)	−0.021(1)	0.007(1)	0.008(1)
C(4)	−0.0070(4)	0.2901(4)	−0.5821(5)	0.059(2)	0.066(2)	0.079(2)	−0.011(2)	−0.008(2)	0.020(2)
H(41)	0.010(4)	0.329(4)	−0.684(5)	0.11(1)					
H(42)	−0.104(4)	0.278(4)	−0.600(4)	0.09(1)					
H(43)	0.004(4)	0.369(4)	−0.486(5)	0.08(1)					
C(4')	0.0840(5)	0.0292(4)	−0.7080(4)	0.080(2)	0.075(2)	0.057(2)	−0.037(2)	−0.000(2)	−0.005(2)
H(44)	−0.000(5)	0.001(4)	−0.727(5)	0.12(1)					
H(45)	0.078(5)	0.068(4)	−0.810(6)	0.12(2)					
H(46)	0.168(5)	−0.056(5)	−0.701(5)	0.11(1)					
C(5)	0.2019(3)	0.2869(3)	−0.1078(3)	0.059(2)	0.047(1)	0.045(1)	−0.010(1)	0.007(1)	0.009(1)
C(6)	0.2450(3)	0.4245(3)	−0.0187(3)	0.059(2)	0.044(1)	0.042(1)	−0.006(1)	0.004(1)	0.003(1)
C(7)	0.2499(4)	0.4905(3)	0.1588(4)	0.087(2)	0.067(2)	0.047(2)	−0.010(2)	0.011(1)	−0.001(1)
H(7)	0.213(4)	0.451(3)	0.254(4)	0.080(9)					
C(8)	0.2994(4)	0.6182(4)	0.2053(4)	0.103(3)	0.068(2)	0.056(2)	−0.015(2)	0.001(2)	−0.022(2)
H(8)	0.302(3)	0.671(3)	0.331(4)	0.069(8)					
C(9)	0.3412(4)	0.6766(3)	0.0807(5)	0.082(2)	0.048(2)	0.079(2)	−0.013(2)	−0.005(2)	−0.013(2)
H(9)	0.375(3)	0.770(3)	0.120(4)	0.074(8)					
C(10)	0.3373(3)	0.6105(3)	−0.0984(4)	0.066(2)	0.043(2)	0.071(2)	−0.013(1)	−0.004(1)	0.005(1)
H(10)	0.375(3)	0.644(3)	−0.196(4)	0.075(9)					
C(11)	0.2891(3)	0.4822(2)	−0.1426(3)	0.054(2)	0.035(1)	0.047(1)	−0.006(1)	−0.004(1)	0.004(1)
C(12)	0.2780(3)	0.3834(2)	−0.3164(3)	0.057(2)	0.043(1)	0.041(1)	−0.009(1)	0.001(1)	0.010(1)

Further details of the structure determination (e.g. structure factors) have been deposited within the relevant database and can be accessed as Collection No. 320268 or ordered from the Fachinformationszentrum Karlsruhe, D-7514 Eggenstein-Leopoldshafen.

References:

1. Easton, C.J., Hutton, C.A., Roselt, P.D., Tiekink, E.R.T.: Synthesis and molecular structure of stable derivatives of (E)- and (Z)-dehydrophenylalanine. Aust. J. Chem. **44** (1991) 687–694.
2. Tan, E.W.: Ph. D. Dissertation, University of Adelaide (1990).

Aust. J. Chem., 1992, **45**, 395–402

Synthesis of Cyclopropyl Amino Acid Derivatives

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Abstract

Derivatives of α,β-methanovaline, α,β-methanophenylalanine and β-methyl-α,β-methanoalanine have been prepared by regioselective side-chain functionalization of suitably protected amino acid derivatives, followed by cyclization with either sodium hydride or 1,8-diazabicyclo[5.4.0]undec-7-ene. The approach used in this work illustrates a method for the synthesis of cyclopropyl amino acid derivatives which is complementary to existing procedures.

Introduction

Natural and synthetic cyclopropyl amino acids (α,β-methano amino acids) and their derivatives are of interest as mechanistic probes in studies of ethylene biosynthesis,[1–5] as enzyme inhibitors,[6,7] and for incorporation into peptides to investigate structure–activity relationships.[8–13] The most common method for the synthesis of cyclopropyl amino acids is the cyclopropanation of α,β-dehydro amino acid derivatives.[13–17] Other syntheses have involved the alkylation and subsequent

[1] Adams, D. O., and Yang, S. F., *Proc. Natl Acad. Sci. U.S.A.*, 1979, **76**, 170.
[2] Lieberman, M., *Annu. Rev. Plant Physiol.*, 1979, **30**, 533.
[3] Pirrung, M. C., and McGeehan, G. M., *J. Org. Chem.*, 1986, **51**, 2103.
[4] Pirrung, M. C., and McGeehan, G. M., *Angew. Chem., Int. Ed. Engl.*, 1985, **24**, 1044.
[5] Hoffman, N. E., Yang, S. F., Ichihara, A., and Sakamura, S., *Plant Physiol.*, 1982, **70**, 195.
[6] Ner, S. K., Suckling, C. J., Bell, A. R., and Wrigglesworth, R., *J. Chem. Soc., Chem. Commun.*, 1987, 480.
[7] Breckenridge, R. J., and Suckling, C. J., *Tetrahedron*, 1986, **42**, 5665.
[8] Mapelli, C., Kimura, H., and Stammer, C. H., *Int. J. Pept. Protein Res.*, 1986, **28**, 347.
[9] Kimura, H., Stammer, C. H., Shimohigashi, Y., Ren-Lin, C., and Stewart, J., *Biochem. Biophys. Res. Commun.*, 1983, **115**, 112.
[10] Stammer, C. H., *Tetrahedron*, 1990, **46**, 2231, and references cited therein.
[11] Mapelli, C., Stammer, C. H., Lok, S., Mierke, D. F., and Goodman, M., *Int. J. Pept. Protein Res.*, 1988, **32**, 484.
[12] Mapelli, C., Newton, M. G., Ringold, C. E., and Stammer, C. H., *Int. J. Pept. Protein, Res.*, 1987, **30**, 498.
[13] King, S. W., Riordan, J. M., Holt, E. M., and Stammer, C. H., *J. Org. Chem.*, 1982, **47**, 3270.
[14] Cativiela, C., Diaz de Villegas, M. D., and Melendez, E., *Tetrahedron*, 1986, **42**, 583.
[15] Arenal, I., Bernabe, M., Fernandez-Alvarez, E., and Penades, S., *Synthesis*, 1985, 773.
[16] Suzuki, M., Gooch, E. E., and Stammer, C. H., *Tetrahedron Lett.*, 1983, **24**, 3839.
[17] Srivastava, V. P., Roberts, M., Holmes, T., and Stammer, C. H., *J. Org. Chem.*, 1989, **54**, 5866.

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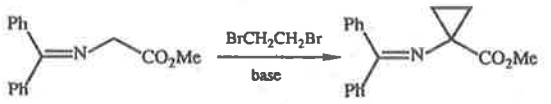
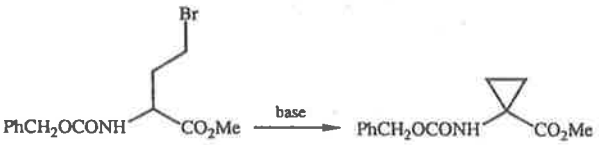
cyclization of certain *N*-substituted glycine derivatives,[18] a simple example of which is shown in Scheme 1. In a related procedure, the α,β-methanoalanine derivative (2) was formed through cyclization of the corresponding γ-substituted α-aminobutyric acid derivative (1) (Scheme 2).[19] In the work described in this report, we have used procedures, complementary to the last approach, for the synthesis of the cyclopropyl amino acid derivatives (3)–(5).

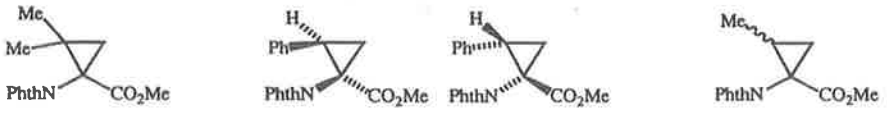


Scheme 1



(1) (2)

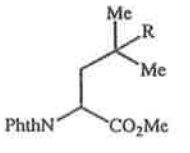
Scheme 2



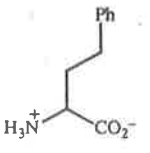
(3) (4) (5)

Results and Discussion

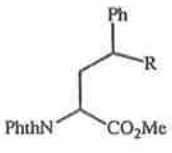
Recent studies[20,21] have shown that treatment of *N*-phthaloyl-protected amino acid derivatives with *N*-bromosuccinimide results in side-chain bromination. Accordingly, reaction of *N*-phthaloylleucine methyl ester (6a) with *N*-bromosuccinimide gave a good yield of the γ-bromoleucine derivative (6b).[21] Treatment of the bromide (6b) with sodium hydride in tetrahydrofuran afforded, after chromatography and crystallization from diethyl ether/light petroleum, the α,β-methanovaline derivative (3) in 67% yield.



(6a) R = H
(6b) R = Br



(7)



(8a) R = H
(8b) R = Br
(8c) R = Cl

In order to obtain the α,β-methanophenylalanine derivative (4) by a route analogous to that used to obtain the α,β-methanovaline derivative (3),

[18] O'Donnell, M. J., Bruder, W. A., Eckrich, T. M., Shullenberger, D. F., and Staten, G. S., *Synthesis*, 1984, 127.
[19] Prochazka, Z., Budesinsky, M., Smolikova, J., Trska, P., and Jost, K., *Collect. Czech. Chem. Commun.*, 1982, **47**, 2291.
[20] Easton, C. J., Tan, E. W., and Hay, M. P., *J. Chem. Soc., Chem. Commun.*, 1989, 385.
[21] Easton, C. J., Hutton, C. A., Rositano, G., and Tan, E. W., *J. Org. Chem.*, 1991, **56**, 5618.

homophenylalanine (7) was used as the starting material. Reaction of the amino acid (7) with phthalic anhydride, followed by treatment with methanol that had been pretreated with thionyl chloride, gave *N*-phthaloylhomophenylalanine methyl ester (8a). This material reacted with *N*-bromosuccinimide to give the crude γ-bromohomophenylalanine derivative (8b), as a 1 : 1 mixture of diastereomers. After chromatography of the mixture and crystallization from dichloromethane/light petroleum, the bromide (8b) was isolated in 69% yield. Bromination at the benzylic position of the amino acid derivative (8a) is consistent with reaction via the most stable radical intermediate.[20,21]

The reaction of the homophenylalanine derivative (8a) with sulfuryl chloride in carbon tetrachloride, under irradiation with ultraviolet light, was investigated as an alternative to bromination. The procedure gave a 1 : 1 mixture of the diastereomers of the chloride (8c), but the yield of purified material was only 16%. The chlorination was much less efficient than the bromination, and it was difficult to separate the chloride (8c) from the unreacted amino acid derivative (8a) by chromatography. Thus, bromination was found to be the better method for side-chain functionalization of the homophenylalanine derivative (8a).

The bromide (8b) was treated with sodium hydride in tetrahydrofuran to give the *cis*-α,β-methanophenylalanine derivative (4) in 38% yield after chromatography and crystallization from dichloromethane/light petroleum. The stereochemistry of the α,β-methanophenylalanine derivative (4) was determined by X-ray crystallographic analysis.[22] Analysis of the ^{1}H n.m.r. spectrum of the crude mixture from reaction of the bromide (8b) indicated that the *cis*-α,β-methanophenylalanine derivative (4) and the corresponding *trans*-isomer (not isolated) were present in the ratio *c.* 30 : 1. Presumably the diastereoselective formation of the *cis*-cyclopropyl amino acid derivative (4) reflects a preferred orientation for cyclization of the anion derived from the bromide (8b), but the basis of this preference is not obvious.

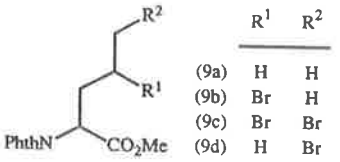
In attempts to improve the yield of the α,β-methanophenylalanine derivative (4), alternatives to the use of sodium hydride were investigated. When the bromide (8b) was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene in tetrahydrofuran, the cyclopropyl amino acid derivative (4) was obtained in 71% yield after isolation and purification.

The approach used in the synthesis of the α,β-methano amino acid derivatives (3) and (4) was found to be unsuitable for the preparation of the β-methyl-α,β-methanoalanine derivative (5). The amino acid derivative (9a), prepared from norvaline as described above for the synthesis of the homophenylalanine derivative (8a), was inert to bromination under the conditions used to prepare the bromides (6b) and (8b). This can be attributed to the inability of the norvaline derivative (9a) to form a radical sufficiently stable to induce hydrogen abstraction by bromine atom.

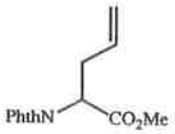
Instead, the cyclopropyl amino acid derivative (5) was synthesized from *N*-phthaloylallylglycine methyl ester (10), which had been prepared from allylglycine as described above for the synthesis of the homophenylalanine derivative (8a). When the allylglycine derivative (10) was dissolved in a freshly prepared solution

[22] Easton, C. J., Mahadevan, I. B., Tiekink, E. R. T., and Ward, C. M., *Z. Kristallogr.*, 1991, in press.

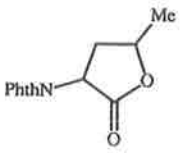
of hydrogen bromide in acetic acid, and the resultant solution was stirred at room temperature for 30 h, a product was obtained which, on chromatography, gave a 2:1 mixture of the diastereomers of the bromide (9b) in 50% yield, and a 4·5:1 mixture of the diastereomers of the lactone (11) in 27% yield.



	R^1	R^2
(9a)	H	H
(9b)	Br	H
(9c)	Br	Br
(9d)	H	Br



(10)



(11)

(12)

With aged solutions of hydrogen bromide in acetic acid, the time taken to produce the bromide (9b) and the lactone (11) increased significantly; indeed, the starting material (10) was consumed faster than the products (9b) and (11) were formed. When a reaction was carried out under these circumstances and monitored by thin-layer chromatography, workup after consumption of the majority of the starting material (10), but prior to the formation of substantial quantities of the bromide (9b) and the lactone (11), gave a 1:1 mixture of the diastereomers of the dibromide (9c), in 26% yield. The formation of the dibromide (9c) can be attributed to the presence of bromine in aged acetic acid solutions of hydrogen bromide. Evidently, the addition of bromine to the allylglycine derivative (10) to give the dibromide (9c) is reversible, which accounts for the eventual formation of the γ-bromide (9b) and the lactone (11).

When the γ-bromonorvaline derivative (9b) was treated with sodium hydride in tetrahydrofuran, the cyclopropyl amino acid derivative (5) was obtained in 74% yield as a 3:1 mixture of diastereomers after chromatography and crystallization from dichloromethane/light petroleum.

The allylglycine derivative (10) was also used in an attempt to produce the cyclobutyl amino acid derivative (12). Thus, treatment of the allylglycine derivative (10) with hydrogen bromide in carbon tetrachloride, under irradiation with ultraviolet light, and in the presence of 2,2′-azobisisobutyronitrile in order to facilitate the free radical addition process, afforded the δ-bromonorvaline derivative (9d), as a colourless oil in 83% yield. The bromide (9d) was treated with sodium hydride in tetrahydrofuran or alternatively with 1,8-diazabicyclo[5.4.0]undec-7-ene in tetrahydrofuran, but failed to afford the cyclobutyl amino acid derivative (12). Only decomposition products and the unreacted bromide (9d) were recovered from these reactions.

On this basis, the approach of side-chain functionalization of amino acid derivatives, followed by cyclization, appears to be unsuitable for the preparation of cyclobutyl amino acid derivatives. However, the method does provide a procedure for the synthesis of cyclopropyl amino acid derivatives, which is complementary to existing methods.

Experimental

General experimental details have been reported previously.[23] Chromatography was performed on silica.

N-*Phthaloyl-α,β-methanovaline Methyl Ester (3)*

A stirred solution of the bromoleucine derivative (6b)[21] (0·50 g, 1·41 mmol) in freshly distilled tetrahydrofuran (20 ml) was treated under nitrogen with sodium hydride (80% in oil; 0·07 g, 2·33 mmol). After 24 h at room temperature, the mixture was concentrated under reduced pressure and the residue dissolved in ethyl acetate and water. The organic layer was separated, washed with water, dried, filtered, and then concentrated under reduced pressure to give an oil which was chromatographed, and crystallized from diethyl ether/light petroleum to afford N-*phthaloyl-α,β-methanovaline methyl ester* (3), as colourless crystals (0·26 g, 67%), m.p. 95–97° (Found: C, 65·9; H, 5·5; N, 5·1. $C_{15}H_{15}NO_4$ requires C, 65·9; H, 5·5; N, 5·1%). ^{1}H n.m.r. ($CDCl_3$) δ 1·20, s, 3H, CCH_3; 1·51, s, 3H, CCH_3; 1·52, d, J 5·9 Hz, 1H, Cβ'–H; 1·89, d, J 5·9 Hz, 1H, Cβ'–H′; 3·65, s, 3H, OCH_3; 7·76, m, 2H, ArH; 7·88, m, 2H, ArH. ^{13}C n.m.r. ($CDCl_3$) δ 19·2, q; 23·3, q; 28·4, t; 29·8, s; 41·0, s; 52·6, q; 123·4, d; 129·3, s; 134·1, d; 168·4, s; 170·4, s. ν_{max} 1770, 1734, 1440, 1302, 1251, 1212, 1143, 1104, 720 cm^{-1}. Mass spectrum m/z 273 (M).

N-*Phthaloylhomophenylalanine Methyl Ester (8a)*

An intimate mixture of homophenylalanine (7) (2·0 g, 11 mmol) and phthalic anhydride (1·7 g, 11 mmol) was heated with stirring for 0·65 h in an oil bath maintained at 140–145°. On cooling, the mixture was dissolved in methanol (30 ml), and the solution was concentrated to 15 ml before being added to methanol (15 ml) that had been pretreated with thionyl chloride (1 ml, 14 mmol). The resultant mixture was stirred overnight at room temperature, then concentrated under reduced pressure, and the residual oil was diluted with methanol. Removal of the solvent gave a product which was chromatographed to yield N-*phthaloylhomophenylalanine methyl ester* (8a) as a pale yellow oil (3·46 g, 96%), b.p. 175°/0·03 mm (block) (Found: C, 70·5; H, 5·3; N, 4·4. $C_{19}H_{17}NO_4$ requires C, 70·6; H, 5·3; N, 4·3%). ^{1}H n.m.r. ($CDCl_3$) δ 2·60, m, 4H, Cβ–H_2, Cγ–H_2; 3·72, s, 3H, OCH_3; 4·88, m, 1H, Cα–H; 7·15, m, 5H, ArH; 7·80, m, 4H, ArH. ν_{max} 1794, 1744, 1720, 1388, 1098, 708 cm^{-1}. Mass spectrum m/z 323 (M).

γ-*Bromo*-N-*phthaloylhomophenylalanine Methyl Ester (8b)*

A mixture of the homophenylalanine derivative (8a) (1·0 g, 3·1 mmol), *N*-bromosuccinimide (0·56 g, 3·1 mmol) and 2,2′-azobisisobutyronitrile (c. 2 mg) in refluxing carbon tetrachloride (50 ml) was irradiated with a 250-W mercury lamp for 2 h. The cooled reaction mixture was diluted with carbon tetrachloride (40 ml), washed with water, dried, and concentrated under reduced pressure, to give crude γ-*bromo*-N-*phthaloylhomophenylalanine methyl ester* (8b), as a 1:1 mixture of diastereomers. ^{1}H n.m.r. ($CDCl_3$) δ 3·15, m, 2H, Cβ–H_2; 3·73, s, 0·5×3H, OCH_3; 3·75, s, 0·5×3H, OCH_3; 4·91, t, J 7·6 Hz, 1H, Cα–H; 5·10, t, J 7·5 Hz, 0·5×1H, Cγ–H; 5·21, dd, J 5·9, 9·2 Hz, 0·5×1H, Cγ–H; 7·20, m, 5H, ArH; 7·80, m, 4H, ArH. ν_{max} 1774, 1744, 1716, 1264, 1216, 722, 704 cm^{-1}. Mass spectrum m/z 322 (M − Br). Chromatography of the mixture and crystallization from dichloromethane/light petroleum gave various mixtures of the diastereomers of the bromide (8b) as colourless crystals (0·86 g, 69%). A 5:1 mixture of the diastereomers of the bromide (8b) had m.p. 112–118° (Found: C, 56·8; H, 4·0; N, 3·5. $C_{19}H_{16}BrNO_4$ requires C, 56·7; H, 4·0; N, 3·5%).

γ-*Chloro*-N-*phthaloylhomophenylalanine Methyl Ester (8c)*

A mixture of the homophenylalanine derivative (8a) (0·25 g, 0·77 mmol), sulfuryl chloride (0·065 ml, 0·81 mmol) and 2,2′-azobisisobutyronitrile (c. 2 mg) in refluxing carbon tetrachloride (15 ml) was irradiated with a 250-W mercury lamp for 2·25 h. The cooled reaction

[23] Easton, C. J., and Peters, S. C., *Aust. J. Chem.*, 1990, **43**, 87.

mixture was washed with saturated aqueous sodium bicarbonate and water, dried, and concentrated under reduced pressure to give a 1:1 mixture of the diastereomers of γ-chloro-*N*-phthaloylhomophenylalanine methyl ester (8c) contaminated with the starting material (8a). Chromatography of the mixture, followed by crystallization from dichloromethane/light petroleum, gave a 3:1 mixture of the diastereomers of the chloride (8c) as colourless crystals (45 mg, 16%), m.p. 100–111° (Found: C, 63·4; H, 4·3; N, 3·9. $C_{19}H_{16}ClNO_4$ requires C, 63·8; H, 4·5; N, 3·9%). ^{1}H n.m.r. ($CDCl_3$) δ 3·00, m, 2H, Cβ–H_2; 3·73, s, 0·25×3H, OCH_3; 3·75, s, 0·75×3H, OCH_3; 4·80, dd, *J* 5·5, 9·2 Hz, 0·75×1H, Cα–H; 5·00, dd, *J* 5·8, 7·8 Hz, 0·25×1H, Cα–H; 5·10, dd, *J* 6·2, 7·7 Hz, 0·25×1H, Cγ–H; 5·25, dd, *J* 4·5, 10·7 Hz, 0·75×1H, Cγ–H; 7·25, m, 5H, ArH; 7·74, m, 2H, ArH; 7·84, m, 2H, ArH. ν_{max} 1774, 1744, 1714, 1268, 1226, 720, 616 cm^{-1}. Mass spectrum *m/z* 322 (M − Cl).

cis-N-*Phthaloyl-α,β-methanophenylalanine Methyl Ester (4)*

(i) Treatment of the γ-bromohomophenylalanine derivatives (8b) (0·50 g, 1·25 mmol) with sodium hydride in tetrahydrofuran, as described above for the preparation of the α,β-methanovaline derivative (3), gave cis-N-*phthaloyl-α,β-methanophenylalanine methyl ester* (4) as colourless crystals (152 mg, 38%), m.p. 131·5–132·5°, after chromatography, and crystallization from dichloromethane/light petroleum (Found: C, 70·9; H, 4·9; N, 4·4. $C_{19}H_{15}NO_4$ requires C, 71·0; H, 4·7; N, 4·4%). ^{1}H n.m.r. ($CDCl_3$) δ 2·27, dd, *J* 6·6, 9·9 Hz, 1H, Cβ′–H; 2·43, dd, *J* 6·6, 8·5 Hz, 1H, Cβ′–H; 3·38, dd, *J* 8·5, 9·9 Hz, 1H, Cβ–H; 3·72, s, 3H, OCH_3; 7·12, m, 5H, ArH; 7·72, m, 4H, ArH. ν_{max} 1782, 1724, 1440, 1404, 1272, 732 cm^{-1}. Mass spectrum *m/z* 321 (M).

The ^{1}H n.m.r. spectrum of the crude mixture obtained from the reaction of the bromide (8b) showed the presence of the *cis*-α,β-methanophenylalanine derivative (4) and the corresponding *trans*-isomer (δ 1·89, dd, *J* 6·2, 9·9 Hz, 1H, Cβ′–H; 2·50, dd, *J* 6·2, 9·1 Hz, 1H, Cβ′–H; 3·18, apparent t, *J* 9·5 Hz, 1H, Cβ–H; 3·72, s, 3H, OCH_3; 7·12, m, 5H, ArH; 7·72, m, 4H, ArH) in the ratio *c.* 30:1.

(ii) A solution of the γ-bromohomophenylalanine derivative (8b) (0·40 g, 0·99 mmol) in tetrahydrofuran (25 ml) was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (0·26 g, 1·71 mmol), and the mixture was heated at reflux for 6 h. The solvent was then removed under reduced pressure, and the residue was dissolved in dichloromethane. The resultant solution was washed with dilute hydrochloric acid followed by water, and then dried, and concentrated under reduced pressure. The residue was chromatographed to give the α,β-methanophenylalanine derivative (4) (0·23 g, 71%), which was identical to the sample obtained as described above.

N-*Phthaloylallylglycine Methyl Ester (10)*

Treatment of allylglycine (2·0 g, 17 mmol) with phthalic anhydride and subsequently with methanol that had been pretreated with thionyl chloride, as described above for the synthesis of the homophenylalanine derivative (8a), gave N-*phthaloylallylglycine methyl ester* (10) as colourless crystals (3·9 g, 87%), m.p. 43·5–45·5°, after chromatography, and crystallization from ethyl acetate/light petroleum [Found: *m/z* 259·085. $C_{14}H_{13}NO_4$ (M) requires *m/z* 259·085]. ^{1}H n.m.r. ($CDCl_3$) δ 3·00, m, 2H, Cβ–H_2; 3·75, s, 3H, OCH_3; 5·00, m, 3H, Cα–H, Cδ–H_2; 5·72, m, 1H, Cγ–H; 7·74, m, 2H, ArH; 7·86, m, 2H, ArH. ν_{max} 1713, 1697, 1254, 1218, 717 cm^{-1}.

*γ-Bromo-*N*-phthaloylnorvaline Methyl Ester (9b) and*
N-*(5-Methyl-2-oxotetrahydrofuran-3-yl)phthalimide (11)*

The allylglycine derivative (10) (1·5 g, 5·8 mmol) was dissolved in a freshly prepared saturated solution of hydrogen bromide in acetic acid (40 ml), and the resultant solution stirred at room temperature for 30 h. The solvent was removed under reduced pressure, and the residue dissolved in ethyl acetate. The solution was then washed with saturated aqueous sodium bicarbonate followed by water, dried, and concentrated under reduced pressure. Chromatography of the residue gave a 2:1 mixture of the diastereomers of *γ-bromo-*N*-phthaloylnorvaline methyl ester* (9b) as a colourless oil (0·98 g, 50%) (Found: C, 49·0; H, 4·2; N, 4·0. $C_{14}H_{14}BrNO_4$ requires C, 49·4; H, 4·2; N, 4·1%). ^{1}H n.m.r. ($CDCl_3$) δ

1·76, d, *J* 6·6 Hz, 0·33×3H, CCH_3; 1·77, d, *J* 6·7 Hz, 0·66×3H, CCH_3; 2·48, ddd, *J* 7·4, 8·5, 15·0 Hz, 0·66×1H, C*β*–H; 2·65, ddd, *J* 3·9, 11·3, 15·3 Hz, 0·33×1H, C*β*–H; 2·87, m, 0·33×1H, C*β*–H′; 2·88, ddd, *J* 5·1, 6·8, 15·0 Hz, 0·66×1H, C*β*–H′; 3·74, s, 0·66×3H, OCH_3; 3·75, s, 0·33×3H, OCH_3; 3·91, m, 0·33×1H, C*γ*–H; 4·32, m, 0·66×1H, C*γ*–H; 5·12, apparent t, *J* 7·0 Hz, 0·66×1H, C*α*–H; 5·29, dd, *J* 3·9, 11·4 Hz, 0·33×1H, C*α*–H; 7·77, m, 2H, ArH; 7·89, m, 2H, ArH. ν_{max} 1776, 1754, 1720, 1442, 1260, 1222, 1088, 720 cm^{-1}. Mass spectrum *m/z* 341, 339 (M).

Chromatography of the reaction mixture also gave a 4·5 : 1 mixture of the diastereomers of N-*(5-methyl-2-oxotetrahydrofuran-3-yl)phthalimide* (11) as colourless crystals (0·39 g, 27%), m.p. 179–185°, after recrystallization from dichloromethane/light petroleum [Found: *m/z* 201·078. $C_{12}H_{11}NO_2$ ($M - CO_2$) requires *m/z* 201·079]. ^1H n.m.r. ($CDCl_3$) *δ* 1·50, d, *J* 6·5 Hz, 0·18×3H, CH_3; 1·58, d, *J* 6·1 Hz, 0·82×3H, CH_3; 2·88, ddd, *J* 3·1, 10·0, 13·0 Hz, 0·18×1H, C4–H; 2·43, ddd, *J* 10·4, 12·0, 12·2 Hz, 0·82×1H, C4–H; 2·66, ddd, *J* 5·7, 9·2, 12·2 Hz, 0·82×1H, C4–H′; 2·79, m, 0·18×1H, C4–H′; 4·70, m, 1H, C5–H; 4·95, m, 0·18×1H, C3–H; 5·18, dd, *J* 9·2, 12·0 Hz, 0·82×1H, C3–H; 7·77, m, 2H, ArH; 7·86, m, 2H, ArH. ν_{max} 1772, 1714, 1210, 720 cm^{-1}.

*γ,δ-Dibromo-*N*-phthaloylnorvaline Methyl Ester (9c)*

Treatment of the allylglycine derivative (10) (0·25 g, 0·97 mmol) with a stored solution of hydrogen bromide in acetic acid, as described above for the preparation of the bromide (9b) and the lactone (11), gave a 1 : 1 mixture of the diastereomers of *γ,δ-dibromo-*N*-phthaloylnorvaline methyl ester* (9c) as colourless crystals (103 mg, 26%), m.p. 88–103°, after chromatography and crystallization from dichloromethane/light petroleum (Found: C, 40·1; H, 3·2; N, 3·4. $C_{14}H_{13}Br_2NO_4$ requires C, 40·1; H, 3·1; N, 3·3%). ^1H n.m.r. ($CDCl_3$) *δ* 2·40, ddd, *J* 6·5, 9·1, 15·4 Hz, 0·5×1H, C*β*–H; 2·64, ddd, *J* 3·6, 11·3, 15·2 Hz, 0·5×1H, C*β*–H; 3·33, m, 1H, C*β*–H′; 3·64, dd, *J* 9·0, 10·7 Hz, 0·5×1H, C*δ*–H; 3·71, dd, *J* 9·7, 10·4 Hz, 0·5×1H, C*δ*–H; 3·76, s, 0·5×3H, OCH_3; 3·78, s, 0·5×3H, OCH_3; 3·85, dd, *J* 4·4, 10·4 Hz, 0·5×1H, C*δ*–H′; 3·89, dd, *J* 4·4, 10·7 Hz, 0·5×1H, C*δ*–H′; 3·98, m, 0·5×1H, C*γ*–H; 4·52, m, 0·5×1H, C*γ*–H; 5·16, apparent t, *J* 6·8 Hz, 0·5×1H, C*α*–H; 5·28, dd, *J* 3·6, 11·8 Hz, 0·5×1H, C*α*–H; 7·78, m, 2H, ArH; 7·90, m, 2H, ArH. ν_{max} 1780, 1744, 1714, 1258, 720 cm^{-1}. Mass spectrum *m/z* 421, 419, 417 (M).

*β-Methyl-*N*-phthaloyl-α,β-methanoalanine Methyl Ester (5)*

Treatment of the *γ*-bromonorvaline derivative (9b) (0·50 g, 1·47 mmol) with sodium hydride in tetrahydrofuran, as described above for the preparation of the *α,β*-methanovaline derivative (3), gave a 3 : 1 mixture of the diastereomers of *β-methyl-*N*-phthaloyl-α,β-methanoalanine methyl ester* (5) as colourless crystals (0·28 g, 74%), m.p. 96–107°, after chromatography and crystallization from dichloromethane/light petroleum (Found: C, 65·0; H, 5·0; N, 5·4. $C_{14}H_{13}NO_4$ requires C, 64·9; H, 5·1; N, 5·4%). ^1H n.m.r. ($CDCl_3$) *δ* 1·13, d, *J* 6·2 Hz, 0·8×3H, CCH_3; 1·38, dd, *J* 5·6, 8·0 Hz, 0·8×1H, C*β*′–H; 1·46, d, *J* 6·0 Hz, 0·2×3H; CCH_3; 1·58, dd, *J* 5·4, 9·4 Hz, 0·2×1H, C*β*′–H; 1·76, dd, *J* 5·4, 8·8 Hz, 0·2×1H, C*β*′–H′; 1·86, m, 0·2×1H, C*β*–H; 1·95, dd, *J* 5·6, 9·5 Hz, 0·8×1H, C*β*′–H′; 2·14, m, 0·8×1H, C*β*–H; 3·66, s, 0·8×3H, OCH_3; 3·67, s, 0·2×3H, OCH_3; 7·77, m, 2H, ArH; 7·88, m, 2H, ArH. ν_{max} 1778, 1722, 1408, 1290, 730, 722 cm^{-1}. Mass spectrum *m/z* 259 (M).

*δ-Bromo-*N*-phthaloylnorvaline Methyl Ester (9d)*

Hydrogen bromide was bubbled through a stirred irradiated (250-W mercury lamp) mixture of the allylglycine derivative (10) (1·0 g, 3·86 mmol) and 2,2′-azobisisobutyronitrile (*c.* 2 mg) in carbon tetrachloride (50 ml) for 0·5 h. Irradition was continued for a further 0·5 h, then the mixture was concentrated under reduced pressure and the residue was dissolved in ethyl acetate and water. The organic layer was washed with dilute aqueous potassium bicarbonate followed by water, and then dried, and concentrated under reduced pressure. Chromatography of the residue gave *δ-bromo-*N*-phthaloylnorvaline methyl ester* (9d) as a viscous oil (1·09 g, 83%) (Found: C, 48·8; H, 4·3; N, 4·1%; $M^{+\bullet}$, 339·009. $C_{14}H_{14}BrNO_4$ requires C, 49·4; H, 4·2; N, 4·1%; $M^{+\bullet}$, 339·011). ^1H n.m.r. ($CDCl_3$) *δ* 1·92, m, 2H, C*β*–H_2; 2·39, m, 2H,

Cγ–H_2; 3·42, t, J 6·7 Hz, 2H, Cδ–H_2; 3·75, s, 3H, CH_3; 4·87, dd, J 5·4, 10·2 Hz, 1H, Cα–H; 7·78, m, 2H, ArH; 7·89, m, 2H, ArH. ν_{max} 1776, 1746, 1720, 1262, 1228, 1106, 720 cm^{-1}. Mass spectrum m/z 341, 339 (M).

Treatment of δ-Bromo-N-phthaloylnorvaline Methyl Ester (9d) with Sodium Hydride and 1,8-Diazabicyclo[5.4.0]undec-7-ene

The bromide (9d) was treated with sodium hydride and 1,8-diazabicyclo[5.4.0]undec-7-ene, as described above for the preparation of the α,β-methanophenylalanine derivative (4). In each case, the only material identified through ^1H n.m.r. spectroscopic and thin-layer chromatographic analysis of the reaction mixture was the starting material (9d).

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Crystal and molecular structure of N-ethyl-1,8-naphthalimide

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Abstract. The crystal structure of N-ethyl-1,8-naphthalimide, ($C_{14}H_{11}NO_2$), has been determined at 180 K. The colourless compound crystallizes in the monoclinic space group $C2/c$ with unit cell dimensions $a = 6.932(1)$, $b = 18.663(4)$, $c = 16.469(2)$ Å, $\beta = 98.04(1)^\circ$, $Z = 8$ and $D_x = 1.418$ Mg m^{-3}. The structure was solved by direct-methods and refined by a full-matrix least-squares procedure on 753 reflections to final $R = 0.056$. The molecule is essentially planar with the exception of the terminal methyl group which lies to one side of the plane and the two methylene H atoms which lie to the other side. The crystal structure determination enables the rationalization of the chemical reactivity of this class of compound.

Introduction

As a part of a study of the synthesis of novel fluorescent probes through free-radical halogenation of N-alkylnaphthalimides we observed that while N-methyl-1,8-naphthalimide reacted with N-bromosuccinimide to give the N-bromomethyl-substituted derivative, the corresponding N-ethyl-naphthalimide was completely inert under the reaction conditions. This observation was unexpected because the naphthalimidomethyl radical, produced as an intermediate in the reaction of N-methyl-1,8-naphthalimide,

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should be less stable and form less readily than the more substituted 1-naphthalimidoethyl radical, expected as an intermediate in the reaction of the N-ethylnaphthalimide.

One explanation to account for this anomaly is that the reaction of N-methylnaphthalimide is under stereoelectronic control (Beckwith, Easton and Serelis, 1980; Beckwith, 1981; Easton, 1983) such that the hydrogen-atom transfer involves homolytic scission of a carbon-hydrogen bond perpendicular to the plane of the naphthalimide ring. This arrangement would provide greatest overlap of the π-orbitals of the naphthalimide group with the semi-occupied p-orbital developing at the radical centre, and hence greatest stabilization of the reaction transition state. The minimum energy conformation of the N-ethylnaphthalimide is likely to be that in which the C–C bond of the substituent is perpendicular to the plane of the naphthalimide ring and steric interactions are likely to prevent the formation of a conformation suitable for hydrogen abstraction under stereoelectronic control. To test this hypothesis, a crystal structure analysis of the title compound, N-ethyl-1,8-naphthalimide, was undertaken to determine the preferred conformation of the molecule.

Experimental

Crystals of N-ethyl-1,8-naphthalimide, prepared by alkylation of potassium 1,8-naphthalimide with iodoethane and recrystallized from ethanol (Devereux and Donahoe, 1960), are weakly scattering at room-temperature. The results presented in this report represent the better of three structure determinations for this compound, namely with a data set collected at 180 K. Intensity data were measured (Enraf-Nonius CAD-4F diffractometer controlled by a PDP8/A computer) employing graphite-monochromated Mo$K\alpha$ radiation ($\lambda = 0.7107$ Å) and the ω:$n/3\theta$ scan technique; the optimum value of n was determined to be 1 on the basis of theta/omega scans performed on representative reflections. Metric crystal data are given in the Abstract. A total of 3196 reflections were measured up to maximum Bragg angle of 25°, of these 1871 were unique and 753 satisfied the $I \geq 2.5\sigma(I)$ criterion of observability. Although a number of direct-method strategies were employed to solve this structure in the space group $C2/c$, none yielded a satisfactory solution. However, E-maps generated for the non-centrosymmetric space group Cc using MITHRIL (Gilmore, 1984) revealed well resolved atomic centres for all of the atoms of the two independent molecules. The atomic coordinates that resulted from a least-squares refinement (Sheldrick, 1976) of this model suggested that the two molecules were centrosymmetrically related, a feature which was supported by strong correlation effects observed between the corresponding parameters of the two molecules during the course of the refinement. A subsequent least-

Table 1. Fractional atomic coordinates and B_{eq} values (Å^2) $B_{eq} = 8\pi^2(U_{11} + U_{22} + U_{33})/3$ for N-ethyl-1,8-naphthalimide.

Atom	x	y	z	B_{eq}
O(11)	−0.2508(6)	0.0079(2)	−0.2742(2)	2.29
O(12)	−0.1200(6)	0.1327(2)	−0.0364(2)	2.71
N(12)	−0.1731(6)	0.0711(2)	−0.1560(3)	1.84
C(1)	−0.2651(7)	0.1300(3)	−0.2863(3)	1.50
C(2)	−0.3125(8)	0.1333(3)	−0.3706(3)	1.90
C(3)	−0.3397(8)	0.1967(3)	−0.4150(4)	2.29
C(4)	−0.3234(9)	0.2609(3)	−0.3765(4)	2.61
C(5)	−0.2545(10)	0.3309(3)	−0.2438(4)	2.45
C(6)	−0.2162(9)	0.3314(3)	−0.1610(4)	2.53
C(7)	−0.1854(10)	0.2671(3)	−0.1177(4)	2.61
C(8)	−0.1986(8)	0.2016(3)	−0.1575(3)	1.90
C(9)	−0.2465(9)	0.2002(3)	−0.2438(3)	1.74
C(10)	−0.2750(8)	0.2656(3)	−0.2883(3)	2.21
C(11)	−0.2313(8)	0.0660(3)	−0.2408(3)	1.58
C(12)	−0.1616(8)	0.1348(3)	−0.1110(3)	1.90
C(13)	−0.1241(9)	0.0032(3)	−0.1111(3)	2.16
C(14)	−0.3001(10)	−0.0277(3)	−0.0782(4)	2.71

Table 2. Selected bond distances (Å) and angles (deg.) for N-ethyl-1,8-naphthalimide.

N(12)−C(11)	1.402(6)	N(12)−C(12)	1.398(6)
N(12)−C(13)	1.482(7)	C(11)−O(11)	1.215(6)
C(12)−O(12)	1.223(6)	C(1)−C(11)	1.461(7)
C(8)−C(12)	1.466(7)	C(13)−C(14)	1.516(8)
C(11)−N(12)−C(12)	125.0(5)	C(11)−N(12)−C(13)	116.9(4)
C(12)−N(12)−C(13)	118.1(4)	N(12)−C(11)−O(11)	120.7(5)
N(12)−C(11)−C(1)	117.1(5)	O(11)−C(11)−C(1)	122.2(5)
N(12)−C(12)−O(12)	119.5(5)	N(12)−C(12)−C(8)	116.9(4)
O(12)−C(12)−C(8)	123.5(5)	N(12)−C(13)−C(14)	111.3(5)

squares refinement in the $C2/c$ space group, after an appropriate shift in origin, confirmed that this was the true space group; the refinement involved 128 parameters and was based on F (Sheldrick, 1976). The N and O atoms as well as the C atoms of the ethyl group were refined with anisotropic thermal parameters; the remaining atoms were refined isotropically. The H atoms were located from a difference map, assigned a common isotropic thermal parameter, and refined. A weighting scheme of the form, $w = k/[\sigma^2(F) + g|F|^2]$ was introduced and the refinement continued until convergence; $R = 0.056$, $wR = 0.060$, $k = 1.85$ and $g = 0.0018$. No extinction correction was applied and the analysis of variance

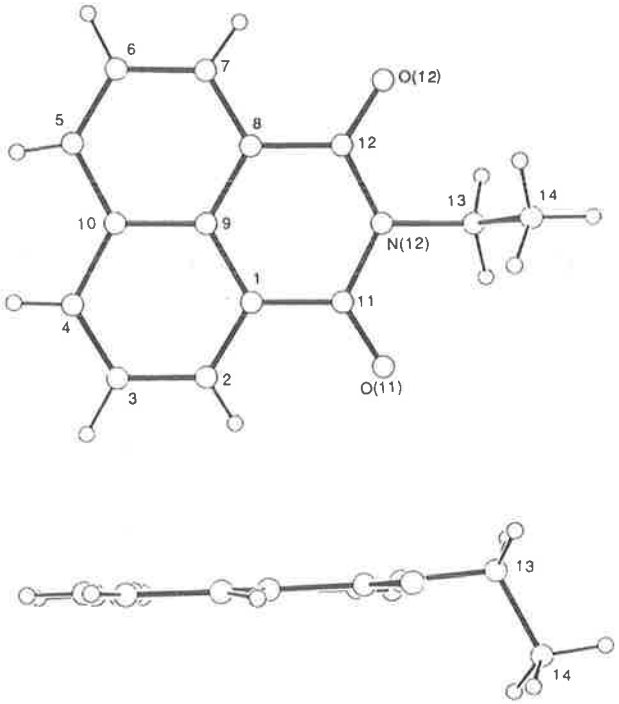

Fig. 1. Two views of the N-ethyl-1,8-naphthalimide molecule showing the crystallographic numbering scheme.

showed no special features. The maximum and minimum residual electron density peaks in the final difference map were $+0.37$ and $-0.26\ e\text{Å}^{-3}$, respectively. Scattering factors for all atoms were as incorporated in SHELX76 (Sheldrick, 1976). Atomic parameters are given in Table 1, selected interatomic parameters in Table 2 and the numbering scheme used is shown in Figure 1 which was drawn with ORTEP (Johnson, 1976) using arbitrary thermal ellipsoids. Listings of thermal parameters, all bond distances and angles and Tables of F_{obs} have been deposited. [1]

Results and discussion

The structure determination confirms the stoichiometry of the compound as N-ethyl-1,8-naphthalimide as shown in Fig. 1 which gives the atomic

[1] Additional material to this paper can be ordered from the Fachinformationszentrum Energie-Physik-Mathematik, D-7514 Eggenstein-Leopoldshafen 2, FRG. Please quote reference no. CSD 55026, the names of the authors and the title of the paper.

numbering scheme. The structure is molecular, there being no significant intermolecular contacts in the crystal lattice. Although the molecule does not possess crystallographic symmetry, there is a pseudo mirror plane bisecting the molecule. As can be seen from the lower view of Figure 1, the three fused six-membered rings are essentially coplanar. The equation for the least-squares plane through these atoms is $0.994X + 0.043Y - 0.010Z = -0.572$ and the maximum deviation from this plane is 0.046(4) Å for the N(12) atom. The O(11) and O(12) atoms lie 0.077(4) and 0.006(4) Å out of this plane in the direction of the C(14) atom. Consistent with the planarity of this system is the extensive delocalization of π-electron density over the heterocyclic portion of the molecule, as indicated by the systematic variation of bond distances and angles about these atoms. Thus, the C(1)–C(11) and C(8)–C(12) bond distances (1.461(7), 1.466(7) Å) are shorter than normal C–C single bond distances (Table 2) and the C=O bond distances (1.215(6), 1.223(6) Å) are longer than expected. While the N(12)–C(13) bond length is normal at 1.482(7) Å, the N(12)–C(11) and N(12)–C(12) bond lengths (1.402(6), 1.398(6) Å) are significantly shorter than this distance. The conjugation does not extend to the ethyl substituent. The remaining bond distances and angles associated with the molecule are as expected and are not discussed further. The approximate *m*-symmetry in the molecule is emphasized in the values of the two torsion angles for C(11)–N(12)–C(13)–C(14) and C(12)–N(12)–C(13)–C(14) of −92.2 and 86.6°, respectively. The main interest in the structure is the disposition of the N-bound ethyl substituent.

In the absence of significant intermolecular contacts (see above), one may conclude that the solution state structure of the molecule resembles closely the structure found in the solid state and therefore it is possible to draw conclusions about the lack of reactivity of the molecule on the basis of the X-ray structure. The C(13) atom lies 0.154(6) Å out of the least-squares plane through the fused rings and the terminal methyl C(14) atom lies 1.213(7) Å out of this plane in a direction opposite to the C(13) atom. Thus, the conformation of the molecule lends support to the hypothesis that the reaction of N-methyl-naphthalimide is under stereoelectronic control. The disposition of the ethyl substituent in the N-ethyl analogue precludes such a mechanism and hence the crystallographic analysis provides a plausible explanation for the observed inertness of this compound.

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References

Beckwith, A. L. J., Easton, C. J., Serelis, A. K.: Some guidelines for radical reactions. J. Chem. Soc., Chem. Commun. (1980) 482–483.

Beckwith, A. L. J.: Regioselectivity and stereoselectivity in radical reactions. Tetrahedron **37** (1981) 3073–3100.

Devereux, M. A., Donahoe, H. B.: *N*-Substituted imides. II. Potassium naphthalimide as a reagent for the identification of alkyl halides. J. Org. Chem. **25** (1960) 457–458.
Easton, C. J.: Stereoelectronic effects in free-radical reactions. Science Progress, Oxford **68** (1983) 503–517.
Gilmore, C. J.: MITHRIL – an integrated direct-methods computer program. J. Appl. Crystallogr. **17** (1984) 42–46.
Johnson, C. K.: ORTEPII. Report ORNL-5138, Oak Ridge National Laboratory, Tennessee (1976).
Sheldrick, G. M.: SHELX76. Program for crystal structure determination. University of Cambridge, England (1976).

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Reactions of α-Substituted Glycine Derivatives with Stannanes

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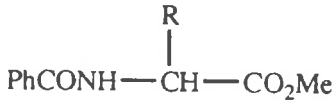
Abstract: α-Benzoyloxy- and α-methoxy-substituted glycine derivatives are more stable than the corresponding bromides, yet they undergo analogous reactions with stannanes. Their reactions with mixtures of hexabutylditin and dialkyl disulphides give α-alkylthio-substituted glycine derivatives, a procedure that is applicable to the synthesis of cross-linked amino acid derivatives.

Several procedures have been reported for the exploitation of α-bromoglycine derivatives in reactions with stannanes. Accordingly, *N*-benzoyl-α-bromoglycine methyl ester (**1a**) gave the reduced product (**1b**) on treatment with tributyltin hydride,[1,2] reacted by allyl group transfer on treatment with allyltributyltin and its derivatives,[2-4] and gave the dimer (**3**) through reaction with hexabutylditin.[5] A recent illustration of the significance of these reactions was their use in the asymmetric synthesis of amino acid derivatives,[6] but their wider application is limited by the inherent instability of α-bromoglycine derivatives. In this report we show that α-benzoyloxy- and α-methoxy-substituted glycine derivatives are stable alternatives to α-bromoglycine derivatives, undergoing similar reactions with stannanes.

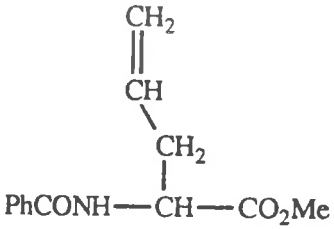
The glycine derivatives (**1c**)[3] and (**1d**)[7] were obtained by treatment of the bromide (**1a**) with benzoic acid and triethylamine, and methanol and triethylamine, respectively. Alternatively, the copper-catalysed reaction of the glycine derivative (**1b**) with *tert*-butylperbenzoate gave the benzoate (**1c**).[8] Each of the glycine derivatives (**1c**) and (**1d**) was obtained as a crystalline solid, and was stable on storage at room temperature for several months, and on distillation and chromatography. By contrast, under these conditions the bromide (**1a**) decomposed completely.

Treatment of the α-benzoyloxyglycine derivative (**1c**) with tributyltin hydride, in benzene at reflux, gave the reduced product (**1b**), in 92% yield. The analogous reaction using tributyltin deuteride gave the α-deuterioglycine derivative (**1e**) in 85% yield, with 84% deuterium incorporation. The latter reaction also afforded benzoyloxytributyltin[9] in 27% yield.



(1)

a) R = Br
b) R = H
c) R = OCOPh
d) R = OMe
e) R = D
f) R = $SCMe_3$
g) R = SCH_2Ph



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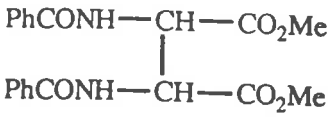

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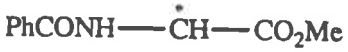

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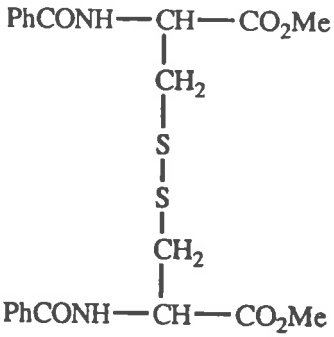
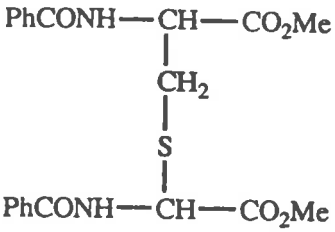


(5)



(6)

When the glycine derivative (**1c**) was treated with allyltributyltin in benzene at reflux, with AIBN to initiate the reaction, the allylglycine derivative (**2**) was produced in 47% yield, together with a 16% yield of a 1:1 mixture of the diastereomers of the dimer (**3**). Similar reactions of the α-methoxyglycine derivative (**1d**) with tributyltin hydride and allyltributyltin gave the glycine derivatives (**1b**), in 91% yield, and (**2**), in 34% yield, respectively. The latter reaction also afforded trace amounts of the diastereomers of the dimer (**3**).

Although related cationic reactions of α-acyloxy-substituted glycine derivatives have been reported,[10] the reactions of the glycine derivatives (**1b**) and (**1c**) are analogous to those of the bromide (**1a**) and are more consistent with a radical mechanism. They indicate that tributyltin radical reacts with the glycine derivatives (**1c**) and (**1d**) by substitution to give the glycyl

radical (**4**) and, in the case of the benzoate (**1c**), benzoyloxytributyltin. In turn, the radical (**4**) reacts by hydrogen or deuterium abstraction, allyl group transfer, or dimerization. While there may be substantial charge development in the transition states leading to formation of the radical (**4**), the production of the dimer (**3**) is convincing evidence that the reactions involve radical rather than ionic intermediates. There has been a previous report[11] of homoloytic substitution reactions of benzoates on treatment with tributyltin hydride, where the efficiency of the process was found to depend on the stability of the radical formed by displacement of the benzoyloxy group. In the reactions of (**1b**) and (**1c**) the product radical (**4**) is particularly stable.[1,12]

Further evidence in support of formation of the glycyl radical (**4**) in reactions of the α-substituted glycine derivatives (**1c**) and (**1d**) with stannanes was obtained from reactions involving hexabutylditin and dialkyl disulphides. Photolysis of an equimolar mixture of the benzoate (**1c**), hexabutylditin and di-*tert*-butyl disulphide, in benzene at reflux for 14 h, gave the α-*tert*-butylthio-substituted glycine derivative (**1f**) [29% yield; m.p. 89-91°C; ^{1}H n.m.r. ($CDCl_3$) δ 1.45 (s, 9H), 3.81 (s, 3H), 5.79 (d, *J* 9 Hz, 1H), 6.83 (d, *J* 9 Hz, 1H), and 7.43-7.82 (m, 5H)],[9] together with a 24% yield of a 1:1 mixture of the diastereomers of the dimer (**3**). Using dibenzyl disulphide instead of di-*tert*-butyl disulphide, only the α-benzylthio-substituted glycine derivative (**1g**)[7] was obtained, in 62% yield. A similar reaction of the methoxide (**1d**) using di-*tert*-butyl disulphide gave the α-*tert*-butylthio-substituted glycine derivative (**1f**), in 19% yield, the dimer (**3**), in 12% yield, and the reduced product (**1b**), in 4% yield, while only the α-benzylthio-substituted glycine derivative (**1g**) was obtained from reaction of the methoxide (**1d**) using dibenzyl disulphide.

The products of these reactions are consistent with formation of the glycyl radical (**4**) by homolytic displacement of the α-substituent from either the benzoate (**1c**) or the methoxide (**1d**). The radical (**4**) reacts either by homolytic substitution on the dialkyl disulphides to give the thioethers (**1f**) and (**1g**), by coupling to give the dimer (**3**), or by hydrogen atom abstraction to give the reduced product (**1b**). The substitution is more facile with dibenzyl disulphide than with di-*tert*-butyl disulphide, due to the comparative steric effects of the disulphides,[13] with the result that only the α-benzylthio-substituted glycine derivative (**1g**) is produced in the reactions using dibenzyl disulphide, whereas alternative reactions of the radical (**4**) to give the dimer (**3**) and the reduced product (**1b**) compete with the substitution reaction of di-*tert*-butyl disulphide to give the α-*tert*-butylthio-substituted glycine derivative (**1f**).

When *N,N'*-dibenzoylcystine dimethyl diester (**5**)[14] was used as the disulphide in a reaction with the benzoate (**1c**) and hexabutylditin, dimethyl 2,5-dibenzamido-3-thiahexanedioate (**6**)[15] was isolated as a 1:1 mixture of diastereomers, in 73% yield. This

reaction demonstrates the potential application of the reactions of α-substituted glycine derivatives with hexabutylditin and dialkyldisulphides in the synthesis of cross-linked amino acid derivatives. Studies of these reactions are continuing in our laboratories.

Acknowledgements: This work was supported by grants from the Australian Research Council and the Australian Wool Corporation.

References and Notes

1. Easton, C. J.; Hay, M. P. *J. Chem. Soc., Chem. Commun.* **1986**, 55.
2. Easton, C. J.; Scharfbillig, I. M.; Tan, E. W. *Tetrahedron Lett.* **1988**, *29*, 1565.
3. Baldwin, J. E.; Adlington, R. M.; Lowe, C.; O'Neil, I. A.; Sanders, G. L.; Schofield C. J.; Sweeney, J. B. *J. Chem. Soc., Chem. Commun.* **1988**, 1030.
4. Easton, C. J.; Scharfbillig, I. M. *J. Org. Chem.* **1990**, *55*, 384.
5. Burgess, V. A.; Easton, C. J.; Hay, M. P.; Steel, P. J. *Aust. J. Chem.* **1988**, *41*, 701.
6. Zhai, D.; Zhai, W.; Williams, R. M. *J. Am. Chem. Soc.* **1988**, *110*, 2501; Hamon, D. P. G.; Razzino, P.; Massy-Westropp, R. A. *J. Chem. Soc., Chem. Commun.* **1991**, 332 and 722.
7. Zoller, U.; Ben-Ishai, D. *Tetrahedron* **1975**, *31*, 863.
8. Easton, C. J.; Kociuba, K.; Peters, S. C. *J. Chem. Soc., Chem. Commun.* **1991**, 1475.
9. All new compounds were fully characterized.
10. O'Donnell, M. J.; Bennett, W. D.; Polt, R. L. *Tetrahedron Lett.* **1985**, *26*, 695.
11. Khoo, L. E.; Lee, H. H. *Tetrahedron Lett.* **1968**, 4351.
12. Burgess, V. A.; Easton, C. J.; Hay, M. P. *J. Am. Chem. Soc.* **1989**, *111*, 1047.
13. Pryor, W. A.; Platt, P. K. *J. Am. Chem. Soc.* **1963**, *85*, 1496; Pryor, W. A.; Guard, H. *J. Am. Chem. Soc.* **1964**, *86*, 1150; Ingold, K. U.; Roberts, B. P. *Free Radical Substitution Reactions;* Wiley and Sons, Inc.: New York, 1971.
14. Gustus, E. L. *J. Org. Chem.* **1967**, *32*, 3425.
15. Easton, C. J.; Peters, S. C. *Aust. J. Chem.* **1990**, *43*, 87.

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Stereoelectronic Effects in the Halogenation of *N*-Alkylnaphthalimides

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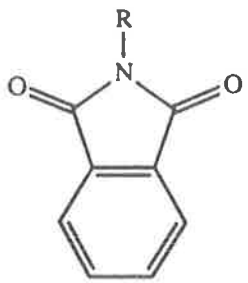
Abstract: The greater rate of bromination of *N*-methylnaphthalimide compared to *N*-ethylnaphthalimide, and the relative ease of chlorination of these compounds, provide convincing evidence of stereoelectronic effects in the free-radical halogenation of *N*-alkylimides.

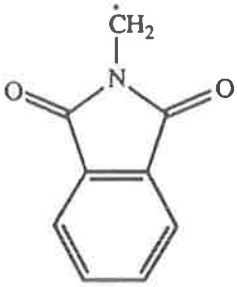
Stereoelectronic effects have been observed in a wide variety of radical reactions[1,2] and interest in this area has continued to increase with the recognition that these effects can be exploited, for example, by using amide-substituted radicals in asymmetric synthesis.[3-5] Recently it was reported[6] that the conformational preferences displayed by alkyl substituents on aromatic systems give rise to stereoelectronic effects in the bromination of alkylaromatics. That disclosure prompts us to report striking examples of stereoelectronic effects in hydrogen atom transfer reactions of *N*-alkyl-1,8-naphthalimides, which also arise from the conformational preferences of alkyl substituents on planar conjugated systems.

Treatment of *N*-methylphthalimide (**1a**)[7] with *N*-bromosuccinimide in carbon tetrachloride at reflux for 4 h, with ultraviolet irradiation to initiate reaction, gave the bromide (**1b**) [^{1}H n.m.r. ($CDCl_3$) δ 5.53 (s, 2H)] in 38% yield.[8] Under identical conditions the reaction of *N*-ethylphthalimide (**1c**) occurred more readily, to give the bromide (**1d**) [^{1}H n.m.r. ($CDCl_3$) δ 2.32 (d, *J* 7 Hz, 3H), 6.41 (q, *J* 7 Hz, 1H)] in 71% yield. This qualitative observation of the greater reactivity of *N*-ethylphthalimide (**1c**) compared with *N*-methylphthalimide (**1a**) was confirmed in competitive experiments. Using mixtures of the phthalimides (**1a**) and (**1c**), with *N*-*tert*-butylbenzamide as an internal standard, the relative rates of consumption of the starting materials (**1a**) and (**1c**), and the relative rates of formation of the products (**1b**) and (**1d**), were each *ca.* 1:4.5, as determined by ^{1}H n.m.r. spectroscopic analysis of crude reaction mixtures. These relative reaction rates reflect the relative ease of formation of the radicals (**2**) and (**3**) and the results are consistent with the expectation that the secondary radical (**3**) should be more stable and form more readily than the primary radical (**2**).

In contrast to the relative rates of reaction of the phthalimides (**1a**) and (**1c**), *N*-methyl-1,8-naphthalimide (**4a**) reacted with *N*-bromosuccinimide to give the bromide (**4b**)



(1)



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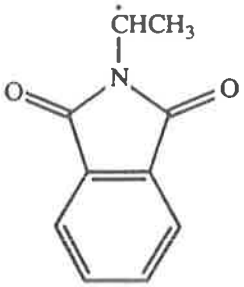
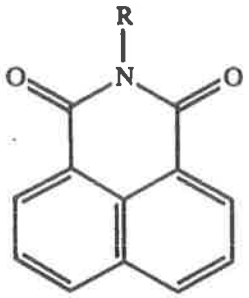


(3)

a) R = CH_3
b) R = CH_2Br
c) R = CH_2CH_3
d) R = $CHBrCH_3$



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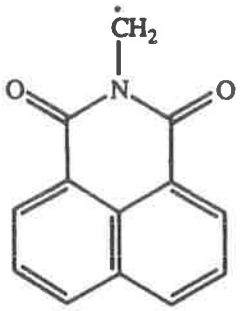

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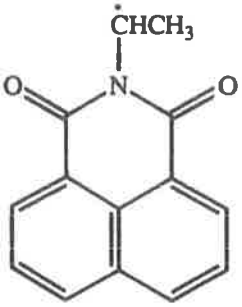

(6)

a) R = CH_3
b) R = CH_2Br
c) R = CH_2CH_3
d) R = CH_2Cl
e) R = $CHClCH_3$
f) R = CH_2CH_2Cl

[^{1}H n.m.r. ($CDCl_3$) δ 5.96 (s, 2H)] in 39% yield after 7.5 h, while the corresponding ethylnaphthalimide (**4c**) was completely inert under identical conditions, and could be recovered in virtually quantitative yield even after extended periods. The anomalously greater reactivity of the methylnaphthalimide (**4a**), which was confirmed using mixtures of the naphthalimides (**4a**) and (**4c**), from which the methylnaphthalimide (**4a**) was consumed while the ethylnaphthalimide remained unchanged, indicates that the primary radical (**5**) is formed more readily than the secondary radical (**6**).

The selective reaction of the methylnaphthalimide (**4a**) can be attributed to a stereoelectronic effect. The preferred conformation of the substrate (**4a**) in the transition state for hydrogen abstraction by bromine atom will be that which is the most stable in the ground state,[9] in which the bond being cleaved is perpendicular to the plane of the naphthalimide ring (Figure 1a). This orientation allows maximum stabilization of the incipient radical (**5**) through interaction with the π system. By contrast, the preferred ground state conformation of the ethylnaphthalimide (**4c**) is that in which the substituent's C-C bond is perpendicular to the plane of the ring (Figure 1b),[9,10] while the stereoelectronically preferred orientations (Figure 1c) will be relatively unstable as a result of steric interactions between the methyl group and the oxygens.

Presumably, the absence of an observable stereoelectronic effect in the reactions of the phthalimides (**1a**) and (**1c**) reflects the relative lack of conformational preference in the phthalimide (**1c**) compared to the naphthalimide (**4c**). Calculations[9] indicate that the energy difference between the ground state (Figure 1b) and stereoelectronically (Figure 1c) preferred conformations of the ethylnaphthalimide (**4c**) is 18.4 kJmol^{-1}, while the corresponding conformations of the ethylphthalimide (**1c**) differ in energy by only 8.8 kJmol^{-1}. In turn, the lower degree of conformational preference of the phthalimide (**1c**) can be attributed to decreased steric interactions

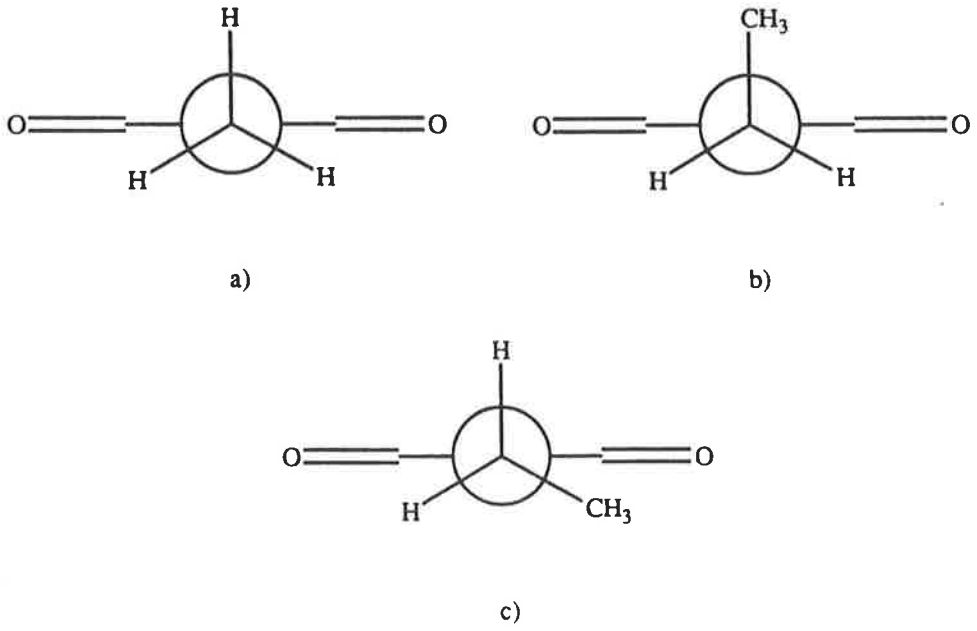

Figure 1. Newman projections along the C(α)-N bond in:
a) the ground state and stereoelectronically preferred conformation of *N*-methylnaphthalimide (**4a**),
b) the ground state preferred conformation of *N*-ethylnaphthalimide (**4c**), and
c) one of the stereoelectronically preferred orientations of *N*-ethylnaphthalimide (**4c**).

between the ethyl substituent and the oxygens, due to their greater separation. In their crystal structures, the average distance between the α-carbon and the oxygens of the ethylnaphthalimide (**4c**) is 2.71 Å,[10] while the comparable average distance in *N*-(2-imidazol-4-ylethyl)phthalimide, a representative phthalimide, is 2.91 Å.[11]

The radical reactions of the naphthalimides (**4a**) and (**4c**) with sulphuryl chloride were also studied. The methylnaphthalimide (**4a**) gave the chloride (**4d**) [^{1}H n.m.r. ($CDCl_3$) δ 5.99 (s, 2H)], while the ethylnaphthalimide afforded an approximately equal mixture of the chlorides (**4e**) [^{1}H n.m.r. ($CDCl_3$) δ 2.21 (d, *J* 7 Hz, 3H), 7.17 (q, *J* 7 Hz, 1H)] and (**4f**) [^{1}H n.m.r. ($CDCl_3$) δ 3.85 (t, *J* 7Hz, 2H), 4.56 (t, *J* 7 Hz, 2H)]. The relative rates of formation of the chlorides (**4d**) and (**4e**) were determined in competitive experiments, using mixtures of the naphthalimides (**4a**) and (**4c**), to be *ca.* 2:1. The slower rate of formation of the chloroethylnaphthalimide (**4e**) compared to the chloromethylnaphthalimide (**4d**) is analogous to, though less marked than, the stereoelectronic effect described above.

In summary, the radical reactions of the naphthalimides (**4a**) and (**4c**) with *N*-bromosuccinimide and sulphuryl chloride provide convincing evidence of stereoelectronic effects in hydrogen atom transfer reactions of these compounds and illustrate the important influence of conformational effects on reactivity in these systems.

Acknowledgements: This work was supported by grants from the Australian Wool Corporation and the Australian Research Council.

References and Notes

1. Beckwith, A. L. J. *Tetrahedron* **1981**, *37*, 3073.
2. Easton, C. J. *Sci. Prog., Oxf.* **1983**, *68*, 503.
3. Curran, D. P.; Shen, W.; Zhang, J.; Heffner, T. A. *J. Am. Chem. Soc.* **1990**, *112*, 6738.
4. Porter, N. A.; Swann, E.; Nally, J.; McPhail, A. T. *J. Am. Chem. Soc.* **1990**, *112*, 6740.
5. Giese, B.; Zehnder, M.; Roth, M.; Zeitz, H.-G. *J. Am. Chem. Soc.* **1990**, *112*, 6741.
6. Baciocchi, E.; Mattioli, M.; Ruzziconi, R. *Tetrahedron Lett.* **1992**, *33*, 1237.
7. All compounds were fully characterized.
8. Corrected yields based on unreacted starting materials were all virtually quantitative.
9. Calculations were performed using PCMODEL, Serena Software, Bloomington, Indiana, **1990**.
10. Easton, C. J.; Gulbis, J. M.; Hoskins, B. F.; Scharfbillig, I. M.; Tiekink, E. R. T. *Acta Cryst. (C)* **1992**, in press.
11. Gerst, K.; Nordman, C. E. *Acta Cryst. (B)* **1977**, *33*, 1588.

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Nickel Peroxide as a Glycine-selective Chemical Model of Peptidylglycine α-Amidating Monooxygenase

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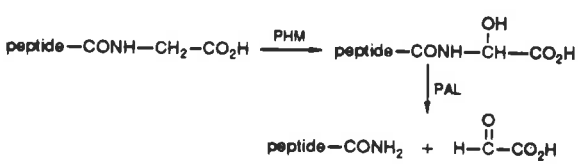
In a process, which is analogous to that catalysed by peptidylglycine α-amidating monooxygenase, nickel peroxide cleaves *N*-benzoylamino acid methyl esters to give benzamide, with a selectivity for reaction of the glycine derivative.

The bioactivation of many peptide hormones and neuropeptides involves oxidative cleavage of carboxy-terminal glycine-extended precursors (Scheme 1).[1,2] The process is catalysed by the enzyme peptidylglycine α-amidating monooxygenase (PAM), which comprises two subunits.[2] One of these, peptidylglycine α-hydroxylating monooxygenase (PHM), requires copper ions, ascorbate and molecular oxygen, and facilitates α-hydroxylation of glycine residues. The other, peptidylhydroxyglycine α-amidating lyase (PAL), cleaves the intermediate α-hydroxyglycine derivatives.

A range of chemical models of PAM has been developed[3] and used to elucidate various features of the enzyme-catalysed reactions. We now report that nickel peroxide[4] is an alternative model for PAM, with the particular feature that it shows selectivity for reaction of glycine residues akin to that displayed by the enzyme.[5] To the best of our knowledge, the basis of this substrate selectivity by PAM has not previously been examined.

When the glycine derivative **1a**[6] (0.05 mol dm^{-3} in benzene) was treated with 2.6 equiv. of nickel peroxide, at reflux under nitrogen for 1 h, filtration of the heterogeneous reaction mixture to remove nickel salts, followed by chromatography on silica, gave benzamide in 39% yield and recovered starting material in 51% yield. By comparison, the derivatives of alanine **1b**[6] and valine **1c**[6] were less reactive. The reaction of the alanine derivative **1b** under the same conditions as those used for the reaction of the glycine derivative **1a** gave benzamide in 13% yield, the dehydroalanine derivative **5**[7] in 9% yield, and 69% recovered starting material, while similar treatment of the valine derivative **1c** gave only a trace of benzamide and 93% recovered starting material. This qualitative observation of the selective reaction of the glycine derivative **1a** was confirmed in competitive experiments using mixtures of the alanine derivative **1b** with either the glycine derivative **1a** or the valine derivative **1c**, and the results are presented in Table 1.



Scheme 1

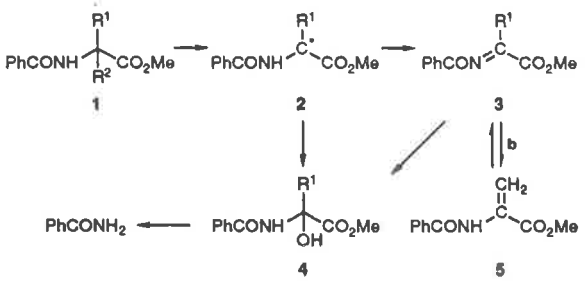
The reactions of the amino acid derivatives **1a–c** to give benzamide may be rationalized as shown in Scheme 2. Following their complexation to nickel, hydrogen-atom transfer from the substrates affords the corresponding α-carbon-centred radicals **2a–c**. Those radicals react to give the corresponding α-hydroxy amino acid derivatives **4a–c**, either directly or indirectly *via* the respective *N*-acylimines **3a–c**. Subsequent hydrolysis of the α-hydroxy amino acid derivatives **4a–c** affords benzamide. Formation of the dehydro amino acid derivative **5** in the reaction of the alanine derivative **1b** may be attributed to tautomerization of the *N*-acylimine **3b**. In a separate experiment, the dehydroalanine derivative **5** gave benzamide on treatment with nickel peroxide, consistent with the proposal that it is an intermediate in the reaction of the alanine derivative **1b**.

In a competitive experiment using 0.025 mol dm^{-3} solutions of each substrate, the glycine derivative **1a** reacted 2.9 ± 0.5

Table 1 Relative rates of reaction of the amino acid derivatives **1a–c** with nickel peroxide[a]

Substrate	Relative reaction rates: At 80 °C, with 0.025 mol dm^{-3} of each substrate	At 80 °C, with 0.0025 mol dm^{-3} of each substrate	At 20 °C, with 0.025 mol dm^{-3} of each substrate
Glycine **1a**	10.0 ± 2.5	4.5 ± 0.4	4.0 ± 0.5
Alanine **1b**	1.0[b]	1.0[b]	1.0[b]
Valine **1c**	0.14 ± 0.03	0.43 ± 0.03	—[c]

[a] Reaction in benzene with *N*-*tert*-butylbenzamide as internal standard. [b] Assigned as unity in each experiment. [c] Absolute reaction rate too slow for relative rate to be determined.



Scheme 2

times faster than the deuteriated analogue **1d**,[6] indicating that α-hydrogen transfer from the amino acid derivatives **1a–c** is a rate-determining step in their reactions with nickel peroxide. It is likely that the ease of complexation of the amino acid derivative **1a–c** with the nickel also affects the reactivity of these species, otherwise it is difficult to rationalize the effects of substrate concentration and reaction temperature on their relative rates of reaction. On this basis, the preferential reaction of the glycine derivative **1a** can be attributed to its selective binding to the nickel surface and subsequent reaction to give the glycyl radical **2a**.

The oxidative cleavage of the amino acid derivatives **1a–c** by nickel peroxide is similar to the process catalysed by PAM, and the selectivity of nickel peroxide for reaction of the glycine derivative **1a** is analogous to the substrate selectivity displayed by the enzyme. Those factors that result in the preferential reaction of glycine derivatives in the present work and earlier studies, that is the preferential complexation of glycine derivatives by metal ions[8] and the relative ease of formation of glycyl radicals,[6] may also contribute to the substrate selectivity shown by PAM. At the least it seems likely that the natural substrates of PAM are synthesized with glycine at the carboxy-terminus because that residue is so easily removed by oxidation.

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References

1 For examples see: A. F. Bradbury, M. D. A. Finnie and D. G. Smyth, *Nature*, 1982, **298**, 686; B. A. Eipper, R. E. Mains and C. C. Glembotski, *Proc. Natl. Acad. Sci. USA*, 1983, **80**, 5144; A. F. Bradbury and D. G. Smyth, *Eur. J. Biochem.*, 1987, **169**, 579; S. E. Ramer, H. Cheng, M. M. Palcic and J. C. Vederas, *J. Am. Chem. Soc.*, 1988, **110**, 8526; S. D. Young and P. P. Tamburini, *J. Am. Chem. Soc.*, 1989, **111**, 1933; T. M. Zabriskie, H. Cheng and J. C. Vederas, *J. Chem. Soc., Chem. Commun.*, 1991, 571; and references cited therein.

2 S. N. Perkins, E. J. Husten and B. A. Eipper, *Biochem. Biophys. Res. Commun.*, 1990, **171**, 926.

3 R. C. Bateman, W. W. Youngblood, W. H. Busby and J. S. Kizer, *J. Biol. Chem.*, 1985, **260**, 9088; K. V. Reddy, S.-J. Jin, P. K. Arora, D. S. Sfeir, S. C. F. Maloney, F. L. Urbach and L. M. Sayre, *J. Am. Chem. Soc.*, 1990, **112**, 2332; P. Capdevielle and M. Maumy, *Tetrahedron Lett.*, 1991, **32**, 3831; D. Ranganathan and S. Saini, *J. Am. Chem. Soc.*, 1991, **113**, 1042.

4 K. Nakagawa, R. Konaka and T. Nakata, *J. Org. Chem.*, 1962, **27**, 1567; R. Konaka, S. Terabe and K. Kurama, *J. Org. Chem.*, 1969, **34**, 1334; M. V. George and K. S. Balachandran, *Chem. Rev.*, 1975, **75**, 491.

5 A. E. N. Landymore-Lim, A. F. Bradbury and D. G. Smyth, *Biochem. Biophys. Res. Commun.*, 1983, **117**, 289; J. S. Kizer, R. C. Bateman, C. R. Miller, J. Humm, W. H. Busby and W. W. Youngblood, *Endrocrinology*, 1986, **118**, 2262.

6 For examples see: V. A. Burgess, C. J. Easton and M. P. Hay, *J. Am. Chem. Soc.*, 1989, **111**, 1047; and references cited therein.

7 V. A. Burgess and C. J. Easton, *Aust. J. Chem.*, 1988, **41**, 1063.

8 C. J. Easton, K. Kociuba and S. C. Peters, *J. Chem. Soc., Chem. Commun.*, 1991, 1475.

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Crystal structure of 2-benzamido-4-hydroxy-2,4,4-trimethylbutanoic acid γ-lactone, $C_{14}H_{17}NO_3$

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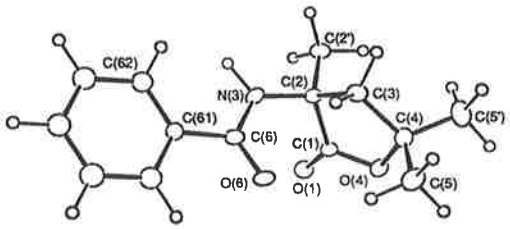
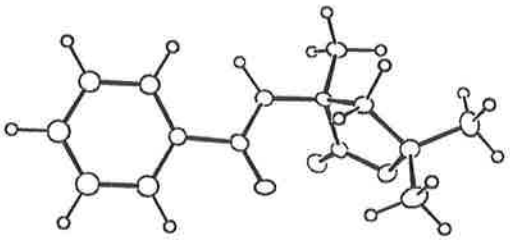


molecule a



molecule b

Source of material: Formed by photolysis of a mixture of N-benzoylalanine methyl ester and di-tert-butyl peroxide in tert-butanol.
There are no significant differences between the two molecules in the asymmetric unit. Significant H-bonding in the lattice involving N(3)-H...O(1)′ leads to the formation of a centrosymmetric ring structure comprised of four connected molecules.

Triclinic, $P\bar{1}$ (no 2), $a = 11.366(2)$, $b = 11.408(1)$, $c = 10.728(1)$ Å, $\alpha = 91.08(1)^\circ$, $\beta = 96.96(1)^\circ$, $\gamma = 106.79(1)^\circ$, $V = 1319.8$ Å^3, $Z = 4$, $R = 0.068$.

Table 1. Parameters used for the X-ray data collection

Diffractometer type:	Enraf-Nonius CAD4	Number of unique reflections:	5433
Wave length:	Cu K_α radiation (1.5418 Å)	Criterion for unobserved reflections:	$F_o < 5\sigma(F_o)$
Crystal characteristics:	transparent crystal, size $0.06 \times 0.10 \times 0.38$ mm	Number of refined parameters:	242
Temperature of measurement:	293 K	Scan mode:	ω:1/3Θ
$2\theta_{max}$:	150°	μ:	6.31 cm^{-1}
		Structure solution program used:	SHELX

Table 2. Final atomic coordinates and displacement parameters (in $Å^2$)

Atom	x	y	z	U_{iso}/U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O(1a)	0.8727(3)	0.2572(3)	0.5856(3)	0.052(2)	0.066(2)	0.044(2)	0.012(2)	0.023(2)	−0.003(2)
O(4a)	0.6861(3)	0.1400(3)	0.6126(3)	0.061(2)	0.053(2)	0.045(2)	0.006(2)	0.018(2)	−0.013(2)
O(6a)	0.6935(3)	0.4242(4)	0.6175(3)	0.060(2)	0.070(3)	0.034(2)	0.020(2)	0.000(2)	−0.003(2)
N(3a)	0.8270(4)	0.4165(4)	0.7859(4)	0.048(2)	0.050(3)	0.039(2)	0.015(2)	−0.003(2)	−0.010(2)
H(3a)	0.8945(4)	0.4613(4)	0.8489(4)	0.097(4)					
C(1a)	0.7887(4)	0.2301(4)	0.6477(4)	0.044(3)	0.046(3)	0.031(2)	0.013(2)	0.007(2)	−0.002(2)
C(2a)	0.7887(4)	0.2841(5)	0.7808(4)	0.045(3)	0.050(3)	0.028(2)	0.016(2)	0.008(2)	−0.003(2)
C(2'a)	0.8798(5)	0.2418(5)	0.8702(5)	0.068(4)	0.063(4)	0.038(3)	0.032(3)	0.008(3)	−0.001(3)
H(21b)	0.8550(5)	0.1529(5)	0.8676(5)	0.097(4)					
H(22b)	0.8809(5)	0.2735(5)	0.9550(5)	0.097(4)					
H(23b)	0.9620(5)	0.2719(5)	0.8452(5)	0.097(4)					
C(3a)	0.6532(5)	0.2292(5)	0.8036(5)	0.051(3)	0.066(4)	0.040(3)	0.021(3)	0.014(3)	0.002(3)
H(31a)	0.6107(5)	0.2918(5)	0.7980(5)	0.097(4)					
H(32a)	0.6500(5)	0.1959(5)	0.8860(5)	0.097(4)					
C(4a)	0.5926(5)	0.1268(5)	0.7008(5)	0.049(3)	0.054(3)	0.053(3)	0.003(2)	0.021(3)	−0.005(3)
C(5a)	0.4788(5)	0.1437(6)	0.6259(7)	0.047(3)	0.095(5)	0.076(4)	0.004(3)	0.007(3)	−0.008(4)
H(51a)	0.4147(5)	0.1359(6)	0.6800(7)	0.097(4)					
H(52a)	0.4494(5)	0.0816(6)	0.5572(7)	0.097(4)					
H(53a)	0.4986(5)	0.2245(6)	0.5923(7)	0.097(4)					
C(5'a)	0.5703(6)	0.0001(6)	0.7491(7)	0.085(5)	0.070(5)	0.103(6)	0.006(4)	0.044(5)	0.008(4)
H(54a)	0.5095(6)	−0.0124(6)	0.8074(7)	0.097(4)					
H(55a)	0.6473(6)	−0.0086(6)	0.7919(7)	0.097(4)					
H(56a)	0.5397(6)	−0.0603(6)	0.6790(7)	0.097(4)					
C(6a)	0.7713(5)	0.4790(5)	0.7054(5)	0.048(3)	0.060(3)	0.032(3)	0.018(3)	0.010(2)	−0.002(3)
C(62a)	0.8787(3)	0.6773(3)	0.8339(3)	0.060(2)					
C(63a)	0.9036(3)	0.8042(3)	0.8495(3)	0.072(2)					
C(64a)	0.8538(3)	0.8678(3)	0.7577(3)	0.073(2)					
C(65a)	0.7792(3)	0.8046(3)	0.6502(3)	0.081(2)					
C(66a)	0.7544(3)	0.6777(3)	0.6346(3)	0.068(2)					
C(61a)	0.8041(3)	0.6141(3)	0.7265(3)	0.045(1)					
H(62a)	0.9133(3)	0.6331(3)	0.8977(3)	0.097(4)					
H(63a)	0.9554(3)	0.8482(3)	0.9242(3)	0.097(4)					
H(64a)	0.8711(3)	0.9560(3)	0.7685(3)	0.097(4)					
H(65a)	0.7447(3)	0.8488(3)	0.5864(3)	0.097(4)					
H(66a)	0.7025(3)	0.6337(3)	0.5599(3)	0.097(4)					
O(1b)	0.1258(3)	−0.4529(3)	0.9052(3)	0.057(2)	0.064(2)	0.049(2)	0.017(2)	0.026(2)	0.006(2)
O(4b)	0.3163(3)	−0.4621(3)	0.8888(3)	0.055(2)	0.073(3)	0.042(2)	0.028(2)	0.018(2)	0.018(2)
O(6b)	0.3039(4)	−0.1839(3)	0.8869(3)	0.073(3)	0.061(2)	0.031(2)	0.021(2)	−0.000(2)	0.002(2)

Table 2. (Continued)

Atom	x	y	z	U_{iso}/U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
N(3b)	0.1842(4)	−0.2703(4)	0.7082(4)	0.047(2)	0.045(2)	0.037(2)	0.012(2)	0.003(2)	0.001(2)
H(3b)	0.1215(4)	−0.2648(4)	0.6404(4)	0.097(4)					
C(1b)	0.2145(5)	−0.4316(5)	0.8492(5)	0.042(3)	0.044(3)	0.041(3)	0.011(2)	0.012(2)	0.000(2)
C(2b)	0.2223(4)	−0.3807(4)	0.7172(4)	0.047(3)	0.042(3)	0.029(2)	0.009(2)	0.009(2)	−0.000(2)
C(2'b)	0.1319(5)	−0.4802(5)	0.6249(5)	0.058(3)	0.054(3)	0.042(3)	0.010(3)	0.011(3)	0.000(3)
H(21b)	0.1561(5)	−0.5550(5)	0.6297(5)	0.097(4)					
H(22b)	0.1341(5)	−0.4525(5)	0.5400(5)	0.097(4)					
H(23b)	0.0485(5)	−0.4958(5)	0.6465(5)	0.097(4)					
C(3b)	0.3582(4)	−0.3592(5)	0.7013(5)	0.049(3)	0.054(3)	0.038(3)	0.014(2)	0.013(2)	0.006(2)
H(31b)	0.3650(4)	−0.3915(5)	0.6190(5)	0.097(4)					
H(32b)	0.4016(4)	−0.2721(5)	0.7103(5)	0.097(4)					
C(4b)	0.4124(5)	−0.4261(5)	0.8038(5)	0.042(3)	0.069(4)	0.046(3)	0.018(3)	0.017(2)	0.011(3)
C(5b)	0.5268(5)	−0.3436(7)	0.8822(6)	0.053(4)	0.109(6)	0.065(4)	0.018(4)	0.005(3)	0.014(4)
H(51b)	0.5932(5)	−0.3175(7)	0.8308(6)	0.097(4)					
H(52b)	0.5525(5)	−0.3880(7)	0.9515(6)	0.097(4)					
H(53b)	0.5079(5)	−0.2723(7)	0.9151(6)	0.097(4)					
C(5'b)	0.4328(6)	−0.5439(6)	0.7553(7)	0.092(5)	0.086(5)	0.084(5)	0.049(4)	0.041(4)	0.017(4)
H(54b)	0.4955(6)	−0.5249(6)	0.6989(7)	0.097(4)					
H(55b)	0.3555(6)	−0.5958(6)	0.7104(7)	0.097(4)					
H(56b)	0.4597(6)	−0.5865(6)	0.8255(7)	0.097(4)					
C(6b)	0.2350(5)	−0.1746(5)	0.7929(5)	0.049(3)	0.045(3)	0.036(3)	0.010(2)	0.016(2)	0.003(2)
C(62b)	0.1449(3)	−0.0349(3)	0.6544(3)	0.056(1)					
C(63b)	0.1178(3)	0.0762(3)	0.6391(3)	0.064(2)					
C(64b)	0.1497(3)	0.1646(3)	0.7388(3)	0.072(2)					
C(65b)	0.2086(3)	0.1420(3)	0.8537(3)	0.077(2)					
C(66b)	0.2356(3)	0.0310(3)	0.8690(3)	0.065(2)					
C(61b)	0.2038(3)	−0.0575(3)	0.7694(3)	0.043(1)					
H(62b)	0.1227(3)	−0.0964(3)	0.5852(3)	0.097(4)					
H(63b)	0.0769(3)	0.0918(3)	0.5592(3)	0.097(4)					
H(64b)	0.1309(3)	0.2418(3)	0.7281(3)	0.097(4)					
H(65b)	0.2307(3)	0.2035(3)	0.9230(3)	0.097(4)					
H(66b)	0.2766(3)	0.0153(3)	0.9489(3)	0.097(4)					

Further details of the structure determination (e.g. structure factors) have been deposited within the relevant database and can be accessed as Collection No. 320440 or ordered from the Fachinformationszentrum Karlsruhe, D-7514 Eggenstein-Leopoldshafen.

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Crystal structure of N-phthaloyl-β-phenylserine methylester, $C_{18}H_{15}NO_5$

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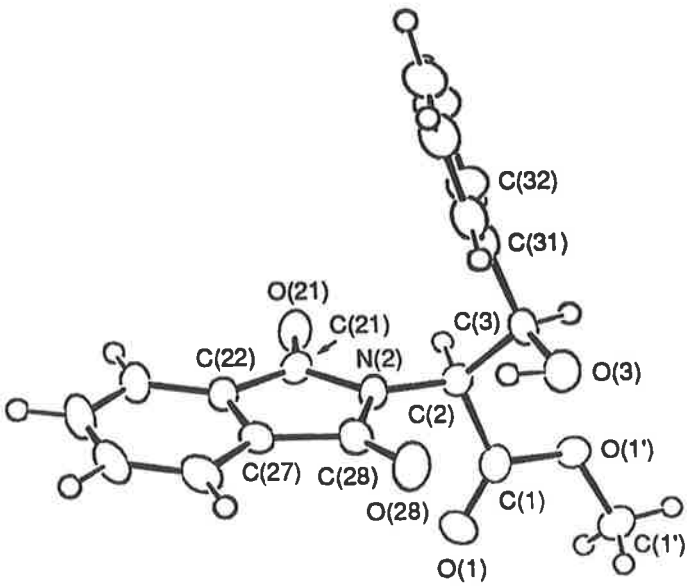

Source of material: see ref. 1.
The structure determination shows the stereochemistry around the C(2) atom to be S and that around the C(3) atom to be R.

Trigonal, $P3_221$ (no 154), $a = 11.810(7)$, $c = 19.556(7)$ Å. $V = 2362.2$ Å^3. $Z = 6$, $R = 0.053$.

Table 1. Parameters used for the X-ray data collection

Diffractometer type:	Enraf-Nonius CAD4	Number of unique reflections:	1254
Wave length:	Mo K_α radiation (0.7107 Å)	Criterion for unobserved reflections:	$F_o < 5\sigma(F_o)$
Crystal characteristics:	spherical crystal of 0.3 mm diameter	Number of refined parameters:	226
Temperature of measurement:	293 K	Scan mode:	ω:2Θ
$2\theta_{max}$:	45°	μ:	0.61 cm^{-1}
		Structure solution program used:	SHELX

Table 2. Final atomic coordinates and displacement parameters (in $Å^2$)

Atom	x	y	z	U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O(1)	0.3069(6)	0.7117(6)	0.0964(3)	0.103(5)	0.072(4)	0.044(3)	0.055(4)	0.004(3)	0.003(3)
O(1')	0.3191(6)	0.6056(5)	0.0047(3)	0.116(5)	0.070(4)	0.055(3)	0.071(4)	−0.023(3)	−0.015(3)
O(3)	0.5018(5)	0.8742(6)	−0.0627(3)	0.048(4)	0.092(5)	0.076(3)	0.041(3)	−0.004(3)	−0.020(3)
H(3)	0.5014	0.9489	−0.045	0.08532					
O(21)	0.0526(5)	0.8027(6)	−0.0003(3)	0.041(3)	0.077(4)	0.095(4)	0.033(3)	−0.016(3)	−0.031(4)
O(28)	0.4879(5)	1.0114(6)	0.0499(3)	0.037(3)	0.057(4)	0.109(4)	0.017(3)	−0.012(3)	−0.026(3)
N(2)	0.2742(5)	0.8844(5)	0.0150(3)	0.039(4)	0.036(3)	0.049(3)	0.020(3)	−0.005(3)	−0.008(3)
C(1)	0.3042(7)	0.6962(7)	0.0361(4)	0.042(5)	0.047(5)	0.069(5)	0.021(4)	0.000(4)	−0.005(4)
C(1')	0.344(1)	0.5208(9)	0.0475(4)	0.114(9)	0.078(7)	0.073(6)	0.070(7)	−0.023(6)	−0.011(5)
H(1'A)	0.353(1)	0.4585(9)	0.0191(4)	0.13(1)					
H(1'B)	0.271(1)	0.4742(9)	0.0789(4)	0.13(1)					
H(1'C)	0.424(1)	0.5726(9)	0.0732(4)	0.13(1)					
C(2)	0.2771(7)	0.7754(7)	−0.0172(3)	0.041(5)	0.042(4)	0.050(4)	0.021(4)	−0.004(4)	−0.007(4)
H(2)	0.189(6)	0.733(6)	−0.041(3)	0.05(2)					
C(3)	0.3674(7)	0.8126(8)	−0.0804(4)	0.048(5)	0.050(5)	0.052(4)	0.027(4)	−0.002(4)	−0.019(4)
H(3)	0.347(5)	0.734(5)	−0.102(2)	0.02(1)					
C(21)	0.1559(7)	0.8851(8)	0.0231(4)	0.040(5)	0.055(5)	0.054(4)	0.028(4)	−0.001(4)	0.002(4)
C(22)	0.1899(7)	1.0072(7)	0.0617(3)	0.054(5)	0.044(5)	0.047(4)	0.031(4)	0.002(4)	−0.000(4)
C(23)	0.1116(8)	1.0574(8)	0.0812(4)	0.060(5)	0.070(6)	0.063(5)	0.043(5)	0.016(4)	0.004(5)
H(23)	0.0194(8)	1.0139(8)	0.0696(4)	0.085(8)					
C(24)	0.171(1)	1.1728(8)	0.1182(4)	0.109(8)	0.051(6)	0.063(5)	0.052(6)	0.026(6)	0.002(5)
H(24)	0.120(1)	1.2122(8)	0.1326(4)	0.085(8)					
C(25)	0.302(1)	1.2331(8)	0.1350(4)	0.102(8)	0.039(5)	0.055(5)	0.031(6)	0.011(5)	−0.009(4)
H(25)	0.340(1)	1.3136(8)	0.1612(4)	0.085(8)					
C(26)	0.3795(8)	1.1828(7)	0.1160(3)	0.066(6)	0.038(4)	0.050(4)	0.013(4)	0.004(4)	−0.003(4)
H(26)	0.4712(8)	1.2255(7)	0.1285(3)	0.085(8)					
C(27)	0.3208(7)	1.0681(6)	0.0781(3)	0.054(5)	0.034(4)	0.038(4)	0.018(4)	0.005(4)	0.004(3)
C(28)	0.3770(8)	0.9899(7)	0.0486(3)	0.047(5)	0.041(5)	0.049(4)	0.017(4)	0.007(4)	0.005(4)
C(31)	0.3348(7)	0.8856(7)	−0.1323(3)	0.046(5)	0.040(4)	0.046(4)	0.022(4)	0.004(4)	−0.007(3)
C(32)	0.2190(8)	0.8223(8)	−0.1679(4)	0.069(6)	0.062(5)	0.059(5)	0.034(5)	−0.008(5)	−0.001(4)
H(32)	0.1550(8)	0.7327(8)	−0.1564(4)	0.085(8)					
C(33)	0.193(1)	0.887(1)	−0.2205(4)	0.098(8)	0.12(1)	0.070(6)	0.073(8)	−0.028(6)	−0.019(7)
H(33)	0.111(1)	0.843(1)	−0.2457(4)	0.085(8)					
C(34)	0.286(1)	1.015(1)	−0.2362(5)	0.16(1)	0.12(1)	0.060(6)	0.10(1)	0.031(7)	0.030(7)
H(34)	0.271(1)	1.060(1)	−0.2734(5)	0.085(8)					
C(35)	0.397(1)	1.077(1)	−0.1990(5)	0.12(1)	0.071(7)	0.083(7)	0.057(7)	0.043(7)	0.023(6)
H(35)	0.460(1)	1.167(1)	−0.2095(5)	0.085(8)					
C(36)	0.4248(9)	1.0164(8)	−0.1476(4)	0.069(6)	0.051(5)	0.064(5)	0.030(5)	0.012(4)	−0.004(4)
H(36)	0.5056(9)	1.0633(8)	−0.1219(4)	0.085(8)					

Further details of the structure determination (e.g. structure factors) have been deposited within the relevant database and can be accessed as Collection No. 320456 or ordered from the Fachinformationszentrum Karlsruhe, D-7514 Eggenstein-Leopoldshafen.

References:

1. Easton, C.J., Hutton, C.A., Tan, E.W., Tiekink, E.R.T.: Synthesis of homochiral hydroxy-α-amino acid derivatives. Tetrahedron Letters **31** (1990) 7059–7062.

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A New α-Haloglycine Template for the Asymmetric Synthesis of Amino Acid Derivatives

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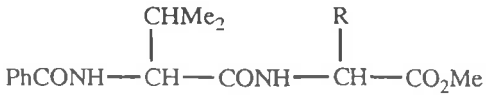
Abstract: Reaction of *(S)-N,N*-diacylvalylglycine anhydride with *N*-bromosuccinimide afforded the corresponding α-bromoglycine derivative, which reacted diastereoselectively with allyltributyltin and deuterium over palladium chloride to give the corresponding α-allyl- and α–deuterio-glycine derivatives, respectively.

There have been many reports of the use of α-haloglycine derivatives in the asymmetric synthesis of amino acids.[1-3] Our recent observation of the selective halogenation of glycine residues in amino acid derivatives[4] resulted in a complementary, though only modestly diastereoselective, method for the elaboration of glycine residues in dipeptides.[5] Thus, for example, reactions of the valyl-α-bromoglycine derivative (**1a**) with allyltributyltin and diethylmalonate anion gave the dipeptide derivatives (**1b**) and (**1c**), respectively, each as a 3:1 mixture of diastereomers. Anticipating that the geometrical constraints imposed by a cyclic system would lead to a greater degree of asymmetric induction, we have now studied analogous reactions of glycine residues in cyclic dipeptides. Prior to this study it had been shown that bis-lactim ethers, such as (**2**), derived from cyclic dipeptides, react with a high degree of asymmetric induction, although they suffer the disadvantage that they are unstable, decomposing by hydrogen chloride elimination with aromatization to give the corresponding pyrazine derivatives.[6]

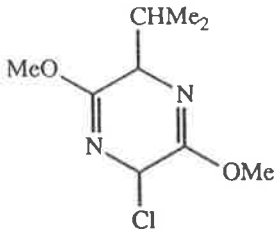
The diketopiperazine (**3b**),[7,8] chosen for this investigation, was obtained by treatment of valylglycine anhydride (**3a**) with excess acetic anhydride. Subsequent treatment with *N*-bromosuccinimide in CCl_4/CH_2Cl_2 (1:1) at reflux under nitrogen, with azobisisobutyronitrile to initiate the reaction, gave the α-bromoglycine derivative (**4**) [^{1}H n.m.r. ($CDCl_3$) δ 0.99 (d, *J* 7 Hz, 3H), 1.19 (d, *J* 7 Hz, 3H), 1.85 (m, 1H), 2.61 (s, 3H), 2.63 (s, 3H), 5.08 (d, *J* 10.5 Hz, 1H) and 6.92 (s, 1H)] as a single diastereomer, in 86% yield. The regioselectivity of this reaction is consistent with the selectivity for reaction of glycine residues observed with acyclic peptides.[4]

When the bromide (**4**) was treated with allyltributyltin in benzene at reflux for 6 h, with azobisisobutyronitrile to initiate the reaction, a single diastereomer of the corresponding allylglycine derivative (**5a**) [^{1}H n.m.r. ($CDCl_3$) δ 0.82 (d, *J* 7 Hz, 3H), 1.00 (d, *J* 7 Hz, 3H), 2.08 (m, 1H), 2.42 (s, 3H), 2.47 (s, 3H), 2.75 (m, 2H), 4.76 (d, *J* 4.5 Hz, 1H), 4.90 (dd, *J* 3.5 and 5.5 Hz, 1H), 5.15 (m, 2H) and 5.60 (m, 1H)] was obtained in 60% yield. Although this material proved to be unsuitable for

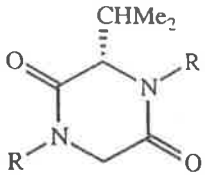


(1)

a) R = Br
b) R = $CH_2CH{=}CH_2$
c) R = $CH(CO_2Et)_2$

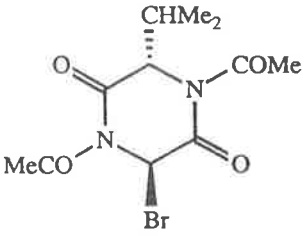


(2)

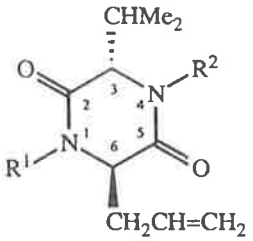


(3)

a) R = H
b) R = COMe

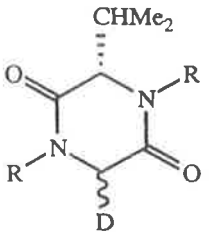


(4)



(5)

a) $R^1 = R^2$ = COMe
b) R^1 = H, R^2 = COMe



(6)

a) R = COMe
b) R = H

X-ray crystallographic analysis, on standing in moist ethyl acetate/light petroleum it underwent hydrolysis to give the monoacyldiketopiperazine derivative (**5b**) [^{1}H n.m.r. ($CDCl_3$) δ 1.00 (d, *J* 7 Hz, 3H), 1.08 (d, *J* 7 Hz, 3H), 2.10 (m, 1H), 2.54 (s, 3H), 2.80 (m, 2H), 4.18 (dd, *J* 3.5 and 9 Hz, 1H), 4.89 (dd, *J* 1.5 and 8.5 Hz, 1H), 5.30 (m, 2H) and 5.70 (m, 1H)], which was shown to be the *trans*-isomer by crystallographic analysis (Figure 1).[9] It is logical to assume that the stereochemistry of this material is the same as that of the diacyldiketopiperazine derivative (**5a**).

In the presence of $Eu(hfc)_3$,[10] the ^{1}H n.m.r. spectrum of the diketopiperazine (**5a**) displayed distinct resonances for each enantiomer. In particular, duplicate signals were observed for one of the methyl groups of the isopropyl substituent and for each *N*-acetyl group. When the above reactions were repeated with the enantiomer of the

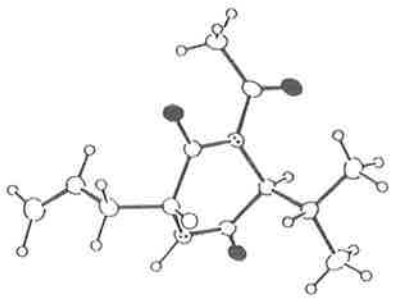


Figure 1. Molecular structure of the monoacyldiketopiperazine derivative (**5b**)

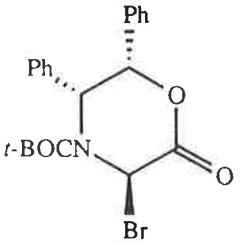
diketopiperazine (**3b**) derived from *(S)*-valine,[11] only one enantiomer of the allylglycine derivative (**5a**) could be detected using this procedure.[12] Thus the elaboration of the glycine derivative (**3b**) occurs without racemization of the valine residue, and the homochiral *(3S,6R)*-diastereomer of the allylglycine derivative (**5a**) is produced under these circumstances.

The bromide (**4**) in THF/D_2O (4:1) was stirred over palladium chloride under an atmosphere of deuterium, at room temperature for 14 h, to give the deuteride (**6a**), with 92% deuterium incorporation, as a 20:1 mixture of diastereomers. That mixture was treated with hydrazine hydrate in DMF[13] to give the deuteriated valylglycine anhydride (**6b**), also as a 20:1 mixture of diastereomers. In the ^1H n.m.r. spectrum (D_2O) of the latter compound, the relative intensity of the resonances at δ 4.20 and 4.37, for the α-hydrogen of the glycine residue in the *cis*- and *trans*-isomers, respectively,[14] showed the *cis*-diastereomer to be predominant. Through correlation, the major diastereomer of the diacyldiketopiperazine derivative (**6a**) can also be assigned the *cis*-stereochemistry.

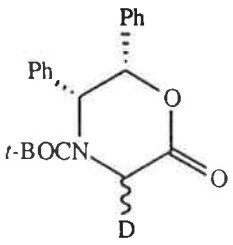
Although the relative stereochemistry of the bromide (**4**) was not separately determined, it is likely that the bromine is incorporated *trans* to the isopropyl substituent. The conversion of the bromide (**4**) to the deuteride (**6a**) would then involve an inversion of configuration.

Treatment of the bromide (**4**) with tributyltin deuteride[5] gave the deuteriated diketopiperazine (**6a**), with 85% deuterium incorporation, as a 2:1 mixture of the *trans*- and *cis*-diastereomers. The predominance of the *trans*-isomers of the diketopiperazines (**5a**) and (**6a**), in the reactions of the bromide (**4**) with allyltributyltin and tributyltin deuteride, respectively, can be attributed to a preference for delivery of deuterium and the allyl group *trans* to the isopropyl group in the radical derived by bromine atom transfer from the halide (**4**).

It is noteworthy that, by analogy with the present work, the palladium-catalysed reaction of the bromide (**7**) with deuterium has been found to be highly stereoselective, affording mainly the deuteride (**8**) through inversion of configuration.[2] The reaction of the bromide (**7**) with tributyltin deuteride was much less stereoselective and gave mainly the deuteride (**8**) resulting from retention of configuration.[2]



(7)



(8)

The synthesis of the bromide (**4**) and its subsequent reactions to give the allylglycine derivative (**5a**) and the deuteriated diketopiperazine (**6a**) illustrate the high degree of diastereoselectivity that can be expected in the elaboration of α-haloglycine derivatives in cyclic dipeptides. On this basis, the bromide (**4**) is likely to have considerable utility as a template for the asymmetric synthesis of amino acid derivatives. In this regard it should be noted that both *(R)*- and *(S)*-valine are inexpensive and readily available, therefore the approach delineated above should prove suitable for the α-substitution of glycine residues to give whichever enantiomer of the product is desired.

Acknowledgement: This work was supported by a grant from the Australian Research Council.

References and Notes

1. For examples see: Kober, R.; Papadopoulos, K.; Miltz, W.; Enders, D.; Steglich, W.; Reuter, H.; Puff, H. *Tetrahedron* **1985**, *41*, 1693; Emert, P.; Meyer, J.; Stucki, C.; Schneebeli, J.; Obrecht, J.-P. *Tetrahedron Lett.* **1988**, *29*, 1265; Bclokon, Y. N.; Popkov, A. N.; Chernoglazova, N. I.; Saporovskaya, M. B.; Bakhmutov, V. I.; Belikov, V. M. *J. Chem. Soc., Chem. Commun.* **1988**, 1336; Hamon, D. P. G.; Razzino, P.; Massy-Westropp, R. A. *J. Chem. Soc., Chem. Commun.* **1991**, 332; Hamon, D. P. G.; Razzino, P.; Massy-Westropp, R. A. *J. Chem. Soc., Chem. Commun.* **1991**, 722; Sinclair, P. J.; Zhai, D.; Reibenspies, J.; Williams, R. M. *J. Am. Chem. Soc.* **1986**, *108*, 1103; Williams, R. M.; Sinclair, P. J.; Zhai, D.; Chen, D. *J. Am. Chem. Soc.* **1988**, *110*, 1547; Williams, R. M.; Sinclair, P. J.; Zhai, W. *J. Am. Chem. Soc.* **1988**, *110*, 482; Zhai, D.; Zhai, W.; Williams, R. M. *J. Am. Chem. Soc.* **1988**, *110*, 2501.
2. Williams, R. M.; Zhai, D.; Sinclair, P. J. *J. Org. Chem.* **1986**, *51*, 5021.
3. For a review see: Williams, R. M. *Synthesis of Optically Active α-Amino Acids, Organic Chemistry Series, Vol. 7*, Baldwin, J. E.; Magnus, P. D. Eds., Pergamon Press, 1989.
4. Easton, C. J.; Hay, M. P. *J. Chem. Soc., Chem. Commun.* **1986**, 55; Burgess, V. A.; Easton, C. J.; Hay, M. P. *J. Am. Chem. Soc.* **1989**, *111*, 1047.
5. Easton, C. J.; Scharfbillig, I. M.; Tan, E. W. *Tetrahedron Lett.* **1988**, *29*, 1565.
6. Schollkopf, U.; Neubauer, H.-J.; Hauptreif, M. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 1066; Schollkopf, U.; Gruttner, S.; Anderskewitz, R.; Egert, E.; Dyrbusch, M. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 683.
7. All new compounds were fully characterized.
8. Initial reactions and the X-ray crystallographic structure determination of the diketopiperazine (**5b**) were performed using racemic materials.
9. Molecular structure of the diketopiperazine (**5b**): monoclinic space group $P2_1/c$, a = 8.778(2), b = 15.416(1), c = 10.012(3) Å, β = 109.24(1)°, R = 0.050 for 1068 reflections.
10. Kessler, H.; Molter, M. *Angew. Chem., Int. Ed. Engl.* **1973**, *12*, 1011; Kessler, H.; Molter, M. *J. Am. Chem. Soc.* **1976**, *98*, 5969.
11. Kanmera, T.; Lee, S.; Aoyagi, H.; Izumiya, N. *Int. J. Peptide Protein Res.* **1980**, *16*, 280.
12. On this basis the e.e. was deemed to be >95%.
13. Fujinaga, M.; Matsushima, Y. *Bull. Chem. Soc. Jpn.* **1966**, *39*, 185.
14. Davies, D. B.; Khaled, M. A. *J. Chem. Soc., Perkin Trans. 2* **1976**, 1238.

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Substituent Effects and Chiral Discrimination in the Complexation of Benzoic, 4-Methylbenzoic and (*RS*)-2-Phenylpropanoic Acids and their Conjugate Bases by β-Cyclodextrin† and 6^A-Amino-6^A-deoxy-β-cyclodextrin in Aqueous Solution: Potentiometric Titration and ^1H Nuclear Magnetic Resonance Spectroscopic Study

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A potentiometric titration study in aqueous solution (I = 0.10 mol dm^{-3}, KCl) of the complexation of benzoic, 4-methylbenzoic and (*RS*)-2-phenylpropanoic acids (HA) and their conjugate bases (A^-) with β-cyclodextrin, βCD, and its substituted analogue, 6^A-amino-6^A-deoxy-β-cyclodextrin, $\beta CDNH_2$, in which a primary hydroxy group is replaced by an amino group which may be protonated to produce a singly charged species, $\beta CDNH_3^+$, is reported. At 298.2 K the stability constants for the complexes have the values (in dm^3 mol^{-1}) shown in parentheses: benzoic acid · βCD (K_{1HA} = 590 ± 60); benzoate · βCD (K_{1A} = 60 ± 10); benzoic acid · $\beta CDNH_3^+$ (K_{2HA} = 340 ± 30); benzoate · $\beta CDNH_3^+$ (K_{2A} = 120 ± 20); benzoate · $\beta CDNH_2$ (K_{3A} = 50 ± 20); 4-methylbenzoic acid · βCD (K_{1HA} = 1680 ± 90); 4-methylbenzoate · βCD (K_{1A} = 110 ± 1); 4-methylbenzoic acid · $\beta CDNH_3^+$ (K_{2HA} = 910 ± 20); 4-methylbenzoate · $\beta CDNH_3^+$ (K_{2A} = 330 ± 20); and 4-methylbenzoate · $\beta CDNH_2$ (K_{3A} = 100 ± 20). These data indicate that for a given cyclodextrin the guest carboxylic acids form complexes of higher stability than do their conjugate base analogues, and that $\beta CDNH_3^+$ forms more stable complexes with the conjugate bases than do βCD and $\beta CDNH_2$. These trends are also observed for the complexation of (*RS*)-2-phenylpropanoic acid and (*RS*)-2-phenylpropanoate where the complexes indicated are characterised by the stability constants (in dm^3 mol^{-1}) shown in parentheses: (*RS*)-2-phenylpropanoic acid · βCD (K_{1RHA} = 1090 ± 30, K_{1SHA} = 1010 ± 40); (*RS*)-2-phenylpropanoate · βCD (K_{1RA} = 63 ± 8, K_{1SA} = 52 ± 5); (*RS*)-2-phenylpropanoic acid · $\beta CDNH_3^+$ (K_{2RHA} = 580 ± 20, K_{2SHA} = 480 ± 10); (*RS*)-2-phenylpropanoate · $\beta CDNH_3^+$ (K_{2RA} = 150 ± 8, K_{2SA} = 110 ± 10); and (*RS*)-2-phenylpropanoate · $\beta CDNH_2$ (K_{3RA} = 36 ± 6, K_{3SA} = 13 ± 7). These data also show that while K_{1RHA} and K_{1SHA}, and K_{1RA} and K_{1SA} are indistinguishable for (*RS*)-2-phenylpropanoic acid · βCD and (*RS*)-2-phenylpropanoate · βCD, chiral discrimination is indicated by $K_{2RHA} > K_{2SHA}$ for (*RS*)-2-phenylpropanoic acid · $\beta CDNH_3^+$, $K_{2RA} > K_{2SA}$ for (*RS*)-2-phenylpropanoate · $\beta CDNH_3^+$, and $K_{3RA} > K_{3SA}$ for (*RS*)-2-phenylpropanoate · $\beta CDNH_2$. The ^1H NMR spectra of the methyl groups of the enantiomers of (*RS*)-2-phenylpropanoic acid appear as two separate doublets, indicating chiral discrimination when complexed by βCD or $\beta CDNH_3^+$, but such chiral discrimination is not observed for (*RS*)-2-phenylpropanoate when complexed by $\beta CDNH_3^+$. The implications of these observations are discussed.

The ability of the chiral α-1,4-linked cyclic oligomers of D-glucopyranose, or cyclodextrins (CDs), to act as host species in the formation of inclusion complexes with a wide range of guest species is well established.[1–9] Because CDs exist only as D enantiomers, two diastereomeric complexes are formed with racemic guest species, which may result in distinct NMR spectra being observed for each of the (*R*) and (*S*) guest enantiomers in solution,[10–12] partial resolution of racemic guests through preferential precipitation of one diastereomeric complex[13–15] and the chromatographic separation of enantiomers on columns where the stationary phase consists of CDs bonded to silica.[16–18] More recently the effect of substitution of CD hydroxy groups by other moieties on the complexation characteristics of CDs has become an area of active study as a consequence of the modification of complexation characteristics and solubilities of CDs which can result from such substitution.[19–24] We are particularly interested in the influence of such substitution, and the effect of substituent charge, on the complexation process and chiral discrimination between enantiomeric guests; an area which has not previously been subjected to systematic study. Accordingly we have selected β-cyclodextrin, βCD, and 6^A-amino-6^A-deoxy-β-cyclodextrin, $\beta CDNH_2$, in which a primary hydroxy group of βCD is replaced by an amino group which may be protonated to produce a positively charged species, $\beta CDNH_3^+$,[20,21] to examine the effect of substitution and charge on the complexation and chiral discrimination characteristics of βCD. The guest species, benzoic acid, 4-methylbenzoic acid, (*RS*)-2-phenylpropanoic acid, and their conjugate bases provide convenient conjugate acid–base pairs to test the effect of changing the guest charge from neutral to negative on complexation by these three CD hosts.

Experimental

βCD (Sigma) and $\beta CDNH_2$, prepared as in the literature,[20,21] were dried to constant weight and stored over P_2O_5 in a vacuum desiccator prior to use. The carboxylic acids (Sigma) were used as received. The enantiomeric purities of (*R*)- and (*S*)-2-phenylpropanoic acid were determined to be >97.5% and >98.5%, respectively, after HPLC analysis of their diastereomeric amides formed with (*S*)-1-phenylethylamine. These purity limits were used in calculations of error limits of the stability constants characterising the complexation of these enantiomers by CDs. Deionised

† Cycloheptamaltaose.

water was purified with a MilliQ-Reagent system to produce water with a specific resistance of >15 MΩ cm, which was then boiled to remove CO_2. All solutions were prepared from this water, and were 0.10 mol dm^{-3} in KCl which acted as the supporting electrolyte. Titrations were performed using a Metrohm Dosimat E665 titrimator, an Orion SA 720 potentiometer and an Orion 8103 Ross combination pH electrode which was filled with 0.10 mol dm^{-3} KCl and calibrated before use with appropriate buffer solutions. Throughout a titration a stream of fine nitrogen bubbles (previously passed through aqueous 0.10 mol dm^{-3} KCl) was passed through the titration solution which was magnetically stirred and thermostatted at 298.2 ± 0.1 K in a water-jacketted titration vessel which was closed to the atmosphere with the exception of a vent which permitted egress of the nitrogen stream. A pK_w value was determined by titration of 1.0×10^{-2} mol dm^{-3} HCl (8.0 or 2.0 cm^3) with standardised 5.0×10^{-2} mol dm^{-3} NaOH. The pK_a values of the carboxylic acids and $\beta CDNH_3^+$ were determined by titration of 2×10^{-3} mol dm^{-3} aqueous solutions (8.0 or 2.0 cm^3) with standardised 5.0×10^{-2} mol dm^{-3} NaOH.

To determine the stability constants for the complexation of a guest carboxylic acid and its conjugate base by βCD, the burette contained a solution of 1.5×10^{-2} mol dm^{-3} βCD at pH 7. The pH of each 2×10^{-3} mol dm^{-3} carboxylic acid–carboxylate solution (2.0 cm^3) in the titration vessel was adjusted to a value near the pK_a of the carboxylic acid. Up to 3 cm^3 of βCD solution were titrated into the carboxylic acid–carboxylate solutions in increments not greater than 0.05 cm^3, and the observed pH increased by 0.4–0.9 pH units in total, depending on the carboxylic acid being studied. At least three similar titrations, with starting pHs in the range 3.9–4.8, were performed for each carboxylic acid system studied.

To determine the stability constants for the complexation of 4-methylbenzoic and (*R*)- and (*S*)-2-phenylpropanoic acids and their conjugate bases by $\beta CDNH_3^+$, the burette contained a solution of 1.6×10^{-2} mol dm^{-3} $\beta CDNH_3^+$ at pH 6. The pH of each 2×10^{-3} mol dm^{-3} carboxylic acid–carboxylate solution (2.0 cm^3) in the titration vessel was adjusted to a value near the pK_a of the carboxylic acid. Up to 3 cm^3 of $\beta CDNH_3^+$ solution were titrated into the carboxylic acid–carboxylate solutions in increments not greater than 0.05 cm^3, and the pH increased by *ca.* 0.4 pH units in total. At least three similar titrations, with starting pHs in the range 3.9–4.5, were performed for each carboxylic acid system studied. To determine the stability constants for the complexation of benzoic acid and benzoate by $\beta CDNH_3^+$, the burette contained a solution of 6.0×10^{-3} mol dm^{-3} benzoic acid–benzoate at pH 4. The pH of each 5×10^{-3} mol dm^{-3} $\beta CDNH_3^+$ solution (2.0 cm^3) in the titration vessel was adjusted to a value near pH 4. Up to 1 cm^3 of benzoic acid–benzoate solution was titrated into the $\beta CDNH_3^+$ solution in increments of 0.01 cm^3, and the observed pH increased by *ca.* 0.3 pH units in total. At least three similar titrations, with starting pHs in the range 3.5–4.5, were performed.

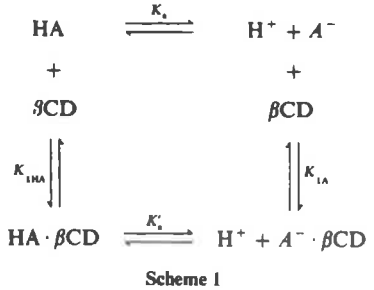
To determine the stability constants for the complexation of a guest carboxylate with $\beta CDNH_3^+$ and its conjugate base $\beta CDNH_2$, the burette contained a solution of 0.01–0.02 mol dm^{-3} carboxylate at pH 7. The pH of each 2×10^{-3} mol dm^{-3} $\beta CDNH_2$ solution (2.0 cm^3) in the titration vessel was adjusted to a value near the pK_a of $\beta CDNH_3^+$. Up to 3 cm^3 of carboxylate solution were added to the $\beta CDNH_3^+$–$\beta CDNH_2$ solutions in increments not greater than 0.05 cm^3, and the pH increased by 0.1–0.3 pH units, depending on the carboxylate being studied. At least three similar titrations, with starting pHs in the range 8.2–8.8, were performed for each carboxylate system studied.

^1H NMR spectra were run in D_2O solution in 5 mm diameter NMR tubes on a Bruker ACP 300 spectrometer. The sweep width was 4000 Hz and typically 100 transients were collected prior to Fourier transformation. Chemical shifts were measured from external sodium 3-trimethylsilylpropane-1-sulfonate (TPS) in D_2O. Solutions of βCD or $\beta CDNH_3^+$ and (*RS*)-2-phenylpropanoic acid were adjusted to pH 1 with DCl–D_2O, and solutions of $\beta CDNH_3^+$ and (*RS*)-2-phenylpropanoate were buffered at pH 6.4 with phosphate buffer (0.20 mol dm^{-3}) made up in D_2O. The concentration of βCD or $\beta CDNH_3^+$ was in the range $(1.0–9.0) \times 10^{-2}$ mol dm^{-3}, and that of (*RS*)-2-phenylpropanoic acid or (*RS*)-2-phenylpropanoate was in the range 1.0×10^{-3}–1.0×10^{-2} mol dm^{-3} so that ≥90% of the guest species was complexed. When separate resonances were observed in the (*RS*)-2-phenylpropanoic acid systems for the guest enantiomers in the two diastereomers, the resonances were assigned by adding resolved (*R*)-2-phenylpropanoic acid to the solution and observing which resonance increased in intensity. The $\beta CDNH_3^+$ and (*RS*)-2-phenylpropanoate solutions were prepared at the higher end of the concentration scales indicated above, which together with the high phosphate buffer concentration, may result in an increased solution viscosity which may explain the broader ^1H resonances observed in these solutions.

Results and Discussion

The complexation of a carboxylic acid, HA, and its conjugate base, A^-, by βCD may be expressed as in Scheme 1, where the acidity constant, K_a, characterises the carboxylic acid, K_{1HA} and K_{1A} are the stability constants for the complexation of HA and A^-, respectively, by βCD, and K'_a characterises HA in the HA · βCD complex.

The variation of the pH of a benzoic acid–benzoate solution in the vicinity of the pK_a ($\equiv -\log K_a$) of benzoic acid as it was titrated with βCD solution (Fig. 1), arises because



Scheme 1

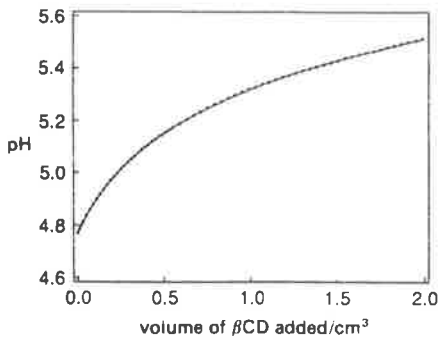
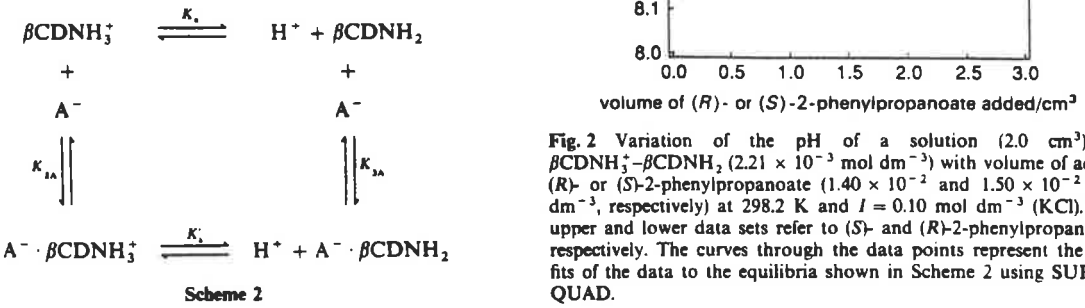


Fig. 1 Variation of the pH of a solution (2.0 cm^3) of benzoic acid–benzoate (1.04×10^{-3} mol dm^{-3}) with volume of added βCD (1.51×10^{-2} mol dm^{-3}) at 298.2 K and $I = 0.10$ mol dm^{-3} (KCl). The curve through the data points represents the best fit of the data to the equilibria shown in Scheme 1 using SUPERQUAD.

$pK_a \neq pK'_a$ or $K_{1HA} \neq K_{1A}$ (and analogous inequalities hold for the other titrations discussed herein). The best fit of the data to the expressions for K_{1HA} and K_{1A}, employing the independently determined value of K_a, using program SUPERQUAD[25] yields the curve through the data points in Fig. 1. (The value of K'_a was subsequently calculated from these three values.) Similar curves were obtained for the titration of 4-methylbenzoic acid–4-methylbenzoate and (*R*)- and (*S*)-2-phenylpropanoic acid–phenylpropanoate by βCD, and the data were fitted in a similar manner. The pK_a and pK'_a values for benzoic, 4-methylbenzoic and (*R*)- and (*S*)-2-phenylpropanoic acid appear in Table 1, as do the K_{1HA} and K_{1A} values for the complexation of these carboxylic acids and their conjugate bases by βCD.

The complexation of a guest carboxylate (A^-) by $\beta CDNH_3^+$ and its conjugate base, $\beta CDNH_2$, may be expressed as in Scheme 2, where K_a is the acidity constant of $\beta CDNH_3^+$, K_{2A} and K_{3A} are the stability constants for the complexation of A^- by $\beta CDNH_3^+$ and $\beta CDNH_2$, respectively, and pK'_a characterises $\beta CDNH_3^+$ in the $A^- \cdot \beta CDNH_3^+$ complex.

The variations of the pH of solutions of $\beta CDNH_3^+$–$\beta CDNH_2$ in the vicinity of the pK_a of $\beta CDNH_3^+$ as they were titrated with a solution of either (*R*)- or (*S*)-2-phenylpropanoate are shown in Fig. 2. The best fit of the data to the expressions for K_{2A} and K_{3A}, employing the independently determined value of K_a, using program SUPERQUAD[25] yields the curve through the data points in Fig. 2. (The value of K'_a was subsequently calculated from these three values.) Similar curves were obtained for the titration of $\beta CDNH_3^+$–$\beta CDNH_2$ by benzoate and 4-methylbenzoate, and the data were similarly fitted. The pK_a and pK'_a values for $\beta CDNH_3^+$ appear in Table 1, and the K_{2A} and K_{3A} values for the complexation of benzoate, 4-methylbenzoate, and (*R*)- and (*S*)-2-phenylpropanoate by $\beta CDNH_3^+$ and $\beta CDNH_2$ also appear in Table 1.



Scheme 2

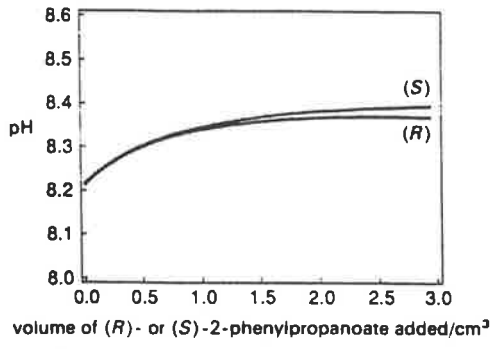
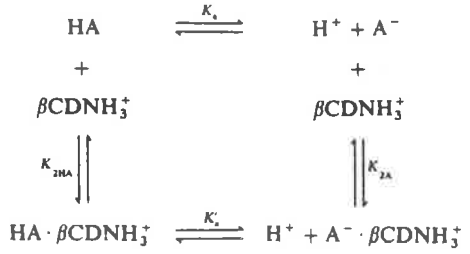


Fig. 2 Variation of the pH of a solution (2.0 cm^3) of $\beta CDNH_3^+$–$\beta CDNH_2$ (2.21×10^{-3} mol dm^{-3}) with volume of added (*R*)- or (*S*)-2-phenylpropanoate (1.40×10^{-2} and 1.50×10^{-2} mol dm^{-3}, respectively) at 298.2 K and $I = 0.10$ mol dm^{-3} (KCl). The upper and lower data sets refer to (*S*)- and (*R*)-2-phenylpropanoate, respectively. The curves through the data points represent the best fits of the data to the equilibria shown in Scheme 2 using SUPERQUAD.

Table 1 K, pK_a and pK'_a values for cyclodextrin complexes, cyclodextrins and guest species at $I = 0.10$ mol dm^{-3} (KCl) and 298.2 K

species	K[a]/dm^3 mol^{-1}	pK_a[b]	pK'_a[c]
benzoic acid		4.06 ± 0.04	
benzoic acid · βCD	K_{1HA} = 590 ± 60		5.1 ± 0.1
benzoate · βCD	K_{1A} = 60 ± 10		
$\beta CDNH_3^+$		8.49 ± 0.01[d]	
benzoic acid · $\beta CDNH_3^+$	K_{2HA} = 340 ± 30		4.5 ± 0.1
benzoate · $\beta CDNH_3^+$	K_{2A} = 120 ± 20		8.9 ± 0.2
benzoate · $\beta CDNH_2$	K_{3A} = 50 ± 20		
4-methylbenzoic acid		4.20 ± 0.08	
4-methylbenzoic acid · βCD	K_{1HA} = 1680 ± 90		5.39 ± 0.09
4-methylbenzoate · βCD	K_{1A} = 110 ± 1		
4-methylbenzoic acid · $\beta CDNH_3^+$	K_{2HA} = 910 ± 20		4.6 ± 0.1
4-methylbenzoate · $\beta CDNH_3^+$	K_{2A} = 330 ± 20		9.0 ± 0.1
4-methylbenzoate · $\beta CDNH_2$	K_{3A} = 100 ± 20		
(*R*)-2-phenylpropanoic acid		4.23 ± 0.05	
(*R*)-2-phenylpropanoic acid · βCD	K_{1RHA} = 1090 ± 30		5.47 ± 0.08
(*R*)-2-phenylpropanoate · βCD	K_{1RA} = 63 ± 8		
(*R*)-2-phenylpropanoic acid · $\beta CDNH_3^+$	K_{2RHA} = 580 ± 20		4.82 ± 0.06
(*R*)-2-phenylpropanoate · $\beta CDNH_3^+$	K_{2RA} = 150 ± 8		9.11 ± 0.08
(*R*)-2-phenylpropanoate · $\beta CDNH_2$	K_{3RA} = 36 ± 6		
(*S*)-2-phenylpropanoic acid		4.23 ± 0.05	
(*S*)-2-phenylpropanoic acid · βCD	K_{1SHA} = 1010 ± 40		5.52 ± 0.07
(*S*)-2-phenylpropanoate · βCD	K_{1SA} = 52 ± 5		
(*S*)-2-phenylpropanoic acid · $\beta CDNH_3^+$	K_{2SHA} = 480 ± 10		4.87 ± 0.07
(*S*)-2-phenylpropanoate · $\beta CDNH_3^+$	K_{2SA} = 110 ± 10		9.4 ± 0.4
(*S*)-2-phenylpropanoate · $\beta CDNH_2$	K_{3SA} = 13 ± 7		

[a] Errors quoted for K (the mean of N runs) represent the standard deviation, $\sigma = \sqrt{\{[\Sigma(K_i - K)^2]/(N-1)\}}$, where K_i is a value from a single run for the best fit of the variation of pH with added volume of cyclodextrin or carboxylic acid–carboxylate titrant obtained through SUPERQUAD, and $i = 1, 2, \ldots, N$. [b] Errors quoted for pK_a are similarly calculated for the best fit of the variation of pH with added volume of NaOH titrant obtained through SUPERQUAD. [c] Errors quoted for pK'_a represent those calculated from the propagation of errors associated with pK_a and K. [d] This pK_a observed for $\beta CDNH_3^+$ is lower than that reported in ref. 21 (8.72 ± 0.02) at a higher I (0.5 mol dm^{-3}).



Scheme 3

The complexation of HA and A^- by $\beta CDNH_3^+$ may be expressed as in Scheme 3, where K_a, K'_a, K_{2HA} and K_{2A} are constants characterising the equilibria, and whose values appear in Table 1. The variations of the pH of (*R*)- and (*S*)-2-phenylpropanoic acid–phenylpropanoate solutions in the vicinity of the pK_a of (*RS*)-2-phenylpropanoic acid as they were titrated with $\beta CDNH_3^+$ solutions are shown in Fig. 3. The best fit of the data to the expressions for K_{2HA} and K_{2A}, employing the independently determined value of K_a, using program SUPERQUAD[25] yields the curve through the data points in Fig. 3. (The value of K'_a was subsequently calculated from these three values.) Similar curves were obtained for the titration of 4-methylbenzoic acid–4-methylbenzoate by $\beta CDNH_3^+$, and for the titration of $\beta CDNH_3^+$ by benzoic acid–benzoate, and the data were similarly fitted. The pK_a and pK'_a values for benzoic, 4-methylbenzoic and (*RS*)-2-phenylpropanoic acid appear in Table 1, and the K_{2HA} and K_{2A} values for the complexation of these carboxylic acids and their conjugate bases by $\beta CDNH_3^+$ also appear in Table 1. A typical plot of the variation of species concentration with pH, calculated from data in Table 1, is shown in Fig. 4.

The formation of a CD inclusion complex involves dipolar, hydrogen bonding and van der Waals interactions to varying degrees depending on the nature of the CD and the guest species, and also solvent interactions with the CD and the guest species.[1–4,26] The general structure of the inclusion complex usually shows the aromatic moiety of the guest species to be in the annulus in the vicinity of the hydrophobic ring delineated by the ether oxygens,[27–30] with the dipole moment of the guest species aligned antiparallel to that of the CD.[31–33] The CD dipole moment is in the range 10–20 D†

† 1 D ≈ $3.335\,64 \times 10^{-30}$ C m.

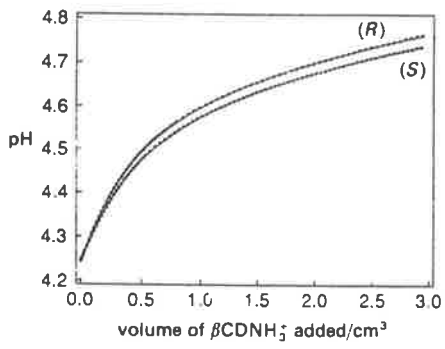


Fig. 3 Variation of the pH of a solution (2.0 cm^3) of (*R*)- or (*S*)-2-phenylpropanoic acid–phenylpropanoate (2.28×10^{-3} and 2.10×10^{-3} mol dm^{-3}, respectively) with volume of added $\beta CDNH_3^+$ (1.58×10^{-2} mol dm^{-3}) at 298.2 K and $I = 0.10$ mol dm^{-3} (KCl). The upper and lower data sets refer to (*R*)- and (*S*)-2-phenylpropanoic acid–phenylpropanoate, respectively. The curves through the data points represent the best fits of the data to the equilibria shown in Scheme 3 using SUPERQUAD.

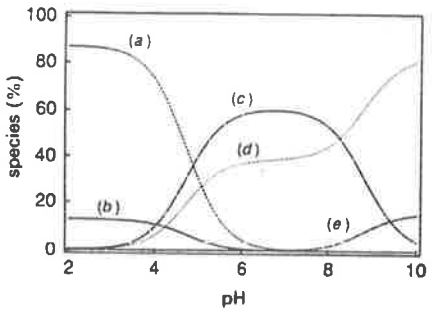


Fig. 4 Speciation plot for the $\beta CDNH_2$–$\beta CDNH_3^+$–(*S*)-2-phenylpropanoic acid–(*S*)-2-phenylpropanoate system calculated from pK_a, K_{2SHA}, K_{2SA} and K_{3SA} (Table 1). The total concentration of (*S*)-2-phenylpropanoic acid–(*S*)-2-phenylpropanoate is 10^{-3} mol dm^{-3} and the total concentration of $\beta CDNH_2$–$\beta CDNH_3^+$ is 1.5×10^{-2} mol dm^{-3}. The total concentration of (*S*)-2-phenylpropanoic acid–(*S*)-2-phenylpropanoate is defined as 100% and the free $\beta CDNH_2$–$\beta CDNH_3^+$ concentration is not shown. The curves represent: (*a*) (*S*)-2-phenylpropanoic acid · $\beta CDNH_3^+$, (*b*) (*S*)-2-phenylpropanoic acid, (*c*) (*S*)-2-phenylpropanoate · $\beta CDNH_3^+$, (*d*) (*S*)-2-phenylpropanoate and (*e*) (*S*)-2-phenylpropanoate · $\beta CDNH_2$.

with the positive and negative poles adjacent to the primary and secondary hydroxy groups delineating the narrow and wide ends of the CD annulus, respectively.[31–33] It has been observed that the carboxylic acid group of benzoic acid is in the vicinity of the primary hydroxy groups of αCD in the benzoic acid · αCD complex consistent with an antiparallel alignment of the αCD and benzoic acid dipole moments,[30] and similar antiparallel dipolar orientations are assumed in the complexes appearing in Table 1.

The magnitude of the CD inclusion complex stability constant reflects the competitive abilities of the CD to complex the guest species and water to solvate it, and accordingly the data in Table 1 indicate the changes in these abilities as both the natures of the host CD and the guest species are varied. Six distinct trends emerge from the data in Table 1 and are now discussed.

(i) For all systems the carboxylic acid complex is of higher stability than the analogous carboxylate complex. This suggests that the negative charge of the carboxylates causes a stronger solvation than in the case of the uncharged conjugate carboxylic acids with the consequence that, although the van der Waals interactions between the aromatic moieties of the guests and the hydrophobic interior of the CD annulus still result in complexation, the stronger solvation of the carboxylates results in a lower stability for their inclusion complexes.

(ii) The stabilities of the carboxylate complexes increase in the sequence $A^- \cdot \beta CDNH_2 \leqslant A^- \cdot \beta CD < A^- \cdot \beta CDNH_3^+$. This trend may be rationalised on the basis that the positive charge on $\beta CDNH_3^+$ increases its dipole moment and provides an increased electrostatic interaction with the carboxylate with a consequent increase in stability of the inclusion complex.

(iii) For a given CD complex, stability increases with the nature of the carboxylic acid in the sequence benzoic acid < (*S*)-2-phenylpropanoic acid ⩽ (*R*)-2-phenylpropanoic acid < 4-methylbenzoic acid. The areas of the hydrophobic surfaces of (*RS*)-2-phenylpropanoic acid and 4-methylbenzoic acid are greater than that of benzoic acid and they probably have more extensive van der Waals interactions with the CD as a consequence. In addition the carboxylic acid group, at which significant solvation may occur, represents a proportionately smaller part of their total surface area than is the case for benzoic acid. It is also possible that the fit of the guest species

to the CD cavity improves in the sequence benzoic acid < (*RS*)-2-phenylpropanoic acid < 4-methylbenzoic acid. The combination of these effects produces a lower stability constant characterising the benzoic acid complex.

(iv) For a given CD complex stability increases with the nature of the carboxylate in the sequence (*S*)-2-phenylpropanoate ≤ benzoate ≤ (*R*)-2-phenylpropanoate < 4-methylbenzoate. Clearly the factors discussed under (iii) apply here also, but it is evident that the increase in solvation arising from the negative carboxylate charge causes this factor to become more important, with the consequence that the stabilities of the benzoate and (*RS*)-2-phenylpropanoate complexes become comparable.

(v) Significant chiral discrimination in favour of complexation of (*R*)-2-phenylpropanoic acid and (*R*)-2-phenylpropanoate over the (*S*)-enantiomers is observed for $\beta CDNH_3^+$ and $\beta CDNH_2$, but not for βCD. The replacement of a primary hydroxy group by NH_3^+ or NH_2 increases the asymmetry of the CD annulus and accordingly $\beta CDNH_3^+$ and $\beta CDNH_2$ are more likely to discriminate between guest enantiomers through the formation of diastereomeric complexes than is βCD.

(vi) For the guest carboxylic acids and for $\beta CDNH_3^+$, $pK_a < pK_a'$, which indicates that in the inclusion complex the conjugate base is destabilised relative to the conjugate acid, by comparison with the situation in the uncomplexed state. Because of its charge the carboxylate will be more strongly solvated than the carboxylic acid, and evidently the decreased solvation resulting from inclusion in the CD annulus has a greater effect on the carboxylate with the consequence that it is destabilised by comparison to the included carboxylic acid. This has the overall effect of decreasing the acidity of the carboxylic acid. In the case of $\beta CDNH_3^+$ the decrease in acidity occurring on formation of the inclusion complex may be a consequence of disruption of the interactions between the NH_3^+ substituent and adjacent hydroxy residues and the ether linkages which are thought to confer the rather low pK_a value on $\beta CDNH_3^+$.[21] In addition, the cancellation of the $\beta CDNH_3^+$ charge by that of the included carboxylate may decrease the acidity of $\beta CDNH_3^+$.

In the presence of βCD in D_2O–DCl at pH 1, the methyl group of (*RS*)-2-phenylpropanoic acid is characterised by two ^{1}H NMR doublet resonances arising from the (*R*)-enantiomer ($\delta = 1.409$, $J_{H-H} = 6.6$ Hz) and the (*S*)-enantiomer ($\delta = 1.421$, $J_{H-H} = 6.9$ Hz), which compares with the doublet observed for (*RS*)-2-phenylpropanoic acid ($\delta = 1.289$, $J_{H-H} = 7.2$ Hz) in the absence of βCD, as seen in Fig. 5. The identification of the enantiomer resonances was made by adding (*R*)-2-phenylpropanoic acid to (*RS*)-2-phenylpropanoic acid in the presence of βCD and noting the relative increase in amplitude of the upfield doublet. In the presence of $\beta CDNH_3^+$ in D_2O–DCl at pH 1, the methyl group of (*RS*)-2-phenylpropanoic acid is characterised by two doublet resonances arising from the (*R*)-enantiomer ($\delta = 1.428$, $J_{H-H} = 6.9$ Hz) and the (*S*)-enantiomer ($\delta = 1.440$, $J_{H-H} = 6.9$ Hz) and the enantiomer resonances were identified as for the previous system. In contrast, in the presence of $\beta CDNH_3^+$ in D_2O–phosphate buffer at pH 6.4 the resonance of the methyl group of (*RS*)-2-phenylpropanoate appears as a doublet ($\delta = 1.315$, $J_{H-H} = 6.9$ Hz), which compares with the doublet observed for (*RS*)-2-phenylpropanoate ($\delta = 1.357$, $J_{H-H} = 7.2$ Hz) in the absence of $\beta CDNH_3^+$. (It was not possible to carry out similar experiments with (*RS*)-2-phenylpropanoate in the presence of βCD or $\beta CDNH_2$ as K_{1RA}, K_{1SA}, K_{3RA} and K_{3SA} are too small to give sufficient concentrations of the inclusion complexes within the solubility limits of the systems.) The observation of separate resonances for (*RS*)-2-phenylpropanoic acid in the presence of βCD and $\beta CDNH_3^+$ indicates that the magnetic environment of the methyl protons in the diastereomeric complexes is different, while the absence of separate resonances for (*RS*)-2-phenylpropanoate in the presence of $\beta CDNH_3^+$ indicates that the magnetic environment of the methyl protons in the diastereomeric complexes is not significantly different. Thus, a spectroscopic chiral discrimination occurs when (*RS*)-2-phenylpropanoic acid is complexed by either βCD or $\beta CDNH_3^+$, but not when (*RS*)-2-phenylpropanoate is complexed by $\beta CDNH_3^+$. In contrast, a significant thermodynamic chiral discrimination occurs in the complexation of both (*RS*)-2-phenylpropanoic acid and (*RS*)-2-phenylpropanoate by $\beta CDNH_3^+$, but no significant thermodynamic chiral discrimination occurs in the complexation of these guests by βCD. This demonstrates that although the titrimetric method may detect a significant thermodynamic chiral discrimination, such discrimination does not necessarily induce sufficient magnetic inequivalence in the diastereomeric complexes to be detectable by ^{1}H NMR spectroscopy. Conversely, although different diastereomeric complexes may be identified by ^{1}H NMR spectroscopy, this does not necessarily imply the existence of a significant thermodynamic chiral discrimination as the energy differences involved in the spectroscopic distinction are small.

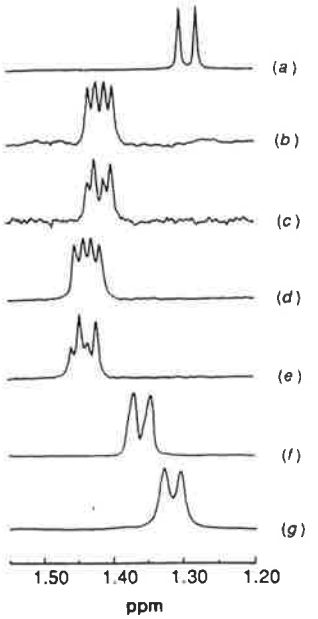


Fig. 5 ^{1}H NMR (300 M Hz) spectra of the methyl groups of: (*a*) (*RS*)-2-phenylpropanoic acid (0.005 mol dm^{-3}) at pH 1, (*b*) (*RS*)-2-phenylpropanoic acid (0.003 mol dm^{-3}) in the presence of βCD (0.011 mol dm^{-3}) at pH 1, (*c*) (*RS*)-2-phenylpropanoic acid (0.001 mol dm^{-3}) with added (*R*)-2-phenylpropanoic aicd (0.0003 mol dm^{-3}) in the presence of βCD (0.011 mol dm^{-3}) at pH 1, (*d*) (*RS*)-2-phenylpropanoic acid (0.005 mol dm^{-3}) in the presence of $\beta CDNH_3^+$ (0.03 mol dm^{-3}) at pH 1, (*e*) (*RS*)-2-phenylpropanoic acid (0.002 mol dm^{-3}) with added (*R*)-2-phenylpropanoic acid (0.002 mol dm^{-3}) in the presence of $\beta CDNH_3^+$ (0.03 mol dm^{-3}) at pH 1, (*f*) (*RS*)-2-phenylpropanoate (0.01 mol dm^{-3}) at pH 6.4, (*g*) (*RS*)-2-phenylpropanoate (0.01 mol dm^{-3}) in the presence of $\beta CDNH_3^+$ (0.09 mol dm^{-3}) at pH 6.4. The chemical shifts are downfield from TPS. The broader resonances observed in (*f*) and (*g*) are thought to be due to the higher viscosity of these solutions.

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References

1 M. L. Bender and M. Komiyama, *Cyclodextrin Chemistry*, Springer, New York, 1977.
2 J. Szejtli, *Cyclodextrins and their Inclusion Complexes*, Akademiai Kiado, Budapest, 1982.
3 W. Saenger, *Inclusion Compounds*, 1984, **2**, 231.
4 R. J. Clarke, J. H. Coates and S. F. Lincoln, *Adv. Carbohyd. Chem. Biochem.*, 1989, **46**, 205.
5 I. M. Brereton, T. M. Spotswood, S. F. Lincoln and E. H. Williams, *J. Chem. Soc., Faraday Trans. 1*, 1984, **80**, 3147.
6 D. L. Pisaniello, S. F. Lincoln and J. H. Coates, *J. Chem. Soc., Faraday Trans. 1*, 1985, **81**, 1247.
7 S. F. Lincoln, A. M. Hounslow, J. H. Coates and B. G. Doddridge, *J. Chem. Soc., Faraday Trans. 1*, 1987, **83**, 2697.
8 S. F. Lincoln, A. M. Hounslow, J. H. Coates, R. P. Villani and R. L. Schiller, *J. Inclusion Phenom.*, 1988, **6**, 183.
9 S. E. Brown, J. H. Coates, C. J. Easton, S. F. Lincoln, Y. Luo and A. K. W. Stephens, *Aust. J. Chem.*, 1991, **44**, 855.
10 D. Greatbanks and R. Pickford, *Magn. Reson. Chem.*, 1987, **25**, 208.
11 N. J. Smith, T. M. Spotswood and S. F. Lincoln, *Carbohydr. Res.*, 1989, **192**, 9.
12 S. E. Brown, J. H. Coates, S. F. Lincoln, D. R. Coghlan and C. J. Easton, *J. Chem. Soc., Faraday Trans.*, 1991, **87**, 2699.
13 F. Cramer and W. Dietsche, *Chem. Ber.*, 1959, **92**, 378.
14 M. Mikolajczyk, J. Drabowicz and F. Cramer, *J. Chem. Soc., Chem. Commun.*, 1971, 317.
15 M. Mikolajczyk and J. Drabowicz, *J. Am. Chem. Soc.*, 1978, **100**, 2510.
16 W. L. Hinze, T. E. Rieh, D. W. Armstrong, W. DeMond, A. Alak and T. Ward, *Anal. Chem.*, 1985, **57**, 237.
17 D. W. Armstrong, T. Ward, R. D. Armstrong and T. E. Beesley, *Science*, 1986, **232**, 1132.
18 N. A. Karnik, R. J. Prankerd and J. H. Perrin, *Chirality*, 1991, **3**, 124.
19 R. P. Villani, S. F. Lincoln and J. H. Coates, *J. Chem. Soc., Faraday Trans. 1*, 1987, **83**, 2751.
20 S. F. Lincoln, J. H. Coates, C. J. Easton, S. J. van Eyk, B. L. May, P. Singh, M. A. Stile and M. L. Williams, *Int. Pat. Appl.*, WO 90/02141.
21 S. E. Brown, J. H. Coates, D. R. Coghlan, C. J. Easton, S. J. van Eyk, W. Janowski, A. Lepore, S. F. Lincoln, Y. Luo, B. L. May, D. S. Schiesser, P. Wang and M. L. Williams, *Aust. J. Chem.*, in the press.
22 J. Pitha, J. Milecki, H. Fales, L. Pennell and K. Uekama, *Int. J. Pharm.*, 1986, **29**, 73.
23 D. Duchêne, *New Trends in Cyclodextrins and Derivatives*, Editions de Santé, Paris, 1991.
24 A. K. Yatsimirsky and A. V. Eliseev, *J. Chem. Soc., Perkin Trans. 2*, 1991, 1769.
25 P. Gans, A. Sabatini and A. Vacca, *J. Chem. Soc., Dalton Trans.*, 1985, 1195.
26 R. I. Gelb, L. M. Schwartz, B. Cardelino, H. S. Fuhrman, R. F. Johnson and D. A. Laufer, *J. Am. Chem. Soc.*, 1981, **103**, 1750.
27 K. Harata, *Bull. Chem. Soc. Jpn.*, 1975, **48**, 2409.
28 K. Harata, *Bull. Chem. Soc. Jpn.*, 1976, **49**, 1493.
29 K. Harata, *Bull. Chem. Soc. Jpn.*, 1976, **49**, 2066.
30 K. Harata, *Bull. Chem. Soc. Jpn.*, 1977, **50**, 1416.
31 M. Kitagawa, H. Hoshi, M. Sakurai, Y. Inoue and R. Chûjô, *Carbohyd. Res.*, 1987, **163**, c1.
32 M. Sakurai, M. Kitagawa, H. Hoshi, Y. Inoue and R. Chûjô, *Chem. Lett.*, 1988, 895.
33 M. Sakurai, M. Kitigawa, H. Hoshi, Y. Inoue and R. Chûjô, *Carbohydr. Res.*, 1990, **198**, 181.

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Synthesis and Properties of 6^A-Amino-6^A-deoxy-α- and -β-cyclodextrin

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Abstract

The monotosylates obtained by treatment of α- and β-cyclodextrin with *p*-methylbenzenesulfonyl chloride reacted with ammonia to give the title compounds. These amines are of unusually low basicity, with pK_a values of 8·70 and 8·72, respectively. In water at 25°, the hydrochloride salt of the amine derived from β-cyclodextrin is approximately 40 times more soluble than β-cyclodextrin and, through complexation, the salt increases the solubility of Nabumetone over 800 times.

Introduction

The ability of the cyclodextrins (1a–c) and their derivatives to act as host molecules in the formation of inclusion complexes is well established.[1–9] As part of a program[10,11] aimed to exploit this behaviour in the administration of pharmaceuticals, we have studied inclusion complexes of a range of modified cyclodextrins. The low solubility of β-cyclodextrin (1b) in water, which is restricted

[1] Bender, M. L., and Komiyama, M., 'Cyclodextrin Chemistry' (Springer: Berlin 1978).
[2] Szejtli, J., 'Cyclodextrins and Their Inclusion Complexes' (Akademio Kaido: Budapest 1982).
[3] Saenger, W., *Inclusion Compd.*, 1984, **2**, 231.
[4] Clarke, R. J., Coates, J. H., and Lincoln, S. F., *Adv. Carbohydr. Chem. Biochem.*, 1989, **46**, 205.
[5] Brereton, I. M., Spotswood, T. M., Lincoln, S. F., and Williams, E. H., *J. Chem. Soc., Faraday Trans. 1*, 1984, **80**, 3147.
[6] Pisaniello, D. L., Lincoln, S. F., and Coates, J. H., *J. Chem. Soc., Faraday Trans. 1*, 1985, **81**, 1247.
[7] Lincoln, S. F., Hounslow, A. M., Coates, J. H., and Doddridge, B. G., *J. Chem. Soc., Faraday Trans. 1*, 1987, **83**, 2697.
[8] Lincoln, S. F., Hounslow, A. M., Coates, J. H., Villani, R. P., and Schiller, R. L., *J. Inclusion Phenom.*, 1988, **6**, 183.
[9] Brown, S. E., Coates, J. H., Easton, C. J., Lincoln, S. F., Luo, Y., and Stephens, A. K. W., *Aust. J. Chem.*, 1991, **44**, 855.
[10] Lincoln, S. F., Coates, J. H., Easton, C. J., van Eyk, S. J., May, B. L., Singh, P., Williams, M. L., and Stile, M. A., International Pat. Appl. PCT/AU89/00359–WO90/02141.
[11] Coates, J. H., Easton, C. J., Lincoln, S. F., van Eyk, S. J., May, B. L., Williams, M. L., Brown, S. E., Lepore, A., Liao, M.-L., Luo, Y., Macolino, V., Schiesser, D. S., Singh, P., Whalland, C. B., and McKensie, I., International Pat. Appl. PCT/AU91/00071–WO91/13100.

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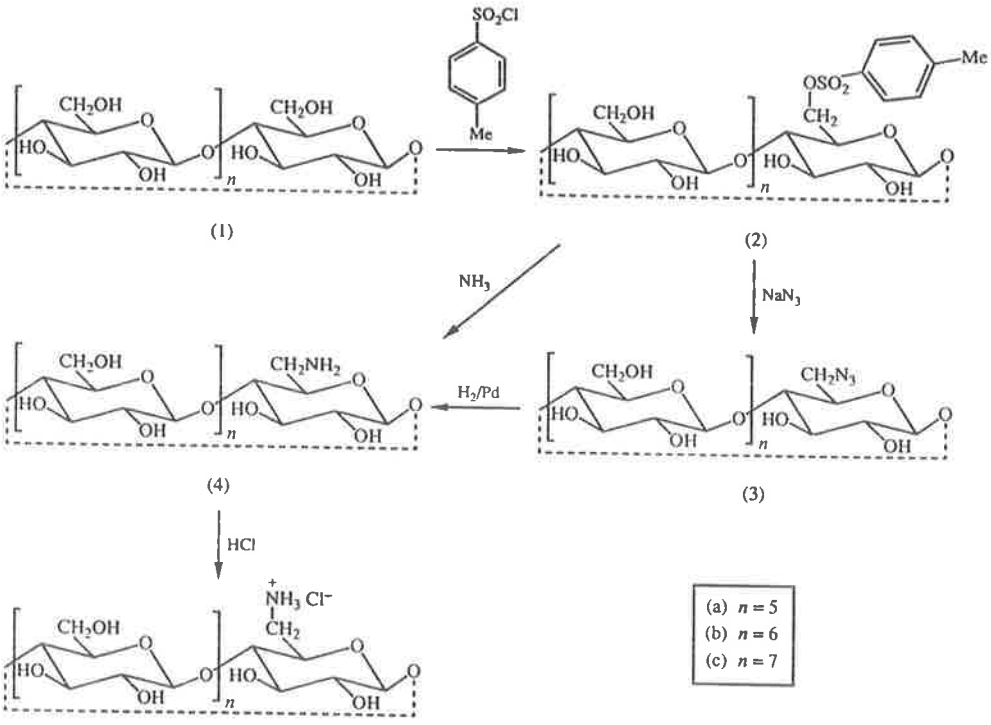
to 1·85 g/100 ml at 25°,[2] is a principal limitation to the study and application of inclusion complexes involving this compound. Derivatives of β-cyclodextrin with enhanced solubility are therefore of particular interest. To be suitable for pharmaceutical applications, the modified cyclodextrins must also be discrete chemical entities, easily obtained pure on a large scale.

At the outset of our work, there had been one report[12] of the synthesis of 6^A-amino-6^A-deoxy-α-cyclodextrin (4a), via the azide (3a) (Scheme 1). A number of groups[13–16] had used a similar approach to obtain the corresponding β-cyclodextrin derivative (4b), although full details were only reported[16] during the course of the present investigation. We set out to obtain the amines (4a,b) in order to study their properties and those of their inclusion complexes, and as starting materials for the synthesis of other modified cyclodextrins. We found that samples obtained in the reported manner were contaminated with residual inorganic azide used to form the azides (3a,b), and with the palladium catalyst used in the reduction of the azides (3a,b) to the corresponding amines (4a,b). In addition, these synthetic procedures are undesirable for large-scale application



Scheme 1

[12] Melton, L. D., and Slessor, K. N., *Carbohydr. Res.*, 1971, **18**, 29.
[13] Ueno, A., Suzuki, I., and Osa, T., *Makromol. Chem., Rapid Commun.*, 1987, **8**, 131.
[14] Nagamine, Y., Sumikawa, M., Omichi, K., and Ikenaka, T., *J. Biochem.*, 1987, **102**, 767.
[15] Ikeda, T., Kojin, R., Yoon, C. J., Ikeda, H., and Iijima, M., *J. Inclusion Phenom.*, 1987, **5**, 93.
[16] Petter, R. C., Salek, J. S., Sikorski, C. T., Kumaravel, G., and Lin, F.-T., *J. Am. Chem. Soc.*, 1990, **112**, 3860.

because of the use of sodium azide and hydrogen under pressure. Consequently, we have developed an alternative method for the synthesis of the amines (4a,b), in pure form, directly from the corresponding tosylates (2a,b). In this report, we describe that synthetic procedure and we give details of the unusual basicity of the amines (4a,b). We report the high solubility of the amino-β-cyclodextrin hydrochloride salt (5b), relative to that of the parent (1b), and we discuss the use of that salt to improve the solubility of pharmaceuticals.

Results and Discussion

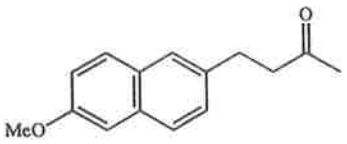
The tosylates (2a,b) were synthesized by a variation of the method of Melton and Slessor[12] for the synthesis of the α-cyclodextrin derivative (2a). Aqueous ammonia was used in an initial attempt to convert the tosylates (2a,b) directly into the corresponding amines (4a,b); however, the tosylates (2a,b) were susceptible to hydrolysis, particularly in the former case. Treatment of the tosylates (2a,b) with anhydrous saturated solutions of ammonia in pyridine or *N,N*-dimethylformamide gave the corresponding amines (4a,b) without hydrolysis but, at atmospheric pressure and room temperature, more than 50% of each of the respective starting materials (2a,b) remained after 2 weeks. Finally, solutions of the tosylates (2a,b) in *N,N*-dimethylformamide were treated with ammonia in a pressure reactor, at room temperature for 18 h, to give the amines (4a,b).

The amines (4a,b) were completely characterized. In particular, the regiospecificity of incorporation of the amino group was confirmed by proton-coupled ^{13}C n.m.r. spectroscopy. The α-cyclodextrin derivative (4a) displayed a triplet at δ 42·8, characteristic for $C6^A$, while the corresponding signal for the β-cyclodextrin derivative (4b) was observed at δ 42·1. As part of the characterization process, the amine (4a) was found to have a pK_a of 8·70±0·02 ($I = 0·500$, 298·2 K), while the β-cyclodextrin derivative (4b) was determined to have a pK_a of 8·72±0·02 under the same conditions. This value for the β-cyclodextrin derivative (4b) is well outside the range of 7·5–8·2 reported[16] during the current work and there is no obvious explanation for the discrepancy. Nevertheless it is clear that both of the compounds (4a,b) have unusually low pK_a values for primary amines. By way of comparison, the pK_a of ethylamine is 10·66, while that of butylamine is 10·70.[17] It may be that the low basicity of the amines (4a,b) results, in each case, from the effect of the hydrophobic cyclodextrin annulus adjacent to the amino group, to limit the extent of stabilization of the protonated form through solvation. Alternatively, hydroxyl residues and the ether linkage in the vicinity of the amino group may affect the pK_a. By analogy, 2-aminoethanol, with a pK_a of 9·62, and 2-methoxyethylamine, with a pK_a of 9·30,[17] are each considerably less basic than ethylamine.

The amine hydrochloride salt (5b) was prepared by titration of an aqueous solution of the amine (4b) with hydrogen chloride. The solubility of the salt (5b) in water at 25° was found to be 70·5 g/100 ml, which is substantially higher than that of either the free base (4b) (3·75 g/100 ml) or the parent cyclodextrin (1b) (1·85 g/100 ml). On this basis, the salt (5b) is particularly suitable for use in studies and applications of cyclodextrin inclusion complexes where a high concentration of the cyclodextrin derivative is required.

[17] Smith, R. M., and Martell, A. E., 'Critical Stability Constants' Vol. 2 (Plenum Press: New York 1975).

One area of application of cyclodextrin inclusion complexes is in the preparation of water-based soluble formulations of pharmaceuticals.[10,11] As an example of the utility of the hydrochloride (5b) in this area, an aqueous solution saturated with the salt (5b) dissolves the nonsteroidal antiinflammatory drug Nabumetone [4-(6-methoxy-2-naphthyl)butan-2-one] (6) to 674 mg/100 ml at 25° whereas, under similar conditions, the solubility of Nabumetone in water saturated with β-cyclodextrin is only 36 mg/100 ml and the solubility of Nabumetone alone in water is even lower at 0·8 mg/100 ml. The relatively greater effect of the amine salt (5b) compared with that of β-cyclodextrin (1b) is not due to a greater tendency of the salt (5b) to bind Nabumetone in an inclusion complex. In fact, the stability constant for the inclusion complex formed between the salt (5b) and Nabumetone is only 1830±185 mol^{-1} dm^3, whereas that of the complex with β-cyclodextrin is 4400±480 mol^{-1} dm^3. Instead, the fact that the salt (5b) increases the solubility of the drug (6) by over 800 times, while the solubility enhancement by β-cyclodextrin (1b) is only a factor of 45, must reflect the greater solubility of the salt (5b) compared with that of β-cyclodextrin (1b).



(6)

Experimental

Ultraviolet spectra were recorded on a Pye Unicam SP8-100 spectrophotometer. ^1H and ^{13}C n.m.r. spectra were recorded on either a Bruker CXP-300 or ACP-300 spectrometer. Fast atom bombardment mass spectra were recorded on a Vacuum Generators ZAB 2HF spectrometer. Thin-layer chromatography (t.l.c) was performed by using Kieselgel 60 F_{254} (Merck) on aluminium-backed plates, eluting with 14:3:3 butanone/methanol/water (solvent A) or 8:1:1 acetic acid/chloroform/water (solvent B), and visualized by wetting with a 1·5% solution of sulfuric acid and heating (the R_F of a cyclodextrin derivative indicates the R_F relative to that of the parent cyclodextrin). High-performance liquid chromatography (h.p.l.c.) was carried out with an ICI LC1500 solvent delivery system coupled to a Knauer differential refractometer. Analytical h.p.l.c. was performed on a Waters carbohydrate analysis column (3·9 by 300 mm), eluting at 1·5 ml min^{-1} with acetonitrile/water (70%, v/v) (the t_r of a cyclodextrin derivative indicates the retention time relative to that of the parent cyclodextrin). Preparative h.p.l.c. was performed on a Waters C_{18} μ-Bondapak column (19 by 150 mm). Solvents were analytical reagent grade and were used as supplied, except that pyridine and *N,N*-dimethylformamide were dried by storage over 4 Å molecular sieves. Ether refers to diethyl ether. The cyclodextrins (1a) and (1b) were supplied by Nihon Shokuhin Kako Co. and contained up to 10% water. They were dried under reduced pressure over phosphorus pentoxide, to constant weight, before use.

6^A-O-(4-Methylphenylsulfonyl)-α-cyclodextrin (2a)

The tosylate (2a) was prepared by a modification of the procedure of Melton and Slessor.[12] α-Cyclodextrin (1a) (8·0 g, 8·22 mmol) was dissolved in pyridine (800 ml) by gentle warming and shaking. 4-Methylbenzenesulfonyl chloride (8·0 g, 42·1 mmol) was then added in one portion and the solution was stirred at room temperature for 2 h. The resultant mixture was poured onto ice-cold acetone/ether (6:1, v/v, 6 litres). The fine white precipitate that formed

was allowed to settle over 1 h, then most of the supernatant was decanted and the solid was subsequently collected by gravity filtration (Whatman No. 1 filter paper). The solid was then washed with cold acetone (100 ml) and allowed to dry overnight, then it was dissolved in aqueous methanol (30%, v/v, 100 ml), and the solution was filtered and loaded in one portion, through the pump, onto the C_{18} h.p.l.c. column. Elution with aqueous methanol (30%, v/v, 100 ml) at 15 ml min^{-1} gave fractions containing α-cyclodextrin (1a) (0–35 min). Continued elution (45–120 min) gave fractions that were concentrated under reduced pressure to give the monotosylate (2a) (1·83 g, 19·7%) as a colourless powder. T.l.c. (solvent A) R_F 1·5. Analytical h.p.l.c. t_r 0·5. Mass spectrum *m/z* 1149 (M+Na), 1127 (M+H). ^{1}H n.m.r. [$(CD_3)_2SO/CDCl_3$] δ 2·45, s, 3H; 3·1–5·6, m, 59H; 7·41, d, *J* 8 Hz, 2H; 7·78, d, *J* 8 Hz, 2H. ^{13}C n.m.r. [$(CD_3)_2SO$] δ 25·2, 64·0, 73·0, 73·7, 75·7, 76·1, 77·1, 77·3, 85·6, 86·1, 105·6, 106·0, 131·7, 134·0, 136·5, 148·9.

The preparative h.p.l.c. column was washed with several volumes of methanol to remove polytosylated cyclodextrins.

6^A-O-(4-Methylphenylsulfonyl)-β-cyclodextrin (2b)

β-Cyclodextrin (13·0 g, 11·45 mmol) was dissolved in pyridine (100 ml). 4-Methylbenzenesulfonyl chloride (1·7 g, 8·94 mmol) was added over a period of 0·75 h, with stirring, and the resultant clear solution was allowed to stand at room temperature for 18 h. The mixture was then concentrated under reduced pressure and the residual oil was triturated with acetone (100 ml). The solid that formed was separated by filtration and twice recrystallized from water to give the monotosylate (2b) (4·5 g, 30·5%) as a colourless powder. T.l.c. (solvent B) R_F 1·6. Analytical h.p.l.c. t_r 0·55. Mass spectrum *m/z* 1311 (M+Na), 1289 (M+H). ^{1}H n.m.r. [$(CD_3)_2SO$] δ 2·47, s, 3H; 3·2–5·1, m, 69H; 7·46, d, *J* 8 Hz, 2H; 7·76, d, *J* 8 Hz, 2H. ^{13}C n.m.r. [$(CD_3)_2SO/D_2O$] δ 25·1, 63·9, 75·8, 76·3, 76·7, 81·9, 82·4, 82·8, 85·6, 105·9, 131·5, 133·5, 136·5, 148·6.

6^A-Amino-6^A-deoxy-α-cyclodextrin (4a)

The monotosylate (2a) (1·0 g, 0·89 mmol) was dissolved in *N,N*-dimethylformamide (50 ml) in a 400 ml Parr pressure reaction vessel. Condensed ammonia (100 ml) was added and the vessel was sealed. The mixture was then allowed to warm to room temperature, while the pressure inside the vessel increased to 10^6 N m^{-2}. After the mixture was stirred for 18 h at room temperature, the pressure was released, allowing the excess ammonia to evaporate, and the residual solution was concentrated under reduced pressure. The residual solid was dissolved in water (10 ml) and the solution was concentrated under reduced pressure, then the process was repeated in order to remove residual *N,N*-dimethylformamide. The remaining solid was dissolved in aqueous ammonia (20%, v/v, 10 ml) and the solution was added dropwise to acetone (200 ml). The precipitate that formed was collected by filtration under reduced pressure and washed with acetone (5 ml) and then with ether (5 ml) to give the amine (4a) as a colourless powder (670 mg, 70%), with spectral and physical properties consistent with those of a sample obtained by using the literature procedure.[12] T.l.c. (solvent A) R_F 0·3. Analytical h.p.l.c. t_r 1·2. Mass spectrum *m/z* 994 (M+Na), 972 (M+H). ^{13}C n.m.r. (D_2O) δ 42·8, 61·7, 73·2, 73·7, 74·7, 82·5, 84·3, 102·8.

6^A-Amino-6^A-deoxy-β-cyclodextrin (4b)

A crude sample of the amine (4b) was prepared from the monotosylate (2b), by using the procedure described above for the synthesis of the amine (4a). The material obtained in that manner contained small quantities of cyclodextrin-based impurities. To remove those impurities, the material was dissolved in water (5 ml) and the solution was added to a stirred suspension of BioRex 70 ion-exchange resin (3 g, acid form) in water (20 ml). The mixture was stirred for 4 h, then the resin was separated by filtration and washed with water. Subsequent elution of the resin with aqueous ammonia (20%, v/v) and concentration of the eluate under reduced pressure gave the pure amine (4b) (450 mg, 54%) as a colourless powder. T.l.c.

(solvent B) R_F 0·6. Analytical h.p.l.c. t_r 1·3. Mass spectrum m/z 1134 (M+H). ^{13}C n.m.r. (D_2O) δ 42·1, 61·2, 72·5, 72·8, 73·0, 74·0, 81·9, 82·1, 83·1, 102·6, 102·8.

6^A-Amino-6^A-deoxy-β-cyclodextrin Hydrochloride (5b)

A solution of the amine (4b) (5 g, 4·4 mmol) in water (20 ml) was adjusted to pH 6 with hydrochloric acid (0·5 M, *c.* 9 ml), then it was filtered and added dropwise to acetone (200 ml). The precipitate that formed was collected by filtration and washed with acetone and ether; then it was allowed to dry. The residual solid was dissolved in water (20 ml) and the solution was concentrated under reduced pressure; then the process was repeated twice. The residue was dried under reduced pressure over phosphorus pentoxide to give the amine hydrochloride (5b) (5 g, 97%) as a colourless powder, with spectral and physical properties consistent with those reported previously.[16]

Determination of the pK_a Values of the Amines (4a,b)

The pK_a values of the amines (4a,b) were determined from pH titrations carried out under CO_2-free nitrogen, either manually or by employing a 665 Dosimat autoburette, with a Ross combination pH electrode (Orion Research Inc. No. 81-03 or 81-72). The pH meter was standardized with a phosphate buffer (pH 6·865) and potassium hydrogen phthalate (pH 4·005) at 298·2 K. Prior to the titrations, solutions of the amines (4a,b) were acidified and purged with nitrogen for 2 h to remove contaminating CO_2. Titrations of the protonated amines (4a,b) were then carried out (I = 0·500, 298·2 K) by addition of standardized 0·100 mol dm^{-3} sodium hydroxide or potassium hydroxide. The pK_a values were computed from the titration data by using the program SUPERQUAD,[18] and by optimizing both the value of pK_w and the value of the purity of the amines (4a,b).

Determination of the Solubility of Nabumetone (6) in Water and in Saturated Aqueous Solutions of β-Cyclodextrin (1b) and the Salt (5b)

Suspensions of Nabumetone (6) in water and in saturated aqueous solutions of β-cyclodextrin (1b) and the salt (5b) were stirred at 25° for 3 days; then they were filtered. The filtrates were analysed by ultraviolet spectroscopy to calculate the concentration of Nabumetone based on the predetermined molar absorption coefficient for Nabumetone at λ_{max} 331 nm, ϵ 1550. No more Nabumetone dissolved when the suspensions were stirred for longer times. The ultraviolet spectrum of Nabumetone was unaffected by the presence of either β-cyclodextrin (1b) or the salt (5b).

Determination of the Stability Constants of the Inclusion Complexes Formed Between Nabumetone (6) and β-Cyclodextrin (1b) and the Salt (5b)

Suspensions of Nabumetone (6) in solutions of β-cyclodextrin (1b) or the salt (5b) in phosphate buffer (0·1 M, pH 7·4), ranging in cyclodextrin concentration from 1×10^{-4} to 3×10^{-3} M, were stirred at 25° for 3 days, then analysed as described above to measure the concentration of dissolved Nabumetone. The solubility of Nabumetone in the buffer was determined in a similar manner. The solubility data were then used to calculate the stability constants of the inclusion complexes.[9]

Acknowledgments

This work was supported by grants from Australian Commercial Research and Development Ltd, the Australian Research Council and the University of Adelaide Research Grants Scheme.

[18] Gans, P., Sabatini, A., and Vacca, A., *J. Chem. Soc., Dalton Trans.*, 1985, 1195.

Zeitschrift für Kristallographie 205, 137–139 (1993)

Crystal structure of 1,4-dimethyl-3-phthalimido-piperazine-2,5-dione, $C_{14}H_{13}N_3O_4$

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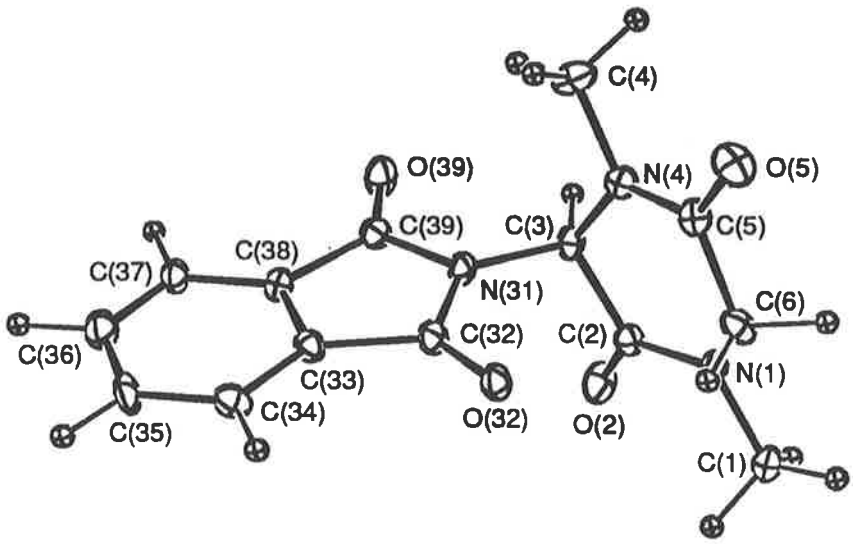
(Received June 29, 1992)



Source of material: see ref. 1.
The structure determination shows the molecule to exist as a dione. The C(39)N(31)C(3)N(4) torsion angle of 116.1° indicates no conjugation in the N(31)-C(3) bond.

Monoclinic, $P12_1/c1$ (no 14), $a = 14.913(7)$, $b = 9.142(2)$, $c = 10.210(3)$ Å, $\beta = 109.63(4)°$, $V = 1311.1$ Å^3, $Z = 4$, $R = 0.064$.

Table 1. Parameters used for the X-ray data collection

Diffractometer type:	Enraf-Nonius CAD4	Number of unique reflections:	1713
Wave length:	Mo K_α radiation (0.7107 Å)	Criterion for unobserved reflections:	$F_o < 6\sigma(F_o)$
Crystal characteristics:	colourless, spherical crystal of approximate diameter 0.30 mm	Number of refined parameters:	230
		Scan mode:	$\omega/2\Theta$
Temperature of measurement:	293 K	μ:	0.68 cm^{-1}
$2\theta_{max}$:	45°	Structure solution program used:	SHELX

Table 2. Final atomic coordinates and displacement parameters (in $Å^2$)

Atom	*x*	*y*	*z*	U_{iso}/U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O(2)	0.1980(4)	0.1532(7)	−0.0617(6)	0.066(4)	0.071(4)	0.074(4)	0.012(3)	0.050(4)	0.022(4)
O(5)	0.0794(4)	−0.1934(6)	0.2681(6)	0.065(4)	0.073(4)	0.053(4)	−0.009(3)	0.027(3)	0.017(4)
O(32)	0.3234(3)	0.0276(6)	0.3021(5)	0.039(3)	0.061(4)	0.044(3)	0.001(3)	0.021(3)	−0.015(3)
O(39)	0.3327(4)	−0.2110(7)	−0.0866(6)	0.048(3)	0.092(5)	0.047(4)	0.010(3)	0.019(3)	−0.024(4)
N(1)	0.1153(4)	0.1269(7)	0.0892(7)	0.040(3)	0.042(4)	0.041(4)	0.007(3)	0.019(3)	0.000(3)
N(4)	0.1538(4)	−0.1711(7)	0.1113(6)	0.039(3)	0.042(4)	0.038(4)	0.000(3)	0.012(3)	−0.001(3)
N(31)	0.3041(4)	−0.0926(6)	0.0960(6)	0.036(3)	0.048(4)	0.037(4)	0.002(3)	0.017(3)	−0.008(3)
C(1)	0.0931(6)	0.284(1)	0.079(1)	0.042(5)	0.056(7)	0.062(7)	0.009(4)	0.016(5)	−0.006(5)
H(1A)	0.044(6)	0.297(9)	0.094(9)	0.069(8)					
H(1B)	0.065(5)	0.317(9)	−0.024(9)	0.069(8)					
H(1C)	0.129(6)	0.342(9)	0.145(9)	0.069(8)					
C(2)	0.1731(5)	0.0793(9)	0.0187(8)	0.032(4)	0.050(5)	0.041(5)	0.001(4)	0.012(4)	0.001(5)
C(3)	0.2005(4)	−0.0817(8)	0.0349(8)	0.026(4)	0.051(5)	0.030(4)	0.007(3)	0.007(3)	0.000(4)
H(3)	0.180(4)	−0.132(7)	−0.070(7)	0.046(5)					
C(4)	0.1692(7)	−0.3293(9)	0.108(1)	0.071(6)	0.039(6)	0.052(7)	0.004(4)	0.009(5)	−0.002(5)
H(4A)	0.166(5)	−0.366(8)	0.000(9)	0.069(8)					
H(4B)	0.127(6)	−0.397(9)	0.138(8)	0.069(8)					
H(4C)	0.233(5)	−0.359(9)	0.173(8)	0.069(8)					
C(5)	0.1103(5)	−0.1172(9)	0.1963(9)	0.040(4)	0.058(6)	0.037(5)	0.002(4)	0.013(4)	0.002(4)
C(6)	0.1011(6)	0.0478(9)	0.2014(9)	0.039(4)	0.060(6)	0.041(5)	−0.004(4)	0.023(4)	0.000(5)
H(6A)	0.152(5)	0.091(7)	0.306(8)	0.057(7)					
H(6B)	0.037(5)	0.066(8)	0.197(7)	0.057(7)					
C(32)	0.3574(5)	−0.0330(8)	0.2253(8)	0.033(4)	0.045(5)	0.044(5)	−0.003(4)	0.012(4)	0.003(4)
C(33)	0.4602(5)	−0.0614(8)	0.2378(8)	0.033(4)	0.045(5)	0.045(5)	0.005(4)	0.013(4)	0.010(4)
C(34)	0.5432(5)	−0.0331(9)	0.3487(9)	0.043(5)	0.055(6)	0.049(6)	−0.009(4)	0.017(4)	−0.009(5)
H(34)	0.540(5)	0.031(8)	0.422(7)	0.046(5)					
C(35)	0.6279(5)	−0.075(1)	0.331(1)	0.029(4)	0.072(6)	0.075(7)	−0.010(4)	0.018(4)	0.004(6)
H(35)	0.676(5)	−0.043(8)	0.407(7)	0.046(5)					
C(36)	0.6293(6)	−0.141(1)	0.210(1)	0.046(5)	0.055(6)	0.092(9)	0.003(4)	0.032(6)	0.005(6)
H(36)	0.699(5)	−0.173(7)	0.199(6)	0.046(5)					
C(37)	0.5460(5)	−0.1706(9)	0.1008(9)	0.046(5)	0.054(5)	0.056(6)	0.007(4)	0.023(5)	−0.003(5)
H(37)	0.545(4)	−0.220(7)	0.009(7)	0.046(5)					
C(38)	0.4609(5)	−0.1310(8)	0.1191(8)	0.034(4)	0.044(5)	0.046(6)	0.009(3)	0.017(4)	0.000(4)
C(39)	0.3625(5)	−0.1535(9)	0.0241(9)	0.040(4)	0.052(5)	0.041(5)	0.003(4)	0.018(4)	−0.006(5)

Further details of the structure determination (e.g. structure factors) have been deposited within the relevant database and can be accessed as Collection No. 320487 or ordered from the Fachinformationszentrum Karlsruhe, D-7514 Eggenstein-Leopoldshafen.

References:

1. Easton, C.J., Scharfbillig, I.M.: Unpublished results.

J. CHEM. SOC. PERKIN TRANS. 1 1993

Synthesis of Very Long Chain Fatty Acid Methyl Esters

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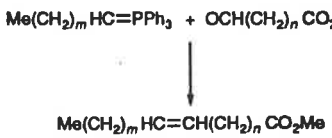
Phosphoranes, produced by treating alkyltriphenylphosphonium bromides with lithium hexamethyldisilazide, reacted with ω-oxo esters to give modest yields of the corresponding methyl *cis*-alkenoates. By an alternative method, treatment of ω-iodo esters with the complexes formed from reactions of alkylcopper(I) and Grignard reagents gave methyl alkanoates, *cis*-alkenoates, and methylene-interrupted *cis,cis*-alka-dienoates and *cis,cis,cis*-trienoates. The stereochemical integrity of the esters was determined by ^{13}C NMR spectroscopy.

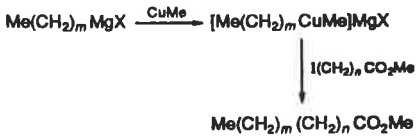
Fatty acids with chain lengths of > 22 carbons (very long chain fatty acids, VLCFA) have been found in a wide variety of species.[1,2] The degree of unsaturation of these compounds varies, depending on the source, but alkenoic VLCFA generally have the *cis*-stereochemistry and the more unsaturated analogues usually comprise methylene-interrupted all-*cis*-polyenes. Ready access to the VLCFA would greatly facilitate studies of their biochemical reactions but, since they generally occur only in trace amounts as components of complex mixtures, isolation of sufficient quantities from natural sources is impractical. Consequently, synthesis appears to be a more viable alternative method to obtain these compounds.

Many of the procedures that have been reported for the synthesis of the shorter chain fatty acids are potentially suitable for the synthesis of VLCFA. In this regard, the Wittig reaction of ω-oxo esters with phosphoranes, to produce *cis*-alkenoates (Scheme 1),[3,4] has been applied in the synthesis of methyl (*Z*)-



Scheme 1

hexacos-9-enoate.[4] Alternatively, fatty acid esters, including ethyl heptacosanoate, have been obtained from reactions between ω-iodo esters and complexes formed from methylcopper(I) and Grignard reagents (Scheme 2).[5] To the best of

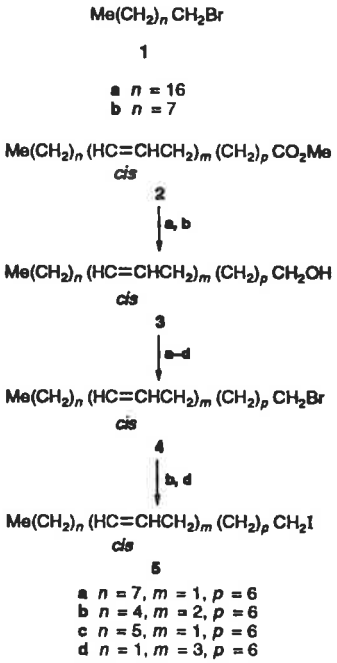


Scheme 2

our knowledge there has been no report of the synthesis of unsaturated VLCFA esters using this method. In order to obtain VLCFA and study their biochemical reactions, we have now examined and compared the general applicability of the two approaches to the synthesis of VLCFA methyl esters, including alkenoates and more highly unsaturated analogues.

Results and Discussion

The alkyl bromides **1a** and **1b**, the esters **2a** and **2b**, the alcohols **3c** and **3d**, and the acids **6a**, **6b** and **8d**, used in this study, were available commercially. The alkenyl bromides **4a–d** were

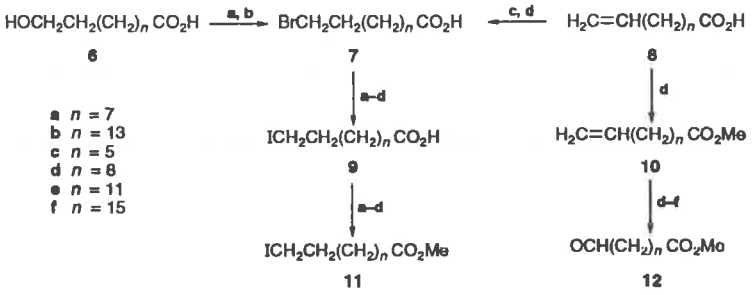


Scheme 3

prepared as shown in Scheme 3, by treatment of the corresponding alcohols **3a–d** with triphenylphosphine/carbon tetrabromide.[6] The alcohols **3a** and **3b** were prepared by reduction of the corresponding esters **2a** and **2b** with lithium aluminium hydride. Treatment of the bromides **4b** and **4d** with sodium iodide in acetone gave the corresponding iodides **5b** and **5d** (Scheme 3).

The ω-iodo esters **11a–d** were obtained as shown in Scheme 4. Treatment of the hydroxy acids **6a** and **6b** with hydrogen bromide in acetic acid gave the corresponding bromides **7a** and **7b**. The unsaturated acids **8c** and **8d** reacted with hydrogen bromide in light petroleum, in the presence of azoisobutyronitrile, to give the bromides **7c** and **7d**, respectively. The acid **8c** was obtained by oxidation of oct-7-en-1-ol.[7] The bromides **7a–d** reacted with sodium iodide in acetone to give the corresponding iodides **9a–d**, which were converted into the respective ω-iodo esters **11a–d** through reaction with methanol that had been pretreated with thionyl chloride.

The ω-oxo esters **12d–f** were obtained by treatment of the corresponding unsaturated esters **10d–f** with ozone,[8] followed by dimethyl sulfide (Scheme 4). The unsaturated ester **10d** was



Scheme 4

prepared by treatment of the acid **8d** with methanol that had been pretreated with thionyl chloride, whereas the unsaturated esters **10e** and **10f** were obtained through cuprate-mediated coupling reactions of the iodo esters **11a** and **11d** with but-3-enyl bromide and hept-6-enyl bromide, respectively.[5] Thus, the Grignard reagents obtained by treatment of but-3-enyl bromide and hept-6-enyl bromide with magnesium, were added to preformed methylcopper(I), to form the corresponding mixed dialkylcuprates, which reacted with the iodo esters **11a** and **11d**, respectively, to give the corresponding coupled products **10e** and **10f**. The yields of the esters **10e** and **10f** depended on the scale of the reactions. Good yields were obtained when reactions were performed on a 10 mmol scale or greater, otherwise the major reaction products were methyl undecanoate and methyl dodecanoate, formed by methyl group transfer to the respective iodo esters **11a** and **11d**. In any event, the ester **10e** was contaminated with a substantial amount of methyl undecanoate. Owing to the closely similar chromatographic behaviour of the unsaturated ester **10e** and methyl undecanoate, they were not separated and the mixture was used without further purification.

The iodo ester **11f** was prepared from the alkene **10f**, by treatment with hydrogen bromide in light petroleum, in the presence of azoisobutyronitrile, to give the bromide **13**, followed by treatment with sodium iodide in acetone.

$BrCH_2CH_2(CH_2)_{15}CO_2Me$

13

$CH_3(CH_2)_n(HC{=}CHCH_2)_m(CH_2)_p CO_2Me$
cis

14

a $n = 16, m = 1, p = 7$
b $n = 7, m = 1, p = 14$
c $n = 7, m = 1, p = 10$
d $n = 17, m = 0, p = 10$
e $n = 7, m = 1, p = 22$
f $n = 5, m = 1, p = 24$
g $n = 4, m = 2, p = 22$
h $n = 4, m = 2, p = 14$
i $n = 1, m = 3, p = 22$

Our examination of the use of the Wittig method in the synthesis of fatty acid methyl esters involved reactions of the phosphoranes, generated from the alkyl bromides **1a** and **1b**, with the ω-oxo esters **12d–f**. The bromides **1a** and **1b** were treated with triphenylphosphine in acetonitrile to produce the corresponding phosphonium salts. The salt derived from the bromide **1a** was obtained as colourless crystals that were stable in air and easily handled. By contrast, the salt of the bromide **1b** was a moisture- and air-sensitive glass. These salts were treated with lithium hexamethyldisilazide to generate the corresponding phosphoranes. The phosphorane derived from the bromide **1a** reacted with the oxo ester **12d** to give the VLCFA ester **14a** (67%) while treatment of the phosphorane derived from the bromide **1b** with the oxo esters **12f** and **12e** gave the esters **14b** (13%) and **14c** (12%) respectively.

The isomeric purity of the esters **14a–c** was determined using ^{13}C NMR spectroscopy (Table 2). It has been reported[9] that allylic carbons of straight chain *cis*-alkenes give rise to NMR signals in the range δ 27.22–27.39, while those of the corresponding *trans*-alkenes appear in the range δ 32.64–32.69. Consistent with that report, the ^{13}C NMR spectrum of methyl (*Z*)-heptadec-10-enoate was found to have signals at δ 27.1(4) and 27.1(7) due to the allylic carbon resonances, whereas the spectrum of methyl (*E*)-heptadec-10-enoate contained a single signal at δ 32.6 attributable to both allylic carbons. The spectrum of the ester **14a** showed peaks at δ 27.1(7) and 27.1(9), due to carbons C-9 and C-12, confirming the *cis*-stereochemistry, and no peaks were observed near δ 32.6 to indicate the presence of any of the corresponding *trans*-isomer. Similarly, in the case of the ester **14b**, a peak at δ 27.1(7) in the ^{13}C NMR spectrum, due to the allylic carbons C-16 and C-19, verified the *cis*-stereochemistry, and no peak was observed near δ 32.6. The spectrum of the ester **14c** contained all the resonances expected for the *cis*-isomer, including a signal at δ 27.2(4), attributable to carbons C-12 and C-15. In addition, there was a signal of relatively low intensity at δ 32.6(2), indicating that the ester **14c** was contaminated with *ca.* 5% of the corresponding *trans*-isomer. The signal arising from carbons C-13 and C-14 of the *cis*-ester **14c** appeared at δ 129.8(9), while the corresponding signal of the *trans*-isomer was characteristically downfield[9] at δ 130.3(5).

The modest yields of the esters **14b** and **14c**, and the lack of stereocontrol in the synthesis of the latter, illustrate limitations of the Wittig method in the synthesis of monoenoic acid esters. The method was found to be even less suitable for the synthesis of more highly unsaturated VLCFA esters, where complex isomeric mixtures always formed.

By contrast, copper-mediated reactions of ω-iodo esters with Grignard derivatives of alkyl and alkenyl bromides proved to be more generally applicable to the synthesis of a range of fatty acid methyl esters, of varying carbon chain length, degree of unsaturation, and series. Accordingly, the mixed dialkylcuprate obtained by treatment of the Grignard derivative of the bromide **1a** with methylcopper(I), reacted with the iodo ester **11d** to give the saturated ester, methyl nonacosanoate **14d** (23%). Similar reactions of the bromides **4a** and **4c** with the iodo esters **11b** and **11f**, respectively, gave the corresponding methyl alkenoates **14e** (13%) and **14f** (10%). The stereochemical integrity of the esters **14e** and **14f** was determined using ^{13}C NMR spectroscopy (Table 2). The spectrum of the ester **14e** showed a signal at δ 27.1(9), due to the allylic carbons C-24 and C-27, while that of the ester **14f** contained a resonance at δ 27.2(1), due to the allylic carbons C-26 and C-29. Neither spectrum showed a signal near δ 32.6, to indicate the presence of the corresponding *trans*-isomer. The mono-unsaturated esters **14b** and **14c** were also obtained using this method, in yields of 14 and 18%, respectively, and with complete stereochemical control within the limits of detection using ^{13}C NMR

Table 1 ^{1}H NMR spectral data of the esters **14**

Ester	Me/*J*	$(CH_2)_n$	C(3)H_2	Allylic	CH_2CO/*J*	Doubly allylic/*J*	OMe	Vinylic/*J*
a	0.88, t, 6.5	1.25, m	1.61, m	2.01, m	2.30, t, 7.5	—	3.67, s	5.34, t, 5.3
b	0.88, t, 6.5	1.25, m	1.62, m	2.01, m	2.30, t, 7.4	—	3.66, s	5.34, t, 4.7
c	0.88, t, 6.5	1.27, m	1.62, m	2.01, m	2.30, t, 7.5	—	3.66, s.	5.35, t, 4.6
d	0.88, t, 6.4	1.25, m	1.60, m	—	2.30, t, 7.5	—	3.67, s	—
e	0.88, t, 6.5	1.25, m	1.60, m	2.01, m	2.30, t, 7.5	—	3.67, s	5.35, t, 4.6
f	0.88, t, 6.2	1.25, m	1.60, m	2.01, m	2.30, t, 7.5	—	3.67, s	5.25, t, 4.6
g	0.89, t, 6.5	1.25, m	1.62, m	2.05, m	2.30, t, 7.5	2.77, t, 5.9	3.66, s	5.35, m
h	0.89, t, 6.6	1.25, m	1.62, m	2.05, m	2.30, t, 7.5	2.78, t, 5.9	3.67, s	5.36, m
i	0.98, t, 7.5	1.25, m	1.62, m	2.06, m	2.30, t, 7.5	2.81, t, 5.6	3.67, s	5.37, m

Table 2 ^{13}C NMR spectral data of the esters **14**

Ester	ω1	ω2	C-3	Doubly allylic	Allylic	$(CH_2)_n$	ω3	C-2	OMe	Vinylic	C-1
a	14.1	22.7	24.9	—	27.1(7), 27.1(9)	29.1–29.8	31.9	34.1	51.4	129.8, 129.9	174.3
b	14.1	22.7	24.9	—	27.1(7)	29.1–29.8	31.9	34.1	51.4	129.8	174.3
c	14.1	22.7	25.0	—	27.2(4)	29.1–29.8	32.0	34.1	51.4	129.9	174.4
e	14.2	22.7	24.9	—	27.1(9)	29.1–29.8	31.9	34.1	51.5	129.9	174.4
f	14.1	22.7	25.0	—	27.2(1)	29.0–29.7	31.8	34.1	51.4	129.9	174.3
g	14.0	22.6	25.0	25.6(1)	27.2(2)	29.1–29.7	31.5	34.1	51.4	127.9, 130.1	174.3
h	14.1	22.6	24.9	25.6(0)	27.1(8), 27.2(2)	29.1–29.7	31.5	34.1	51.5	127.9, 130.2	174.4
i	14.3	—[a]	25.0	25.5(2), 25.6(0)	20.5(5), 27.2(5)	29.2–29.7	—[a]	34.1	51.4	127.6, 128.2, 130.4	174.3

[a] Carbons ω2 and ω3 are allylic and vinylic, respectively, in the ester **14i**.

spectroscopy, through reactions of the bromide **4a** with the iodo ester **11c** and methyl 4-iodobutyrate.

The procedure was extended to the synthesis of alkadienoates and trienoates. Reaction of the bromide **4b** with the iodo ester **11b** gave the ester **14g** (22%), while analogous reactions of the bromides **4b** and **4d** with the iodo-esters **11c** and **11b**, respectively, gave the corresponding coupled products **14h** and **14i**.

As described above for the synthesis of the esters **10e** and **10f**, the copper-mediated reactions to give the esters **14b–i** were accompanied by methyl group transfer to the iodo esters **11b**, **11c**, **11f** and methyl 4-iodobutyrate. As particular examples, methyl nonanoate and methyl heptadecanoate were isolated in yields of 58 and 43%, respectively, from the reactions to form the esters **14b** and **14e**. Although the experience with the reactions to give the esters **10e** and **10f** indicated that this type of side reaction would be less significant with reactions carried out on a larger scale, the scale of synthesis of the esters **14b–i** is restricted by the cost of, and limited access to, the starting materials.

Fortunately, the esters **14b–g** were easily separable by chromatography from the products of methyl group transfer reactions, but the esters **14h** and **14i** were contaminated with methyl nonanoate and methyl heptadecanoate, respectively, which could not be separated in this way. Instead, (*Z*,*Z*)-octadeca-9,12-dienyl- and (*Z*,*Z*,*Z*)-octadeca-9,12,15-trienylcopper(I) were used in place of methylcopper(I), to prevent methyl group transfer reactions, in order to obtain uncontaminated samples of the esters **14h** and **14i**, respectively. Thus, the iodide **5b** was treated with *tert*-butyllithium and cuprous iodide to give (*Z*,*Z*)-octadeca-9,12-dienylcopper(I), which reacted with the Grignard reagent derived from the bromide **4b** to give the corresponding dialkylcopper(I) complex. Reaction of this complex with the iodo ester **11c** gave the pure ester **14h** (12%). A similar procedure using the iodide **5d**, the bromide **4d**, and the iodo ester **11b**, gave the trienoate **14i** (10%).

^{13}C NMR spectroscopy was used to confirm the stereochemistry of the esters **14g–i** (Table 2). The spectrum of the dienoate **14g** showed signals at δ 27.2(2) and 25.6(1), characteristic of the allylic carbons and the doubly allylic carbon, respectively, in a methylene-interrupted *cis*, *cis*-diene.[9] In a similar fashion, the spectrum of the ester **14h** contained signals at δ 27.1(8) and 27.2(2), attributable to the allylic carbons C-16 and C-22, and at δ 25.6(0) for the doubly allylic carbon C-19, while the spectrum of the trienoate **14i** included resonances at δ 25.5(2) and 25.6(0) for the doubly allylic carbons C-27 and C-30, and at δ 20.5(5) and 27.2(5) for carbons C-33 and C-24, respectively. The chemical shift of C-33 is affected by the proximity of that carbon to the end of the carbon chain and is consistent with values reported for fatty acids of the $n - 3$ series, to which the ester **14i** belongs.

Based on the above results, copper-mediated coupling reactions of ω-iodo esters with Grignard reagents, derived from alkyl and alkenyl bromides, are more generally suitable than the reactions of ω-oxo esters with phosphoranes, for the synthesis of VLCFA esters. Nevertheless, compounds of this type are accessible using either approach, and from a range of starting materials, as used in this study.

Experimental

General.—M.p.s were determined on a Kofler hot-stage apparatus and are uncorrected. B.p.s are quoted as the block temperature required for distillation. IR spectra were recorded as liquid films or as solutions in chloroform, on a Hitachi 270-30 spectrometer. ^{1}H NMR spectra were recorded in chloroform, unless otherwise stated, using Me_4Si as internal standard, on either a Varian T-60, a Bruker CXP-300, or a Bruker ACP-300 spectrometer. ^{13}C NMR spectra were recorded in chloroform using Me_4Si as internal standard, on either a Bruker CXP-300 or a Bruker ACP-300 spectrometer. NMR spectral data of the esters (**14a–i**) are listed in Tables 1 (^{1}H) and 2 (^{13}C). *J*-Values are given in Hz. Electron impact (EI) mass spectra were recorded on an AEI MS-30 double focussing spectrometer, operating at 70 eV. Elemental analyses were performed by Canadian Microanalytical Service Ltd., New Westminster, British Columbia, Canada, or by Chemical and Microanalytical Services Pty. Ltd., North Essendon, Victoria, Australia.

All solvents were purified and dried using standard methods. Light petroleum refers to the fraction with b.p. 66–68 °C. Ether

refers to diethyl ether. Flash-column chromatography[10] was performed on Merck Kieselgel 60 (230–400 mesh ASTM).

1-Bromooctadecane **1a**, 1-bromononane **1b**, methyl (*Z,Z*)-octadeca-9,12-dienoate **2b**, 10-hydroxydecanoic acid **6a**, 16-hydroxyhexadecanoic acid **6b**, undec-10-enoic acid **8d**, and but-3-enyl bromide were purchased from Aldrich Chemical Co. Methyl (*Z*)-octadec-9-enoate **2a** was purchased from Koch-Light Laboratories. (*Z*)-Hexadec-9-en-1-ol **3c** and (*Z,Z,Z*)-octadeca-9,12,15-trien-1-ol **3d** were obtained from Nu-Chek-Prep, Inc., Elysian, Minnesota, USA.

(Z)-*Octadec-9-en-1-ol* **3a**.—A solution of the ester **2a** (3.0 g, 10.1 mmol) in ether (20 cm^3) was added slowly to a stirred suspension of lithium aluminium hydride (0.77 g, 20.3 mmol) in ether (20 cm^3). After the vigorous reaction had subsided, the mixture was heated at reflux for 3 h and then cooled and poured into saturated aqueous ammonium chloride (100 cm^3) and water (20 cm^3). The layers that formed were separated and the aqueous layer was extracted with ether (3 × 20 cm^3). The organic layer and the ether extracts were combined and the mixture was dried ($MgSO_4$) and concentrated under reduced pressure. The residual oil distilled to give the title alcohol **3a** as a colourless oil (2.1 g, 78%), b.p. 200 °C/0.03 mmHg (block) (lit.,[11] b.p. 177–183 °C/3 mmHg); $\delta_H(CCl_4)$ 0.89 (m, 3 H, CH_3), 1.33 (m, 24 H, CH_2), 1.52 (br s, 1 H, OH), 2.00 (m, 4 H, allylic), 3.53 (t, *J* 6.0, 2 H, CH_2OH) and 5.29 (t, *J* 4.5, 2 H, vinylic).

(Z,Z)-*Octadeca-9,12-dien-1-ol* **3b**. This compound, prepared from the ester **2b** as described for the synthesis of the alcohol **3a**, was obtained as a colourless oil (1.47 g, 81%), b.p. 230 °C/0.03 mmHg (block) (lit.,[12] b.p. 148–150 °C/1 mmHg); δ_H 0.86 (m, 3 H, CH_3), 1.47 (m, 18 H, CH_2), 2.08 (br s, 1 H, OH), 2.30 (m, 4 H, allylic), 2.75 (m, 2 H, doubly-allylic), 3.62 (t, *J* 6.0, 2 H, CH_2OH) and 5.35 (m, 4 H, vinylic).

(Z)-*Octadec-9-enyl Bromide* **4a**.—Carbon tetrabromide (1.45 g, 4.37 mmol) was added in small portions to a solution of the alcohol **3a** (1.17 g, 4.37 mmol) and triphenylphosphine (1.16 g, 4.42 mmol) in dichloromethane (8 cm^3), cooled in ice.[6] The mixture was stirred at room temperature for 16 h and then concentrated under reduced pressure. The residual solid was extracted with light petroleum and the extracts were concentrated under reduced pressure. Chromatography of the residual oil, with light petroleum as eluent, gave the title bromide **4a** as a colourless oil (1.32 g, 91%); $\delta_H(CCl_4)$ 0.88 (m, 3 H, CH_3), 1.65 (m, 28 H, CH_2), 3.31 (t, *J* 6.5, 2 H, CH_2Br) and 5.24 (t, *J* 4.5, 2 H, vinylic). The spectral properties of the bromide **4a** are consistent with those reported previously.[13]

(Z,Z)-*Octadeca-9,12-dienyl bromide* **4b**. This compound, prepared from the alcohol **3b** as described for the synthesis of the bromide **4a**, was obtained as a colourless oil (1.60 g, 95%); $\delta_H(CCl_4)$ 0.90 (m, 3 H, CH_3), 1.67 (m, 22 H, CH_2), 2.72 (m, 2 H, doubly allylic), 3.34 (t, *J* 6.5, 2 H, CH_2Br) and 5.29 (m, 4 H, vinylic); ν_{max}/cm^{-1} 3008, 2924, 2852, 1466, 724 and 660; *m/z* (EI) 330 (M^+), 328 (M^+), 137, 123, 109 and 95.

(Z)-*Hexadec-9-enyl bromide* **4c**. This compound, prepared from the alcohol **3c** as described for the synthesis of the bromide **4a**, was obtained as a colourless oil (1.27 g, 94%); $\delta_H(CCl_4)$ 0.89 (m, 3 H, CH_3), 1.65 (m, 24 H, CH_2), 3.32 (t, *J* 6.5, 2 H, CH_2Br) and 5.25 (t, *J* 4.5, 2 H, vinylic); ν_{max}/cm^{-1} 3000, 2924, 2848, 1650, 1466, 725 and 658; *m/z* (EI) 304 (M^+), 302 (M^+), 150, 148, 111, 97, 83, 69 and 55.

(Z,Z,Z)-*Octadeca-9,12,15-trienyl bromide* **4d**. This compound, prepared from the alcohol **3d** as described for the synthesis of the bromide **4a**, was obtained as a colourless oil (1.31 g, 92%); $\delta_H(CDCl_3)$ 0.97 (t, *J* 7.5, 3 H, CH_3), 1.68 (m, 16 H, CH_2), 2.73 (m, 4 H, doubly allylic), 3.30 (t, *J* 6.5, 2 H, CH_2Br) and 5.26 (m, 6 H, vinylic); ν_{max}/cm^{-1} 3008, 2924, 2852, 1650, 1464, 720 and 658; *m/z* (EI) 328 (M^+), 326 (M^+), 272, 270, 135, 121, 108, 95, 93, 79 and 67.

(Z,Z)-*Octadeca-9,12-dienyl Iodide* **5b**.—A solution of the bromide **4b** (0.50 g, 1.52 mmol) and sodium iodide (0.57 g, 3.80 mmol) in acetone (10 cm^3) was heated at reflux for 16 h and then cooled to room temperature and poured onto water (200 cm^3). The mixture was extracted with dichloromethane (3 × 30 cm^3) and the combined extracts were washed with 10% aqueous sodium thiosulfate (30 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography of the residual oil, with light petroleum as eluent, gave the title iodide **5b** (0.45 g, 78%); $\delta_H(CCl_4)$ 0.90 (m, 3 H, CH_3), 1.67 (m, 22 H, CH_2), 2.71 (m, 2 H, doubly allylic), 3.12 (t, *J* 6.5, 2 H, CH_2I) and 5.25 (m, 4 H, vinylic); ν_{max}/cm^{-1} 3004, 2924, 2848, 1650, 1464 and 722; *m/z* (EI) 376 (M^+), 196, 156, 110, 95, 81, 67, 55 and 41; *m/z* (EI) 376.162 (M^+) [Calc. for $C_{18}H_{33}I$ (M^+): *m/z* 376.163].

(Z,Z,Z)-*Octadeca-9,12,15-trienyl iodide* **5d**. This compound was prepared from the bromide **4d** as described above for the synthesis of the iodide **5b** (0.40 g, 70%); δ_H 0.98 (t, *J* 7.5, 3 H, CH_3), 1.75 (m, 16 H, CH_2), 2.75 (m, 4 H, doubly allylic), 3.12 (t, *J* 6.5, 2 H, CH_2I) and 5.29 (m, 6 H, vinylic); ν_{max}/cm^{-1} 3008, 2924, 2848, 1650, 1464 and 718; *m/z* (EI) 374 (M^+), 345, 317, 183, 155, 136, 121, 109, 107, 94, 91, 79 and 55; *m/z* (EI) 374.146 (M^+) [Calc. for $C_{18}H_{31}I$ (M^+) *m/z* 374.147].

10-*Bromodecanoic Acid* **7a**.—A suspension of the hydroxy acid **6a** (5.03 g, 26.8 mmol) in a solution of 33% hydrogen bromide in acetic acid (70 cm^3) was stirred at room temperature for 16 h and then heated at 100 °C for 4 h. It was then cooled and concentrated under reduced pressure. The residual oil was dissolved in dichloromethane (40 cm^3) and the resultant solution was washed with water (2 × 20 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure, to give a solid which recrystallized from light petroleum to give the title bromo acid **7a** as a colourless solid (5.06 g, 75%), m.p. 36.5 °C (lit.,[14] m.p. 37–38 °C); $\delta_H(CCl_4)$ 1.85 (m, 16 H, CH_2), 3.34 (t, *J* 6.5, 2 H, CH_2Br) and 11.77 (br s, 1 H, CO_2H).

16-*Bromohexadecanoic acid* **7b**. This compound, prepared from the hydroxy acid **6b** as described for the synthesis of the bromo acid **7a**, was obtained as a colourless solid (2.69 g, 87%), m.p. 68–69 °C (lit.,[15] m.p. 70–70.5 °C); δ_H 1.60 (m, 26 H, CH_2), 2.37 (m, 2 H, CH_2CO), 3.43 (t, *J* 6.5, 2 H, CH_2Br) and 10.50 (br s, 1 H, CO_2H).

8-*Bromooctanoic Acid* **7c**.—Hydrogen bromide gas was passed through a solution of oct-7-enoic acid **8c** (2.46 g, 17.3 mmol) and azoisobutyronitrile (*ca.* 20 mg) in light petroleum (25 cm^3), for 15 min whilst the mixture was illuminated with a 300 W UV lamp. The mixture was irradiated for a further 15 min and then cooled to −10 °C. The resulting precipitate was filtered off and recrystallized from light petroleum to give the title bromide **7c** as colourless crystals (2.66 g, 69%), m.p. 33–36 °C (lit.,[14] m.p. 36–37 °C); δ_H 1.85 (m, 12 H, CH_2), 3.41 (t, *J* 6.5, 2 H, CH_2Br) and 10.42 (br s, 1 H, CO_2H).

11-*Bromoundecanoic acid* **7d**. This compound, prepared from undec-10-enoic acid **8d** as described for the synthesis of the bromo acid **7c**, was obtained as colourless crystals (19.25 g, 67%), m.p. 44–47 °C (lit.,[16] m.p. 49–50 °C); δ_H 1.65 (m, 16 H, CH_2), 2.37 (m, 2 H, CH_2CO), 3.44 (t, *J* 6.5, 2 H, CH_2Br) and 10.40 (br s, 1 H, CO_2H).

10-*Iododecanoic acid* **9a**. This compound, prepared from the bromo acid **7a** as described for the synthesis of the iodide **5b** and recrystallized from light petroleum, was obtained as colourless crystals (5.36 g, 90%), m.p. 49–50 °C (lit.,[14] m.p. 49–50 °C); $\delta_H(CCl_4)$ 1.60 (m, 14 H, CH_2), 2.30 (m, 2 H, CH_2CO), 3.13 (t, *J* 6.5, 2 H, CH_2I) and 11.71 (br s, 1 H, CO_2H).

16-*Iodohexadecanoic acid* **9b**. This compound, prepared from the bromo acid **7b** as described for the synthesis of the iodide **9a**, was obtained as colourless crystals (5.18 g, 90%), m.p. 71–73 °C (lit.,[17] m.p. 76 °C); δ_H 1.60 (m, 26 H, CH_2), 2.37 (m, 2 H,

CH_2CO), 3.21 (t, J 6.5, 2 H, CH_2I) and 11.30 (br s, 1 H, CO_2H).

8-Iodooctanoic acid **9c**. This compound, prepared from the bromo acid **7c** as described for the synthesis of the iodo acid **9a**, was obtained as colourless crystals (2.51 g, 83%), m.p. 42.5–44 °C; δ_H 1.85 (m, 12 H, CH_2), 3.18 (t, J 6.5, 2 H, CH_2I) and 9.05 (br s, 1 H, CO_2H); ν_{max}/cm^{-1} 3450–2400, 2932, 2856 and 1710; m/z (EI) 270 (M^+), 252, 143, 125, 97, 83 and 55.

11-Iodoundecanoic acid **9d**. This compound prepared from the bromo acid **7d** (10.0 g, 38 mmol) as described for the synthesis of the iodide **9a**, was obtained as colourless crystals (8.88 g, 75%), m.p. 64.5–65 °C (lit.,[14] m.p. 64–65 °C); δ_H 1.70 (m, 16 H, CH_2), 2.32 (m, 2 H, CH_2CO), 3.15 (t, J 6.5, 2 H, CH_2I) and 11.83 (br s, 1 H, CO_2H).

Methyl Undec-10-enoate **10d**.—Undec-10-enoic acid **8d** (3.02 g, 16.4 mmol) was added to methanol (200 cm^3) that had been pretreated with thionyl chloride (1.0 cm^3), and the mixture was stirred at room temperature for 4 h. The solvent was then removed under reduced pressure and the residue was dissolved in dichloromethane (50 cm^3). The resultant solution was washed with saturated aqueous sodium hydrogen carbonate (50 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. The residual oil distilled to give the title ester **10d** as a colourless oil (2.60 g, 80%), b.p. 130 °C/0.05 mmHg (block) (lit.,[18] b.p. 248 °C); δ_H 1.80 (m, 16 H, CH_2), 3.70 (s, 3 H, OCH_3), 5.00 (m, 2 H, ω1-vinylic) and 5.85 (ddt, J 16.5, 9.0, 6.5, 1 H, ω2-vinylic).

Methyl 10-iododecanoate **11a**. This compound, prepared from the iodo acid **9a** as described for the synthesis of the ester **10d**, was obtained as a colourless oil (1.12 g, 89%), b.p. 140 °C/0.03 mmHg (block) (lit.,[19] b.p. 139–141 °C/0.15 mmHg); δ_H 1.60 (m, 14 H, CH_2), 2.31 (m, 2 H, CH_2CO), 3.18 (t, J 6.5, 2 H, CH_2I) and 3.67 (s, 3 H, OCH_3).

Methyl 16-iodohexadecanoate **11b**. This compound, prepared from the iodo acid **9b** as described for the synthesis of the ester **10d**, was obtained as colourless crystals after recrystallization from methanol (4.38 g, 84%), m.p. 37.5–38 °C; δ_H 1.62 (m, 26 H, CH_2), 2.31 (m, 2 H, CH_2CO), 3.22 (t, J 6.5, 2 H, CH_2I) and 3.70 (s, 3 H, OCH_3); ν_{max}/cm^{-1} 2928, 2852, 1730 and 1174; m/z (EI) 396 (M^+), 364, 259, 226 and 208.

Methyl 8-iodooctanoate **11c**. This compound, prepared from the iodo acid **9c** as described for the synthesis of the ester **10d**, was obtained as a colourless oil (2.22 g, 88%), b.p. 150 °C/0.2 mmHg (block); $\delta_H(CCl_4)$ 1.80 (m, 12 H, CH_2), 3.15 (t, J 6.5, 2 H, CH_2I) and 3.64 (s, 3 H, OCH_3); ν_{max}/cm^{-1} 2928, 2852, 1738, 1436 and 1174; m/z (EI) 285 ($M^+ + 1$), 253, 183, 169, 157, 125, 97 and 83.

Methyl 11-iodoundecanoate **11d**. This compound, prepared from the iodo acid **9d** as described for the synthesis of the ester **10d**, was obtained as a colourless oil (4.59 g, 95%), b.p. 180 °C/0.04 mmHg (block) (lit.,[5] b.p. 98–102 °C/0.15 mmHg), which solidified with time, m.p. 24–25 °C; δ_H 1.60 (m, 16 H, CH_2), 2.32 (m, 2 H, CH_2CO), 3.20 (t, J 6.5, 2 H, CH_2I) and 3.69 (s, 3 H, OCH_3).

Methyl Octadec-17-enoate **10f**.—By the method of Bergbreiter and Whitesides,[5] a solution of methyllithium in ether (1.2 mol dm^{-3}; 3.2 cm^3, 3.84 mmol) was added slowly to a suspension of cuprous iodide (0.867 g, 4.55 mmol) in tetrahydrofuran (4.5 cm^3), while the temperature was maintained between −60 and −78 °C. The resultant mixture was stirred at −78 °C for 1 h after which it was slowly allowed to warm to 0 °C, whereupon a bright yellow suspension formed. The mixture was immediately cooled to −78 °C and a solution of hept-6-enylmagnesium bromide [formed by the addition of 1-bromohept-6-ene[20] (1.61 g, 9.10 mmol) to magnesium (0.24 g, 9.87 mmol) in tetrahydrofuran (7 cm^3), under an atmosphere of nitrogen] was added, while the temperature was maintained below −60 °C. The mixture thus obtained was stirred at −78 °C for 1 h and then allowed to warm to 0–10 °C, whereupon a distinct purple colouration appeared. The mixture was then cooled to −78 °C and a solution of methyl 11-iodoundecanoate **11d** (1.44 g, 4.42 mmol) in tetrahydrofuran (15 cm^3) was added, while the temperature was maintained < −60 °C. That mixture was stirred at −78 °C for 1 h and then allowed to warm to room temperature whereupon it was stirred for 2 h, before being poured into saturated aqueous ammonium chloride (20 cm^3). The layers that formed were separated and the aqueous layer was extracted with ether (3 × 15 cm^3). The organic layer and the ether extracts were combined and the mixture was washed with brine (30 cm^3), dried ($MgSO_4$), and concentrated under pressure. Chromatography of the residual oil, with ether–light petroleum as eluent gave the title ester **10f** as colourless crystals (0.837 g, 74%), m.p. 24–26 °C; δ_H 1.31 (m, 26 H, CH_2), 2.22 (m, 4 H, CH_2CO and CH_2C=), 3.63 (s, 3 H, OCH_3), 4.98 (m, 2 H, ω1-vinylic), and 5.83 (ddt, J 16.5, 9.0, 6.5, 1 H, ω2-vinylic); ν_{max}/cm^{-1} 3076, 2924, 2848, 1742, 1642, 1466, 1438, 1176 and 910; m/z (EI) 296 (M^+), 265, 264, 222, 87 and 74.

For complete characterization, a sample of the ester **10f** was heated with dilute aqueous sodium hydroxide at reflux for 24 h,[5] to give octadec-17-enoic acid, m.p. 56–56.5 °C (lit.,[21] m.p. 55.5–56.1 °C), as colourless crystals from methanol.

Methyl 10-Oxodecanoate **12d**.—Ozone-containing oxygen was bubbled through a solution of the ester **10d** (1.0 g, 5.04 mmol) in chloroform (50 cm^3) for 5 h, while the mixture was maintained between −10 and −20 °C. Dimethyl sulfide (0.31 cm^3, 7.21 mmol) was then added to the mixture which was then stirred at room temperature for 16 h before concentration under reduced pressure. The residual oil was dissolved in dichloromethane (30 cm^3) and the solution was washed with water (2 × 20 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography of the resultant oil, with ethyl acetate–light petroleum as eluent, gave the title ω-oxo ester **12d** as a colourless oil (0.49 g, 49%); δ_H 1.52 (m, 12 H, CH_2), 2.30 (m, 4 H, CH_2CO), 3.65 (s, 3 H, OCH_3) and 9.85 (t, J 1.8, 1 H, CHO). The spectral properties of the oxo ester **12d** are consistent with those reported previously.[22]

Methyl 13-Oxotridecanoate **12e**.—Treatment of the cuprate prepared from but-3-enyl bromide and the iodoester **11a**, as described for the synthesis of the ester **10f**, gave a 5:2 mixture of the unsaturated ester **10e** and methyl undecanoate as a colourless oil. The ester **10e** had δ_H 1.30 (s, 18 H, CH_2), 2.20 (m, 4 H, CH_2CO and CH_2C=), 3.65 (s, 3 H, OCH_3) 4.95 (m, 2 H, ω1-vinylic) and 5.83 (ddt, J 16.5, 9.0, 6.5, 1 H, ω2-vinylic). Methyl undecanoate had δ_H 0.87 (m, 3 H, CCH_3), 1.27 (m, 16 H, CH_2), 2.29 (m, 2 H, CH_2CO) and 3.66 (s, 3 H, OCH_3). A portion of the mixture was treated with ozone-containing oxygen followed by dimethyl sulfide, as described for the synthesis of the oxo ester **12d**, to give the title ω-oxo ester **12e** as a colourless oil (0.36 g, 68%); δ_H 1.85 (m, 22 H, CH_2), 3.67 (s, 3 H, OCH_3) and 9.85 (t, J 1.8, 1 H, CHO); ν_{max}/cm^{-1} 2924, 2852, 2716, 1740, 1466, 1438 and 1172; m/z (EI) 242 (M^+), 214, 211, 199, 167 and 54; m/z (EI) 242.187 (M^+) [Calc. for $C_{14}H_{26}O_3$ (M^+) m/z 242.188]. The spectral properties of the oxo ester **12e** are consistent with those reported previously.[23]

Methyl 17-oxoheptadecanoate **12f**. This compound, prepared from the unsaturated ester **10f** as described for the synthesis of the oxo ester **12d**, was obtained as a colourless solid (67%), m.p. 38–39.5 °C, after recrystallization from light petroleum; δ_H 1.55 (m, 26 H, CH_2), 2.33 (m, 4 H, CH_2CO), 3.65 (s, 3 H, OCH_3) and 9.83 (t, J 1.8, 1 H, CHO); ν_{max}/cm^{-1} 2928, 2852, 2728, 1724, 1409 and 1176; m/z (EI) 298 (M^+), 266, 254, 222, 122, 98 and 74; m/z (EI) 298.250 (M^+) [Calc. for $C_{18}H_{34}O_3$ (M^+); m/z 298.251].

Methyl 18-*bromooctadecanoate* **13**. This compound, prepared from the ester **10f** as described for the synthesis of the bromide **7c**, was obtained as colourless crystals (1.02 g, 66%), m.p. 36–37 °C (lit.,[24] m.p. 35–36 °C); δ_H 1.45 (m, 30 H, CH_2), 2.28 (m, 2 H, CH_2CO), 3.38 (t, *J* 6.5, 2 H, CH_2Br) and 3.65 (s, 3 H, OCH_3).

Methyl 18-*iodooctadecanoate* **11f**. This compound, prepared from the bromo ester **13** as described for the synthesis of the iodide **5b**, was obtained as colourless crystals after recrystallization from methanol (0.82 g, 73%), m.p. 44–44.5 °C; δ_H 1.55 (m, 30 H, CH_2), 2.28 (m, 2 H, CH_2CO), 3.22 (t, *J* 6.5, 2 H, CH_2I) and 3.71 (s, 3 H, OCH_3); ν_{max}/cm^{-1} 2924, 2852 and 1730; *m/z* (EI) 424 (M^+), 393, 293, 265, 247 and 155.

Octadecyltriphenylphosphonium Bromide.—A mixture of the bromide **1a** (2.05 g, 6.15 mmol), triphenylphosphine (3.78 g, 14.4 mmol) and acetonitrile (20 cm^3) was heated at reflux for 16 h. The resultant mixture was cooled and concentrated under reduced pressure to afford a colourless solid, which was washed several times with ethyl acetate and then recrystallized from dichloromethane–light petroleum to give the title salt as colourless crystals (1.98 g, 54%), m.p. 98–99.5 °C (lit.,[25] m.p. 99–100 °C).

Methyl (Z)-*Octacos*-10-*enoate* **14a**.—A solution of lithium hexamethyldisilazide, generated by the addition of butyllithium (1.5 mol dm^{-3} in hexane; 1.35 cm^3, 2.03 mmol) to hexamethyldisilazane (0.43 cm^3, 2.04 mmol) in tetrahydrofuran (1.6 cm^3) at 0 °C, was added to a suspension of octadecyltriphenylphosphonium bromide (1.20 g, 2.01 mmol) in tetrahydrofuran–hexamethylphosphoramide (4:1; 3 cm^3) cooled to 0 °C. The resultant orange solution was stirred for 10 min at 0 °C and then cooled to −78 °C when a solution of the oxo ester **12d** (0.20 g, 1.00 mmol) in tetrahydrofuran (3 cm^3) was added to it at that temperature. The mixture was subsequently allowed to warm to 0 °C and was stirred at that temperature for 1 h; it was then poured into saturated aqueous ammonium chloride (20 cm^3) and extracted with ethyl acetate (3 × 15 cm^3). The combined organic extracts were washed with water (20 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure to give an oil, which was chromatographed with ether–light petroleum as eluent, to give the title ester **14a** as colourless crystals after recrystallization from acetone (0.29 g, 67%), m.p. 36–37 °C; ν_{max}/cm^{-1} 2996, 2928, 2848, 1730, 1466, 1440 and 1176; *m/z* (EI) 437 ($M^+ + 1$), 436 (M^+), 405, 404, 362, 320, 228, 213, 199, 185, 171, 111, 97, 83, 69, 55, 43 and 41; *m/z* (EI) 436.426 (M^+) [Calc. for $C_{29}H_{56}O_2$ (M^+); *m/z* 436.428] (Found: C, 79.7; H, 13.2. Calc. for $C_{29}H_{56}O_2$: C, 79.8; H, 12.9%).

Nonyltriphenylphosphonium Bromide.—A mixture of the bromide **1b** (1.0 g, 4.83 mmol), triphenylphosphine (1.40 g, 5.34 mmol) and acetonitrile (4 cm^3) was heated at reflux for 36 h and then cooled and concentrated under reduced pressure to afford a colourless oil. This was washed several times with ether and then dried under reduced pressure to give the title salt as a hygroscopic, colourless glass. This material was used without characterization or purification.

Methyl (Z)-*hexacos*-17-*enoate* **14b**. This compound, prepared from the bromide **4a** and the ω-iodo ester **11c** as described for the synthesis of the ester **10f**, was obtained as a colourless oil (58 mg, 14%); ν_{max}/cm^{-1} 3004, 2924, 2852, 1744, 1650, 1468 and 1264; *m/z* (EI) 409 ($M^+ + 1$), 408 (M^+), 377, 376, 334, 292, 172, 143, 141, 129, 119, 117 87, 74, 55, 43 and 41; *m/z* (EI) 408.396 (M^+) [Calc. for $C_{27}H_{52}O_2$ (M^+) *m/z* 408.397] (Found: C, 79.1; H, 12.4. Calc. for $C_{27}H_{52}O_2$: C, 79.3; H, 12.8%).

The ester **14b** was also prepared from nonyltriphenylphosphonium bromide and the oxo ester **12f**, as described for the synthesis of the ester **14a**. After chromatography of the crude material, with ether–light petroleum as eluent, the product **14b** (16 mg, 13%) had physical and spectral properties identical with those described above.

Methyl (Z)-*docos*-13-*enoate* **14c**. This compound, prepared from the bromide **4a** and methyl 4-iodobutyrate, as described for the synthesis of the ester **10f**, was obtained as a colourless oil (18%); ν_{max}/cm^{-1} 3004, 2920, 2842, 1744, 1650, 1466 and 1170; *m/z* (EI) 353 ($M^+ + 1$), 352 (M^+), 321, 320, 278, 253, 236, 157, 125 and 97; *m/z* (EI) 352.336 (M^+) [Calc. for $C_{23}H_{44}O_2$ (M^+) *m/z* 352.334].

The ester **14c** was also prepared from nonyltriphenylphosphonium bromide and the oxo ester **12e**, as described for the synthesis of the ester **14a**. After chromatography of the crude material, with ether–light petroleum as eluent, the product **14c** (19 mg, 12%) contaminated with *ca.* 5% of the corresponding *trans*-isomer had physical and spectral properties similar to those described above. The ^{13}C NMR spectrum showed all the expected signals for the ester **14c**, with additional signals at δ 32.6(2) and 130.3(5) for the corresponding *trans*-isomer. The spectral properties of the ester **14c** are consistent with those reported.[26]

Methyl nonacosanoate **14d**. This compound, prepared from the bromide **1a** and the ω-iodo ester **11d** as described for the synthesis of the ester **10f**, was obtained as colourless crystals after recrystallization from light petroleum (0.11 g, 23%), m.p. 69–70 °C (lit.,[27] m.p. 68.8 °C); ν_{max}/cm^{-1} 2924, 2852, 1730, 1468 and 1194; *m/z* (EI) 452 (M^+), 420, 409, 395, 381, 367, 353, 199, 185, 143, 129, 87 and 74.

Methyl (Z)-*tetratriacont*-25-*enoate* **14e**. This compound, prepared from the bromide **4a** and the ω-iodo ester **11b** as described for the synthesis of the ester **10f**, was obtained as a colourless wax (63 mg, 13%), m.p. 45.5–46 °C; ν_{max}/cm^{-1} 3004, 2928, 2848, 1742, 1650, 1468 and 1172; *m/z* (EI) 521 ($M^+ + 1$), 520 (M^+), 489, 488, 446, 415, 97, 83, 74, 69, 57 and 55; *m/z* (EI) 520.519 (M^+) [Calc. for $C_{35}H_{68}O_2$ (M^+) *m/z* 520.522] (Found: C, 81.1; H, 13.7. Calc. for $C_{35}H_{68}O_2$: C, 80.7; H, 13.2%).

Methyl (Z)-*tetratriacont*-27-*enoate* **14f**. This compound, prepared from the bromide **4c** and the ω-iodo ester **11f** as described for the synthesis of the ester **10f**, was obtained as a colourless wax (35 mg, 10%), m.p. 46–47 °C; ν_{max}/cm^{-1} 3008, 2924, 2852, 1742, 1650, 1468, 1262, 1172, 1116 and 1014; *m/z* (EI) 521 ($M^+ + 1$), 520 (M^+), 489, 488, 446, 404, 143, 125, 111, 97, 83, 74, 69, 57, 55 and 43; *m/z* (EI) 520.520 (M^+) [Calc. for $C_{35}H_{68}O_2$ (M^+) *m/z* 520.522] (Found: C, 80.8; H, 13.7. Calc. for $C_{35}H_{68}O_2$: C, 80.7; H, 13.2%).

Methyl (Z,Z)-*tetratriaconta*-25,28-*dienoate* **14g**. This compound, prepared from the bromide **4b** and the ω-iodo ester **11b** as described for the synthesis of the ester **10f**, was obtained as a colourless wax (107 mg, 22%), m.p. 43–43.5 °C; ν_{max}/cm^{-1} 3008, 2924, 2848, 1742, 1650, 1468 and 1172; *m/z* (EI) 519 ($M^+ + 1$), 518 (M^+), 517, 487, 486, 284, 279, 253, 241, 227, 199, 185, 143, 129 and 87; *m/z* (EI) 518.504 (M^+) [Calc. for $C_{35}H_{66}O_2$ (M^+) *m/z* 518.506].

Methyl (Z,Z)-*Hexacosa*-17,20-*dienoate* **14h**.—A solution of *tert*-butyllithium in pentane (1.6 mol dm^{-3}; 1.0 cm^3, 1.60 mmol) was added to a solution of the iodide **5b** (0.30 g, 0.80 mmol) in ether (0.80 cm^3) at −78 °C, and the mixture was stirred at that temperature for 15 min. Cuprous iodide (0.15 g, 0.80 mmol) was then added to it and a black solid formed. Stirring was continued at −78 °C for 1 h, after which the temperature of the mixture was allowed to warm to 0 °C. The mixture was then cooled to −78 °C and a solution of the Grignard reagent generated from the bromide **4b** (0.32 g, 0.97 mmol) and magnesium (0.06 g, 2.47 mmol) in tetrahydrofuran (1.5 cm^3) was added to it at −78 °C and stirring was continued for 1 h. The resultant mixture was warmed to 0 °C, whereupon a black suspension formed, and then recooled to −78 °C and treated with a solution of the ω-iodo ester **11c** (0.41 g, 1.44 mmol) in

tetrahydrofuran (1 cm^3). That mixture was stirred for 1 h at −78 °C and then allowed to warm to room temperature over 3 h; it was then stirred at room temperature for a further 2 h. The mixture thus obtained was poured into aqueous ammonium chloride (20 cm^3). The layers that formed were separated and the aqueous layer was extracted with ether (3 × 10 cm^3). The organic layer and the ether extracts were combined, washed with brine (20 cm^3), dried ($MgSO_4$), and concentrated under reduced pressure. Chromatography of the residual oil, with ether–light petroleum as eluent, gave the title ester **14h** (37 mg, 12%); ν_{max}/cm^{-1} 3008, 2928, 2852, 1742, 1650, 1466 and 1172; *m/z* (EI) 407 ($M^+ + 1$), 406 (M^+), 375, 374, 123, 109, 85, 81, 67, 55 and 41; *m/z* (EI) 406.380 (M^+) [Calc. for $C_{27}H_{50}O_2$ (M^+) *m/z* 406.381].

Methyl (Z,Z,Z)-*tetratriaconta*-25,28,31-*trienoate* **14i**. This compound prepared from the bromide **4d**, the iodide **5d**, and the ω-iodo ester **11b** as described for the synthesis of the ester **14h**, was obtained as a colourless wax (48 mg, 10%), m.p. 39–41 °C; ν_{max}/cm^{-1} 3008, 2924, 2848, 1742, 1650, 1466 and 1172; *m/z* (EI) 516 (M^+), 515, 284, 270, 253, 141, 227, 199, 185, 143 and 129; *m/z* (EI) 516.488 (M^+) [Calc. for $C_{35}H_{64}O_2$ (M^+) *m/z* 516.491] (Found: C, 81.2; H, 12.8. Calc. for $C_{35}H_{64}O_2$: C, 81.3; H, 12.5%).

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References

1 For a review see, T. Rezanka, *Progr. Lipid Res.*, 1989, **28**, 147.
2 For examples see, J. Templier, C. Largeau and E. Casadevall, *Phytochemistry*, 1987, **26**, 277; C. Litchfield, A. J. Greenberg, G. Noto and R. W. Morales, *Lipids*, 1976, **11**, 567; H. R. Schulten, N. Smmleit and H. H. Rump, *Chem. Phys. Lipids*, 1986, **41**, 209; M. I. Alvedano and H. Sprecher, *J. Biol. Chem.*, 1987, **262**, 1180; A. Poulos, P. Sharp, D. Johnson, I. White and A. Fellenberg, *Biochem. J.*, 1986, **240**, 891; A. Poulos, D. W. Johnson, K. Beckman, I. G. White and C. Easton, *Biochem. J.* 1987, **248**, 961; A. Poulos, P. Sharp, D. Johnson and C. Easton, *Biochem. J.*, 1988, **253**, 645.
3 C. Prakash, S. Saleh, B. J. Sweetman, D. F. Taber and I. A. Bair, *J. Labelled Compd. Radiopharm.*, 1989, **27**, 539; Y. Leblanc, B. J. Fitzsimmons, J. Adams, F. Perez and J. Rokach, *J. Org. Chem.*, 1986, **51**, 789; M. Labelle, J.-P. Falgueyret, D. Riendeau and J. Rokach, *Tetrahedron*, 1990, **46**, 6301.
4 L. D. Bergelson and M. M. Shemyakin, *Angew. Chem., Int. Ed. Eng.*, 1964, **3**, 250; L. D. Bergelson and M. M. Shemyakin, *Tetrahedron*, 1963, **19**, 149.
5 D. E. Bergbreiter and G. M. Whitesides, *J. Org. Chem.*, 1975, **40**, 779.
6 J. Hooz and S. S. H. Gilani, *Can. J. Chem.*, 1968, **46**, 86.
7 H. Matsuda, A. Yamamoto, N. Iwamoto and S. Matsuda, *J. Org. Chem.*, 1978, **43**, 4567.
8 P. D. Gokhali, V. S. Dalavoy, A. S. C. Prakasa Rao, U. R. Nayak and S. Dev, *Synthesis*, 1974, 718.
9 F. D. Gunstone, M. R. Pollard, C. M. Scrimgeour and H. S. Vedanayagam, *Chem. Phys. Lipids*, 1977, **18**, 115.
10 W. C. Still, M. Kahn and A. Mitra, *J. Org. Chem.*, 1978, **43**, 2923.
11 E. E. Reid, F. O. Cockerille, J. D. Meyer, W. M. Cox and J. R. Ruhoff, *Org. Synth.*, Coll. Vol. II, 1943, 468.
12 O. Turpeinen, *J. Am. Chem. Soc.*, 1938, **60**, 56.
13 E. Ucciani and R. Piantoni, *Chem. Phys. Lipids*, 1968, **2**, 256.
14 F. L. M. Pattison, J. B. Stothers and R. G. Woolford, *J. Am. Chem. Soc.*, 1956, **78**, 2255.
15 P. Chuit and J. Hausser, *Helv. Chim. Acta*, 1929, **12**, 463.
16 M. S. Raasch, *J. Am. Chem. Soc.*, 1953, **75**, 2956.
17 J. Bougault, *Compt. Rend.*, 1910, **150**, 874.
18 G. H. Jeffery and A. I. Vogel, *J. Chem. Soc.*, 1948, 658.
19 N. R. De Leu and H. C. Brown, *Synthesis*, 1976, 114.
20 P. Gaubert, R. P. Linstead and H. N. Rydon, *J. Chem. Soc.*, 1937, 1971.
21 W. F. Huber, *J. Am. Chem. Soc.*, 1951, **73**, 2730.
22 J.-H. Fuhrhop and M. Baccouche, *Justus Liebigs Ann. Chem.*, 1976, 2058.
23 K. M. Waugh and K. D. Berlin, *J. Org. Chem.*, 1984, **49**, 873.
24 U. Jensen-Korte and H.-J. Schafer, *Liebigs Ann. Chem.*, 1982, 1532.
25 T. Kauffmann, E. Rauch and J. Schulz, *Chem. Ber.*, 1973, **106**, 1612.
26 J. M. Bernassau and M. Fetizon, *Tetrahedron*, 1981, **37**, 2105.
27 F. Francis and S. H. Piper, *J. Am. Chem. Soc.*, 1939, **61**, 577.

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Formic acid is a product of the α-oxidation of fatty acids by human skin fibroblasts: deficiency of formic acid production in peroxisome-deficient fibroblasts

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Human skin fibroblasts in culture can oxidize β-methyl fatty acids, such as phytanic acid and 3-methylhexadecanoic acid, to CO_2 and water-soluble products. The latter are released largely into the culture medium. The major water-soluble product formed from [1-^{14}C]phytanic and [1-^{14}C]3-methylhexadecanoic acids is [^{14}C]formic acid. As phytanic acid and 3-methylhexadecanoic acids contain β-methyl groups and theoretically cannot be degraded by β-oxidation, we postulate that formic acid is formed from fatty acids by α-oxidation. The marked reduction in formic acid production from β-methyl fatty acids in peroxisome-deficient skin fibroblasts suggests that peroxisomes are involved in the generation of C_1 units.

INTRODUCTION

Fatty acid oxidation is an important biological process and takes place in most mammalian cells. Three separate oxidative pathways have been described, i.e. α-, β- and ω-oxidation. β-Oxidation has been studied in detail and is known to take place in mitochondria and peroxisomes. Different enzyme proteins are involved in mitochondrial and peroxisomal β-oxidation (Schulz, 1991). Acetyl-CoA is formed from fatty acids by β-oxidation in mitochondria and peroxisomes (Singh and Poulos, 1986), and is further metabolized in mitochondria via the citric acid cycle or is used in biosynthetic reactions in the cell. The intracellular site of α-oxidation is controversial. Subcellular fractionation studies carried out on rat, monkey and human liver suggest that α-oxidation takes place in mitochondria (Skjeldal and Stokke, 1987; Watkins et al., 1990; Wanders et al., 1991) but the occurrence of patients with abnormalities in peroxisomal assembly whose skin fibroblasts show marked reductions in their ability to α-oxidize fatty acids, suggests that α-oxidation takes place in peroxisomes in human skin fibroblasts (Poulos et al., 1986a; Skjeldal et al., 1986). The primary non-lipid product of α-oxidation is believed to be carbon dioxide, formed by decarboxylation after the introduction of a hydroxy group at the α-position of the fatty acid (Herndon et al., 1969; Steinberg, 1972). These early studies were based on experiments performed with [U-^{14}C]phytanic acid. However, more recent studies carried out with [1-^{14}C]phytanic acid have failed to show the presence of the expected α-hydroxylated intermediate and have therefore raised doubts about the postulated pathway (Skjeldal and Stokke, 1988).

We report here on the production of formic acid from β-methyl fatty acids by human skin fibroblasts in culture. These findings suggest that formic acid is an α-oxidation product of fatty acids.

MATERIALS AND METHODS

Materials

Analytical grade solvents were purchased from May and Baker Pty. Ltd., Melbourne, Victoria, Australia, or from Ajax Chemicals, Sydney, Australia. Basal modified Eagle's medium (BME) was purchased from Flow Laboratories, Irvine, Scotland, U.K., and fetal calf serum was obtained from Gibco New Zealand Ltd. Formate dehydrogenase (from *Candida boidii*) was purchased from Boehringer Mannheim, Mannheim, Germany. Sodium [^{14}C]cyanide (47 mCi/mmol), [1-^{14}C]acetic acid (55 mCi/mmol), [^{14}C]formic acid (52.5 mCi/mmol), and NCS tissue solubilizer were purchased from Amersham Australia Pty. Ltd., Sydney, Australia. All other chemicals were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A.

Methods

[1-^{14}C]Phytanic acid (47 mCi/mmol) and [1-^{14}C]pristanic acid (47 mCi/mmol) were synthesized as described by Poulos et al. (1980) and by Johnson and Poulos (1989) respectively. [1-^{14}C]3-Methylhexadecanoic acid (47 mCi/mmol) was synthesized from 2-methylpentadecanoic acid via a reaction sequence involving a Favorsky rearrangement (Johnson and Poulos, 1989). The 2-methyl acid was then converted to [1-^{14}C]3-methylhexadecanoic acid by a chain elongation procedure involving methylation, reduction, mesylation, conversion to a [^{14}C]nitrile and alkaline hydrolysis. The structures of all radiolabelled fatty acids used in these studies was confirmed by g.c./m.s. (Poulos et al., 1988). The radiochemical purities of [1-^{14}C]phytanic acid, β-methylhexadecanoic acid and pristanic acid were determined by reverse-phase t.l.c. essentially as described by Street et al. (1989), except that the non-esterified fatty acids rather than their corresponding methyl esters were chromatographed. Briefly, the fatty acids (200000–300000 d.p.m.) were applied to reverse phase KC-18 thin layer plates (Whatman Inc., Clifton, NJ, U.S.A.) and chromatograms were developed twice in the same direction in acetonitrile/tetrahydrofuran/acetic acid (90:10:1, by vol.). Under these conditions fatty acids were separated according to carbon chain length and degree of unsaturation. Autoradiographs were prepared by exposing the plates to Hyperfilm-^{3}H (Amersham) for 5 days. After development of autoradiographs, the chromatograms were divided into 1–2 cm zones and each

Abbreviation used: BME, basal modified Eagle's medium.

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zone was scraped from the plate and into scintillation vials containing 1 ml of water. The radioactivity content was determined after adding 10 ml of Optiphase Hisafe 3 (LKB, Loughborough, Leics., U.K.) and scintillation counting. The radiochemical purities of [1-^{14}C]phytanic acid, β-methylhexadecanoic acid and pristanic acid were > 99.3%, 99.9% and 98.0% respectively.

Skin fibroblast cultures were established in the Department of Chemical Pathology, Adelaide Children's Hospital, from skin biopsies of patients with peroxisomal disorders, and from individuals with no prior evidence of abnormality. The diagnoses of Zellweger's syndrome, infantile Refsum's disease and classical Refsum's disease were based on case histories and on clinical and biochemical investigations (Poulos et al., 1986a). Human skin fibroblasts were routinely grown under sterile conditions in 25 cm^2 culture flasks in BME containing 10% fetal calf serum until confluent. The medium was then removed and replaced with 3 ml of BME containing 0.5% fetal calf serum. Endogenous phytanic acid, as determined by gas chromatographic analysis (Poulos et al., 1988), could not be detected either in the BME or in the fetal calf serum used for cell culture. After 24 h the medium was replaced with BME containing 0.5–1.0% fetal calf serum and 0.05–0.06 μCi of [1-^{14}C]phytanic acid. $^{14}CO_2$ production was measured after 4 days as reported earlier (Poulos, 1981; Poulos et al., 1986b). After collection of $^{14}CO_2$, the acidified culture medium was extracted by the Bligh and Dyer (1959) method. The radioactivity in the upper aqueous phase, which contained the water-soluble metabolites, was determined by liquid scintillation counting. For studies involving the characterization of the water-soluble product, larger amounts of radiolabelled substrate were added to the skin fibroblast cultures (0.33 μCi of [1-^{14}C]phytanic acid, 0.33 μCi of [1-^{14}C]pristanic acid and 0.30 μCi of [1-^{14}C]3-methylhexadecanoic acid) and incubated at 37 °C for 4 days, except for the cultures containing [1-^{14}C]pristanic acid which were incubated for 1 day (Singh et al., 1990). Fatty acid analysis of the culture medium was carried out as described by Poulos et al. (1988).

Characterization of radiolabelled water-soluble product

Ion-exchange chromatography

The cell culture medium after incubation with radiolabelled substrates was acidified and partitioned according to the Bligh and Dyer (1959) method. The radioactive product present in the upper aqueous phase was applied to a Dowex 50W-X8 (200–400 mesh; hydrogen form) column. The column was eluted with 5 column vol. of 0.1 M HCl. The non-retained material was neutralized and applied to a column of Bio-Rad AG1 X8 (100–200 mesh; propionate form) and washed first with 6 column vol. of water and then eluted with 4 vol. of 1.5 M HCl.

H.p.l.c

After incubation with the radiolabelled fatty acids, the culture medium was acidified and evaporated to dryness under vacuum, and the volatile material was collected in an alcohol/solid CO_2 trap. The volatile fractions were made alkaline with the addition of 50 μl of 1 M NaOH and lyophilized. The residue was dissolved in 250 μl of 0.1 M HCl, the pH was adjusted to pH < 3, and 100 μl was injected into a Beckman single pump h.p.l.c. apparatus fitted with a Bio-Rad Aminex ion-exclusion HPX-87H column (300 mm × 7.8 mm). The column was eluted with 6 mM H_2SO_4 at a flow rate of 0.5 ml/min and the eluate was monitored at 210 nm using a Beckman 163 variable wavelength detector. The various absorbing fractions, as well as the fractions in between, were collected manually and their radioactivity content was determined by liquid scintillation counting. For some experiments reverse-phase h.p.l.c. was also carried out using an SGE ODS C18 column (250 mm × 4 mm), both on its own and in tandem with the ion-exclusion column (Buchanan and Thoene, 1982), using 6 mM H_2SO_4 as the elution solvent.

Recovery of formic acid was assessed by adding [^{14}C]formic acid (83500 d.p.m.; 52.5 mCi/mmol) and unlabelled formic acid (40 nmol) to 2 ml of culture medium (1% fetal calf serum in BME), trapping the volatile fractions and counting the radioactivity. Under the conditions described, > 90% of the added label was recovered in the volatile fraction and < 5% of the remaining label was lost after lyophilization. More than 85% of the [^{14}C]formic acid applied to the ion-exclusion column was recovered in a peak eluting with unlabelled formic acid. The overall recovery was 65–70%.

Enzymic analysis

Enzymic analysis of the water-soluble products was carried out by a modification of the method outlined by Morand et al. (1988). The incubation mixtures contained an aliquot of the volatile fraction (containing 16000–19000 d.p.m. of volatile product) or [^{14}C]formic acid (78800 d.p.m.; 52.5 mCi/mmol), KCl (110 mM), NaCl (550 mM), unlabelled formic acid (400 μM), NAD^+ (7.5 mM) and formate dehydrogenase (0.5 mg; 0.47 unit/mg) in 2.0 ml of 100 mM sodium phosphate buffer, pH 6.8. Incubations were carried out for 2 h at 37 °C in 25 cm^2 culture flasks sealed with a rubber stopper (Suba seal). At the end of this period 1 ml of 1 M H_2SO_4 was added and $^{14}CO_2$ released was collected by passing nitrogen through the culture flask via a syringe needle and trapping in 2 ml of NCS. $^{14}CO_2$ release was also measured in a control incubation from which formate dehydrogenase was omitted. Under these conditions $^{14}CO_2$ was released from [^{14}C]formic acid by formate dehydrogenase, but not from [1-^{14}C]acetic acid; 77.5% of the label from the volatile product (mean of duplicate incubations) was recovered as $^{14}CO_2$.

Table 1 Oxidation of [1-^{14}C]phytanic acid by skin fibroblasts in culture

The production of radiolabelled CO_2 and water-soluble products from [1-^{14}C]phytanic acid (0.05–0.06 μCi) by skin fibroblasts in culture was measured as outlined in the Materials and methods section. The data represent the means of duplicate assays. The protein content of the cell pellet from individual culture flasks varied from 179 to 451 μg. Endogenous phytanic acid (i.e. esterified and non-esterified) could not be detected in the culture medium added to the cells.

	Radiolabelled products formed (nmol) per mg of protein	
Cell line	CO_2	Water-soluble metabolites
Controls		
1	0.30	3.1
2	0.43	2.8
3	0.46	2.7
4	0.67	7.7
Refsum's disease	0.02	0.02
Infantile Refsum's disease	0.02	0.04
Zellweger's syndrome		
1	0.01	0.05
2	0.01	0.05

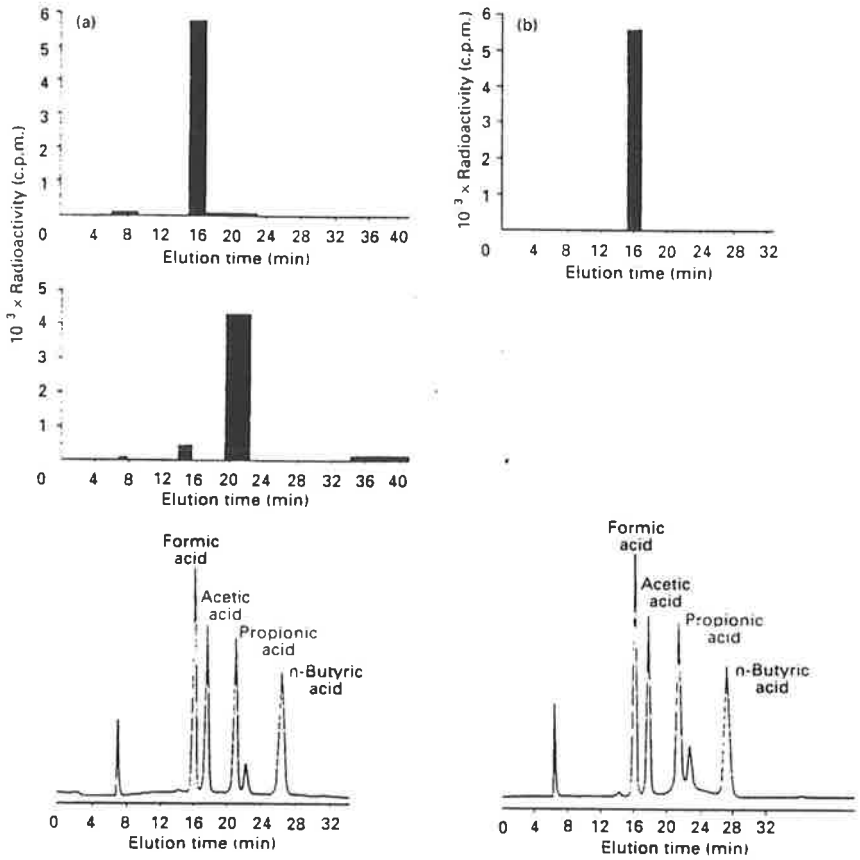

Figure 1 Volatile ^{14}C-labelled metabolites produced from [1-^{14}C]fatty acids by skin fibroblasts

(**a**) The volatile fatty acids produced from [1-^{14}C]fatty acids were subjected to h.p.l.c. as described in the Materials and methods section. The top two panels show the ion-exclusion h.p.l.c. radioactivity profile obtained with the volatile fractions produced from [1-^{14}C]phytanic acid (0.33 μCi) (top panel) and [1-^{14}C]pristanic acid (0.33 μCi) (middle panel). Chromatography was carried out on a Bio-Rad Aminex ion-exclusion HPX-87H column as outlined in the Materials and methods section. The bottom panel shows the profile obtained by chromatography of a mixture of unlabelled short-chain acids under the same conditions, with monitoring of the eluate at 210 nm. (**b**) The top panel shows the radioactivity profile obtained with the volatile products obtained from [1-^{14}C]3-methylhexadecanoic acid (0.30 μCi), while the bottom panel shows the profile obtained from a standard mixture of unlabelled short-chain acids. The chromatographic conditions were the same as those used in (**a**).

Chemical analysis

Chemical oxidation of the radiolabelled water-soluble product present in non-acidified medium was carried out as described by Hefetz and Blum (1978), except for the substitution of mercuric acetate for mercuric chloride. For these experiments 0.5 ml of culture medium (obtained after incubation of control skin fibroblasts with [1-^{14}C]phytanic acid) was treated with mercuric acetate and released $^{14}CO_2$ was trapped in NCS (as above). $^{14}CO_2$ was formed from [^{14}C]formic acid, but not from [1-^{14}C]phytanic acid, under these conditions.

Mass spectrometry

Mass spectrometric analysis of the volatile fraction isolated from the culture medium of cells incubated in the presence of [1-^{14}C]phytanic acid was performed on a JEOL DX-303 mass spectrometer operating in electron impact mode with the gas chromatograph inlet blanked off. The source pressure was 1.2×10^{-4} Pa (9×10^{7} Torr). Aqueous samples (5 μl; equivalent to the volatile products from a single culture flask) were injected into the heated inlet system with a reservoir temperature of 70 °C. The mass spectra of the various solutions were obtained at an electron multiplier setting of 1 kV, and the intensities of the common ions were measured relative to m/z 40 (argon). A 0.9% aqueous solution of formic acid in water afforded ions at m/z 48 [$(M+2)^+$, 0.000], 47 [$(M+1)^+$, 0.050], 46 [M^+, 1.83] and 45 [$(M-1)^+$, 1.50]. [^{14}C]Formic acid gave a series of ions that were 2 mass units higher than that observed for the unlabelled acid.

RESULTS AND DISCUSSION

Human skin fibroblasts in culture converted [1-^{14}C]phytanic acid to $^{14}CO_2$ (Table 1). In addition, up to 20% of the radioactivity from added [1-^{14}C]phytanic acid was converted into radiolabelled water-soluble products and released into the culture medium (Table 1). Most of the water-soluble products were volatile under acidic conditions, as around 75% of the radioactivity was lost under a nitrogen stream at 40 °C. However, little loss occurred if the evaporation was carried out under alkaline conditions. The

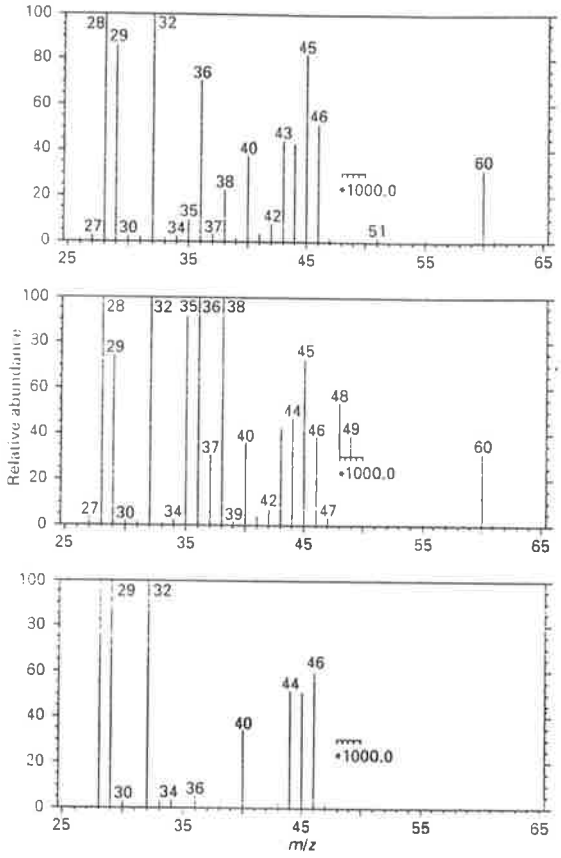

Figure 2 Mass spectrometric identification of [^{14}C]formic acid in the volatile fraction isolated from the cell culture medium

Normal fibroblasts were incubated with culture medium containing unlabelled phytanic acid (7 nmol) and [1-^{14}C]phytanic acid (0.33 μCi) and the volatile products were isolated (see the Materials and methods section). Mass spectra of volatile fractions produced from unlabelled phytanic acid (upper panel), [1-^{14}C]phytanic acid (middle panel), and an unlabelled formic acid standard (lower panel) are shown. Identical source conditions and electron multiplier settings were used.

amount of water-soluble products formed from [1-^{14}C]phytanic acid by fibroblasts of patients with disorders of peroxisomal assembly (Zellweger's syndrome and infantile Refsum's disease), and of patients with Refsum's disease, was markedly depressed compared with control cells (Table 1). These cells showed the expected reductions in $^{14}CO_2$ production, an indicator of abnormal phytanic acid oxidase activity.

These observations suggested that the volatile water-soluble product was a short-chain acid and was not formed in peroxisome-deficient cells. Further supporting evidence for this hypothesis was provided by the demonstration that most (70–80%) of the water-soluble product was retained on AG1 X8 (propionate form) anion-exchange columns and was eluted with 1.5 M HCl. Because of the difficulties inherent in the characterization of the volatile water-soluble product in the presence of the relatively large concentrations of hydrophilic substances, the culture medium was evaporated to dryness under vacuum and the volatile products were recovered in an alcohol/solid CO_2 trap. Of the total radioactivity added to the cells, 21.6% (mean of four incubations) was recovered in the volatile fraction isolated from the acidified culture medium. This figure correlated closely with the amount of volatile material (determined by counting aliquots of the acidified culture after air drying). Between 85 and 90% of the volatile radioactivity eluted from ion-exclusion (Figure 1) and reverse-phase h.p.l.c. (results not shown) columns as a single peak which co-eluted with formic acid.

Small amounts of unlabelled formic acid and acetic acid, together with a number of other unidentified substances, were detected by ion-exclusion h.p.l.c. of the volatile fractions isolated from the culture medium (results not shown).

The mass spectra, obtained under identical conditions, of the volatile fractions recovered from human skin fibroblasts which had been cultured in the presence of unlabelled phytanic acid and [1-^{14}C]phytanic acid respectively, were compared (Figure 2). In both cases, formic acid (M^+: m/z 46) and acetic acid (M^+: m/z 60) were the major identifiable components (present in the culture medium). The mass spectrum derived from the [1-^{14}C]phytanic acid culture also showed a small but significant ion at m/z 48 which had been observed in the mass spectrum of [1-^{14}C]formic acid. Formate dehydrogenase treatment of the radioactive water-soluble product released $^{14}CO_2$. Also, treatment of the radiolabelled water-soluble product with mercuric acetate resulted in the formation of $^{14}CO_2$. These observations provide evidence that an α-oxidation product of phytanic acid is formic acid.

To determine whether the [^{14}C]formic acid produced was specific for phytanic acid, we compared the volatile radioactive products formed from [1-^{14}C]phytanic acid and two other branched-chain fatty acids, [1-^{14}C]pristanic acid and [1-^{14}C]3-methylhexadecanoic acid. The data shown in Figure 1 demonstrate that there are differences in the short-chain fatty acids produced from the three substrates. The major volatile radiolabelled product formed from the fatty acids containing a β-methyl group, i.e. [1-^{14}C]phytanic and [^{14}C]3-methylhexadecanoic acids, was [^{14}C]formic acid. In contrast, [^{14}C]propionic acid was the major volatile product formed from [1-^{14}C]pristanic acid (a fatty acid that can undergo β-oxidation). As [^{14}C]formic acid was also detected in cultures incubated with [1-^{14}C]3-methylhexadecanoic acid, this suggests that any fatty acid which cannot be degraded by β-oxidation is degraded by α-oxidation, and one oxidation product formed by this pathway is formic acid.

To our knowledge, this is the first report showing that formic acid can be produced from fatty acids by human skin fibroblasts. Activated single-carbon units, normally linked to tetrahydrofolic acid, are formed during the catabolism of purines and amino acids (Rabinowitz and Pricer, 1956; Hefetz and Blum, 1978), and enzymes which can cleave these compounds to release free formic acid have been described in bacterial, insect and mammalian tissues (Whiteley, 1960; Rader and Huenneken, 1973; Hefetz and Blum, 1978), although the synthesis of free formic acid in high concentrations is more a feature of certain specialized tissues such as the poison glands of ants (Hefetz and Blum, 1978). Formic acid can also be produced by irradiation of 12-(1-pyrene)dodecanoic acid-photosensitized mammalian cells (Morand et al., 1988). Our data indicate that formic acid production is linked to the α-oxidation of β-methyl fatty acids. Whether it is formed directly, or indirectly from CO_2, is not known. As formic acid production is several-fold greater than CO_2 production, we speculate that CO_2 is formed from formic acid. This hypothesis is supported by preliminary experiments indicating that fibroblasts in culture, or in suspension, can generate $^{14}CO_2$ from [^{14}C]formic acid. Early studies on the α-oxidation of phytanic acid indicated that the first step involved is an α-hydroxylation of phytanic acid, followed by a subsequent

decarboxylation of the hydroxylated product to give pristanic acid (Herndon et al., 1969). CO_2 is believed to be the sole non-lipid product of α-oxidation. Whether a single enzyme is responsible for the hydroxylation and decarboxylation, or whether more than one enzyme is involved in the process, is not clear. The production of formic acid from phytanic acid raises some doubts as to the proposed sequence of reactions (Herndon et al., 1969).

The oxidation of fatty acids is a major peroxisomal function. There is now good evidence that the acetate released by peroxisomal β-oxidation is utilized in biosynthetic reactions (Street et al., 1990). Our data showing the generating of formic acid via the α-oxidation of fatty acids believed to be oxidized predominantly in peroxisomes suggest that peroxisomes are involved in the production of C_1 units which can be utilized for biosynthetic processes within the cell.

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REFERENCES

Bligh, E. G. and Dyer, W. J. (1959) Can. J. Biochem. Physiol. **37**, 911–917
Buchanan, D. N. and Tocher, J. G. (1982) Anal. Biochem. **124**, 106–116
Hietala, A. and Rilm, M. S. (1978) Biochim. Biophys. Acta **543**, 484–496
Herndon, J. H., Steinberg, D., Uhlendorf, B. W. and Fales, H. M. (1969) J. Clin. Invest. **48**, 1017–1031
Johnson, D. W. and Poulos, A. (1989) Biomed. Environ. Mass Spectrom. **18**, 603–612
Morand, O. H., Zoeller, R. A. and Raetz, C. R. H. (1988) J. Biol. Chem. **263**, 11597–11606
Poulos, A. (1981) Clin. Genet. **20**, 247–253
Poulos, A., Barone, E. and Johnson, D. W. (1980) Lipids **15**, 19–21
Poulos, A., Singh, H., Paton, B., Sharp, P. and Derwas, N. (1986a) Clin. Genet. **29**, 397–408
Poulos, A., van Crugten, C., Sharp, P., Carey, W. F., Robertson, E. F., Becroft, D. M. O., Saudubray, J. M., Poll-The, B. T., Christensen, E. and Brandt, N. (1986b) Eur. J. Pediatr. **145**, 507–510
Poulos, A., Sharp, P., Fellenberg, A. J. and Johnson, D. W. (1988) Eur. J. Pediatr. **147**, 143–147
Rabinowitz, J. C. and Pricer, W. E. (1956) J. Biol. Chem. **218**, 189–194
Rader, J. I. and Huenneken, F. M. (1973) Enzymes 3rd Ed. **11**, 207–223
Robertson, E. F., Poulos, A., Sharp, P., Manson, J., Wise, G., Jaunzems, A. and Carter, R. F. (1988) Eur. J. Pediatr. **147**, 133–142
Schulz, H. (1991) Biochim. Biophys. Acta **1081**, 109–120
Singh, H. and Poulos, A. (1986) Arch. Biochem. Biophys. **250**, 171–179
Singh, H., Usher, S., Johnson, D. and Poulos, A. (1990) J. Lipid Res. **31**, 217–225
Skjeldal, O. H. and Stokke, O. (1987) Biochim. Biophys. Acta **921**, 38–42
Skjeldal, O. H. and Stokke, O. (1988) Scand. J. Clin. Invest. **48**, 97–102
Skjeldal, O. H., Stokke, O., Norseth, J. and Lie, S. O. (1986) Scand. J. Clin. Invest. **46**, 283–287
Steinberg, D. (1972) in The Metabolic Basis of Inherited Disease (Stansbury, J. B., Wyngaarden, J. B. and Frederickson, D. S., eds.), 3rd edn., pp. 833–853, McGraw-Hill Book Co., New York
Street, J. M., Johnson, D. W., Singh, H. and Poulos, A. (1989) Biochem. J. **260**, 647–655
Street, J. M., Singh, H. and Poulos, A. (1990) Biochem. J. **269**, 671–677
Wanders, R. J. A., van Roermund, C. W. T., Jakobs, C. and Ten Brink, H. J. (1991) J. Inher. Metab. Dis. **14**, 349–352
Watkins, P. A., Mihalik, S. J. and Skjeldal, O. H. (1990) Biochem. Biophys. Res. Commun. **167**, 586–590
Whiteley, H. R. (1960) Comp. Biochem. Physiol. **1**, 227–247

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Crystal structure of 1-acetyl-6-(1-methylethyl)-3-(prop-2-enyl)-2,5-piperazinedione, $C_{12}H_{18}N_2O_3$

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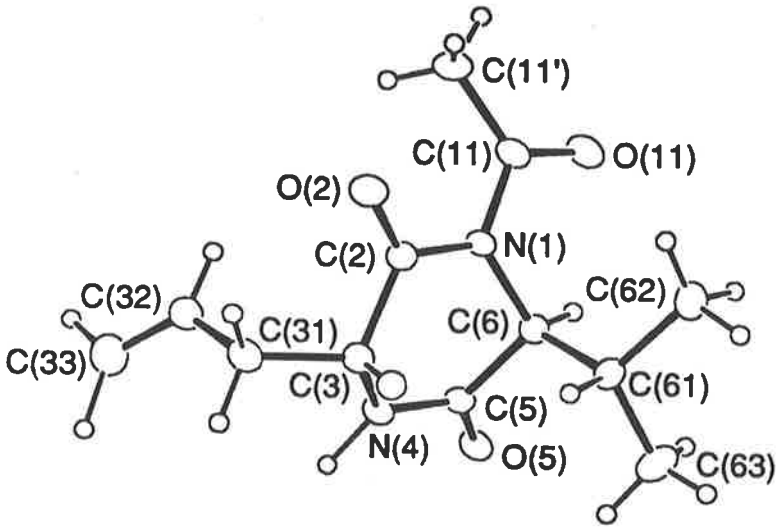
(Received November 28, 1992)



Source of material: see ref. 1.
The title compound was prepared from the partial hydrolysis of the corresponding 1,4-diacyldiketopiperazine; structure analysis was employed to determine the regioselectivity of deacylation, and the stereochemistry at C(3) and C(6).

Monoclinic, $P12_1/c1$ (No. 14), $a = 8.778(2)$ Å, $b = 15.416(1)$ Å, $c = 10.012(2)$ Å, $\beta = 109.24(1)°$, V = 1279.2 Å^3, Z = 4, R = 0.046.

Table 1. Parameters used for the X-ray data collection

Diffractometer type:	Enraf-Nonius CAD4	Criterion for unobserved reflections:	$F_o < 5\ \sigma(F_o)$
Wave length:	Cu K_α radiation (1.5418 Å)	Number of refined parameters:	226
Crystal characteristics:	colourless block, size 0.02 x 0.05 x 0.06 mm	Scan mode:	$\omega/2\theta$
Temperature of measurement:	295 K	μ:	6.52 cm^{-1}
$2\theta_{max}$:	130°	Structure solution program used:	SHELX
Number of unique reflections:	1993		

Table 2. Final atomic coordinates and displacement parameters (in $Å^2$)

Atom	*x*	*y*	*z*	U_{iso} /U_{11}	U_{22}	U_{33}	U_{12}	U_{13}	U_{23}
O(2)	0.3338(3)	0.2480(2)	-0.4042(3)	0.0897(8)	0.0595(8)	0.0658(8)	-0.0006(8)	-0.0057(8)	0.0203(7)
O(5)	0.6123(3)	0.0920(2)	0.0854(2)	0.0756(8)	0.0503(7)	0.0497(7)	-0.0096(7)	0.0147(7)	0.0050(7)
O(11)	0.5833(3)	0.3752(2)	-0.0435(3)	0.0899(8)	0.0495(7)	0.0832(8)	-0.0077(8)	0.0335(7)	-0.0150(8)
N(1)	0.5073(3)	0.2543(2)	-0.1760(3)	0.0525(8)	0.0365(8)	0.0511(8)	-0.0002(7)	0.0179(7)	0.0030(7)
N(4)	0.4643(3)	0.0802(2)	-0.1464(3)	0.0625(8)	0.0437(8)	0.0441(8)	-0.0051(8)	0.0101(7)	0.0058(7)
C(2)	0.4129(4)	0.2113(2)	-0.2975(3)	0.0528(8)	0.0500(8)	0.0581(8)	-0.0016(8)	0.0150(8)	0.0119(8)
C(3)	0.4180(4)	0.1127(2)	-0.2904(3)	0.0556(8)	0.0500(8)	0.0455(8)	-0.0003(8)	0.0157(8)	0.0037(8)
C(6)	0.6365(4)	0.2051(2)	-0.0706(3)	0.0502(8)	0.0510(8)	0.0424(8)	-0.0013(8)	0.0121(7)	0.0051(8)
C(5)	0.5690(4)	0.1210(2)	-0.0368(3)	0.0554(8)	0.0409(8)	0.0459(8)	0.0022(8)	0.0170(7)	0.0069(8)
C(11)	0.4877(4)	0.3429(2)	-0.1463(4)	0.0690(8)	0.0445(8)	0.0712(8)	-0.0027(8)	0.0360(8)	0.0034(8)
C(11')	0.3451(4)	0.3906(2)	-0.2377(4)	0.0813(8)	0.0553(8)	0.1049(9)	0.0148(8)	0.0417(8)	0.0187(8)
C(31)	0.2575(4)	0.0736(2)	-0.3780(4)	0.0724(8)	0.0560(8)	0.0521(8)	-0.0060(8)	0.0174(8)	-0.0053(8)
C(32)	0.1257(4)	0.0892(3)	-0.3198(4)	0.0642(8)	0.0602(8)	0.0722(8)	-0.0026(8)	0.0130(8)	-0.0015(8)
C(33)	0.0331(5)	0.0296(3)	-0.2984(4)	0.0795(8)	0.0929(9)	0.0968(9)	-0.0112(8)	0.0372(8)	-0.0121(9)
C(61)	0.7856(4)	0.1906(2)	-0.1155(3)	0.0474(8)	0.0641(8)	0.0525(8)	0.0038(8)	0.0130(8)	0.0086(8)
C(62)	0.8581(4)	0.2761(3)	-0.1396(4)	0.0558(8)	0.0883(9)	0.0808(8)	-0.0085(8)	0.0189(8)	0.0184(8)
C(63)	0.9106(4)	0.1359(3)	-0.0080(4)	0.0678(8)	0.1064(9)	0.0819(9)	0.0270(8)	0.0269(8)	0.0209(8)
H(3)	0.4950(9)	0.1004(8)	-0.3297(9)	0.0455(9)					
H(4)	0.4206(9)	0.0190(9)	-0.1179(9)	0.2168(9)					
H(6)	0.6740(9)	0.2367(8)	0.0172(9)	0.0507(9)					
H(11a)	0.3484(9)	0.4018(9)	-0.3314(9)	0.0868(9)					
H(11b)	0.2526(9)	0.3571(8)	-0.2464(9)	0.0591(9)					
H(11c)	0.3463(9)	0.4454(9)	-0.1897(9)	0.1062(9)					
H(31a)	0.2277(9)	0.1001(9)	-0.4743(9)	0.0899(9)					
H(31b)	0.2694(9)	0.0101(9)	-0.3878(9)	0.0680(9)					
H(32a)	0.1171(9)	0.1502(9)	-0.2957(9)	0.1081(9)					
H(33a)	-0.0602(9)	0.0425(8)	-0.2616(9)	0.0561(9)					
H(33b)	0.0556(9)	-0.0343(8)	-0.3232(9)	0.0574(9)					
H(61)	0.7491(9)	0.1578(8)	-0.2048(9)	0.0686(9)					
H(62a)	0.9077(9)	0.3049(9)	-0.0382(9)	0.1102(9)					
H(62b)	0.9569(9)	0.2618(8)	-0.1706(9)	0.0871(9)					

Table 2. (Continued)

Atom	x	y	z	U_{iso}
H(62c)	0.7851(9)	0.3134(8)	-0.2046(9)	0.0601(9)
H(63a)	0.9330(9)	0.1647(9)	0.0825(9)	0.0879(9)
H(63b)	1.0067(9)	0.1262(9)	-0.0294(9)	0.0813(9)
H(63c)	0.8696(9)	0.0762(9)	-0.0003(9)	0.1136(9)

Further details of the structure determination (e.g. structure factors) have been deposited within the relevant database and can be accessed as Collection No. 400021 or ordered from the Fachinformationszentrum Karlsruhe, D-76344 Eggenstein-Leopoldshafen, Federal Republic of Germany.

References:

1. Badran, T.W. et.al.: A new a-haloglycine template for the asymmetric synthesis of amino acid derivatives. Tetrahedron Asymmetry **4** (1993) 197-200.

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Tryptophan Anion Complexes of β-Cyclodextrin (Cyclomaltaheptaose), an Aminopropylamino-β-cyclodextrin and its Enantioselective Nickel(II) Complex

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6^{I}-(3-Aminopropylamino)-6^{I}-deoxy-cyclomaltaheptaose (βCDpn) exhibits enhanced complexation of tryptophan anion, by comparison with βCD, while the nickel(II) complex of βCDpn complexes tryptophan anion even more strongly and exhibits a tenfold enantioselectivity in favour of the (*S*)-tryptophan anion.

As part of a study of the complexation characteristics of modified cyclodextrins we have prepared 6^{I}-(3-aminopropylamino)-6^{I}-deoxy-cyclomaltaheptaose (βCDpn) and its nickel(II) complex {[Ni(βCDpn)]$^{2+}$} and investigated the complexation of tryptophan anion (trp^{-}) by these species. The complexation characteristics of unmodified cyclodextrins and their ability to discriminate between enantiomers are well documented.[1–5] Substituents on a cyclodextrin are known to affect the extent of complexation and chiral discrimination. Thus, by comparison with βCD, 6^{I}-amino-6^{I}-deoxy-cyclomaltaheptaose shows greater enantioselectivity in its complexation of sodium 2-phenylpropanoate, although the complexes with βCD are more stable.[4] The aminopropylamino substituent of βCDpn offers greater structural flexibility for interaction with guests, and provides an opportunity for chelation of metal ions. Such metal complexes, or metallocyclodextrins, have been studied as metalloprotein models[6] and recently their enantiomeric complexation characteristics have attracted attention.[7,8] We now report that βCDpn, by comparison with βCD, exhibits enhanced complexation of trp^{-}, while [Ni(βCDpn)]$^{2+}$ exhibits a further enhancement in complexation and also a tenfold enantioselectivity between (*R*)-trp^{-} and (*S*)-trp^{-}, which is much higher than reported previously for a metallocyclodextrin.[7,8]

βCDpn [^{13}C NMR (D_2O): 32.5 (C2′), 39.6 (C3′), 47.4 (C1′) and 50.4 (C6^{I})] was prepared by treatment of 6^{I}-*O*-(4-methylphenylsulfonyl)-cyclomaltaheptaose[9] with 1,3-diaminopropane (1.5 equiv.) in *N*,*N*-dimethylformamide at 313 K for 24 h, and isolated in 93% yield after recrystallization from water–acetone of the precipitate obtained by diluting the cooled reaction mixture with acetone.

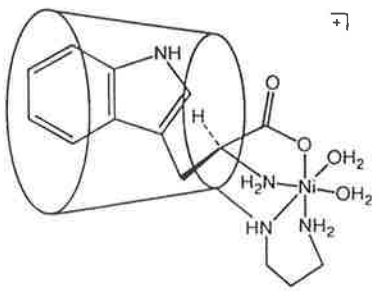

Fig. 1 A possible structure for [Ni(βCDpn)(*S*)-trp]$^{+}$, where the cyclodextrin annulus is shown as a truncated cone with the narrow and wide ends representing the circles delineated by the primary and secondary hydroxy groups, respectively

Table 1 Stability constants (K) for cyclodextrin and tryptophan anion complexes in aqueous solution at 298.2 K and I = 0.10 ($NaClO_4$)

Complexation	log (K/dm^3 mol^{-1})
βCD + (*R*)-trp^{-} ⇌ βCD(*R*)-trp^{-}	2.33 ± 0.06
βCD + (*S*)-trp^{-} ⇌ βCD(*S*)-trp^{-}	2.33 ± 0.08
βCDpn + (*R*)-trp^{-} ⇌ βCDpn(*R*)-trp^{-}	3.41 ± 0.05
βCDpn + (*S*)-trp^{-} ⇌ βCDpn(*S*)-trp^{-}	3.40 ± 0.07
βCDpn + Ni^{2+} ⇌ [Ni(βCDpn)]$^{2+}$	5.2 ± 0.1
[Ni(βCDpn)]$^{2+}$ + (*R*)-trp^{-} ⇌ [Ni(βCDpn)(*R*)-trp]$^{+}$	4.1 ± 0.2
[Ni(βCDpn)]$^{2+}$ + (*S*)-trp^{-} ⇌ [Ni(βCDpn)(*S*)-trp]$^{+}$	5.1 ± 0.2
Ni^{2+} + trp^{-} ⇌ [Ni(trp)]$^{+}$	5.42 ± 0.03

The stability constants for complexation of Ni^{2+} by βCDpn, and (*R*)-trp^{-} and (*S*)-trp^{-} by Ni^{2+}, βCD, βCDpn and [Ni(βCDpn)]$^{2+}$, determined using standard automated pH titration procedures,[4] are presented in Table 1. Neither βCDpn nor βCD discriminate between the enantiomers of trp^{-}, whereas the metallocyclodextrin [Ni(βCDpn)]$^{2+}$ complexes (*S*)-trp^{-} enantioselectively. It appears that the metal is important for chiral discrimination and, since the chirality of βCDpn is essential to the enantioselectivity displayed by [Ni(βCDpn)]$^{2+}$, it seems likely that [Ni(βCDpn)(*S*)-trp]$^{+}$ has both (*S*)-trp^{-} and βCDpn coordinated to Ni^{2+} as shown in Fig. 1. A similar structure is anticipated for [Ni(βCDpn)(*R*)-trp]$^{+}$. It has been suggested that discrimination between the enantiomers of trp^{-} by metallocyclodextrins requires the indole moiety of the more strongly bound enantiomer to be inside the cyclodextrin annulus while that of the other enantiomer is excluded from it.[7,8] We have no evidence for such a major structural difference in our system.

The stronger complexation of trp^{-} by the metallocyclodextrin [Ni(βCDpn)]$^{2+}$, relative to that by βCDpn and βCD, is consistent with bidentate coordination of trp^{-} stabilizing its complexation. However, this stronger complexation does not result from a simple combination of the effects of the cyclodextrin annulus and Ni^{2+}, as is apparent from the observation that the stability constant for [Ni(trp)]$^{+}$ is greater than that for either [Ni(βCDpn)(*R*)-trp]$^{+}$ or [Ni(βCDpn)(*S*)-trp]$^{+}$.

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References

1 R. J. Clarke, J. H. Coates and S. F. Lincoln, *Adv. Carbohydr. Chem. Biochem.*, 1989, **46**, 205.
2 J. Szejtli, *Cyclodextrin Technology*, Kluwer, Dordrecht, 1988.
3 S. E. Brown, J. H. Coates, S. F. Lincoln, D. R. Coghlan and C. J. Easton, *J. Chem. Soc., Faraday Trans.*, 1991, **87**, 2699.
4 S. E. Brown, J. H. Coates, P. A. Duckworth, S. F. Lincoln, C. J. Easton and B. L. May, *J. Chem. Soc., Faraday Trans.*, 1993, **89**, 1035.
5 K. B. Lipkowitz, S. Raghothama and J. Yang, *J. Am. Chem. Soc.*, 1992, **114**, 1554.
6 I. Tabushi, *Coord. Chem. Rev.*, 1988, **86**, 1.
7 G. Impellizzeri, G. Maccarrone, E. Rizzarelli, G. Vecchio, R. Corradini and R. Marchelli, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 1348.
8 V. Cucinotta, F. D'Alessandro, G. Impellizzeri, G. Maccarrone and G. Vecchio, *J. Chem. Soc., Chem. Commun.*, 1992, 1743.
9 Y. Chao, PhD Thesis, Columbia University, 1972; M. Shiono, T. Shibanuma and T. Mukaiyama, *Chem. Lett.*, 1976, 1041.

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Metallocyclodextrins of 6^A-(3-Aminopropylamino)-6^A-deoxy-β-cyclodextrin†: Their Formation and Enantioselective Complexation of (*R*)- and (*S*)-Tryptophan Anions in Aqueous Solution

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From a pH titration study, the complexation of divalent metal ions (M^{2+}) by 6^A-(3-aminopropylamino)-6^A-deoxy-β-cyclodextrin (βCDpn) to form the metallocyclodextrins, $[M(\beta CDpn)]^{2+}$, is characterized by $\log(K_2/dm^3\ mol^{-1}) = 4.22 \pm 0.02$, 5.2 ± 0.1, 7.35 ± 0.04 and 4.96 ± 0.08 when $M^{2+} = Co^{2+}$, Ni^{2+}, Cu^{2+} and Zn^{2+}, respectively, in aqueous solution at $I = 0.10$ ($NaClO_4$) and 298.2 K. The complexation of the tryptophan anion (Trp^-) by $[M(\beta CDpn)]^{2+}$ is enantioselective for (*S*)-Trp^- as indicated by $\log(K_{eR}/dm^3\ mol^{-1})$ and $\log(K_{eS}/dm^3\ mol^{-1}) = 4.04 \pm 0.03$ and 4.32 ± 0.05, 4.1 ± 0.2 and 5.1 ± 0.2, and 7.85 ± 0.07 and 8.09 ± 0.05, where the first and second magnitudes refer to the stability constants for $[M(\beta CDpn)(R)\text{-}Trp]^+$ and $[M(\beta CDpn)(S)\text{-}Trp]^+$, respectively, when $M^{2+} = Co^{2+}$, Ni^{2+} and Cu^{2+}, respectively. The corresponding magnitudes for $M^{2+} = Zn^{2+}$ are both 5.3 ± 0.1, indicating no enantioselectivity. The role of M^{2+} and other factors affecting complexation and enantioselectivity are discussed.

Natural and modified cyclodextrins exist in single enantiomeric forms and, when acting as host molecules, may preferentially complex one enantiomer of a chiral guest to produce two diastereomeric host–guest complexes of differing thermodynamic stability.[1–7] The degree of enantioselectivity varies substantially with the nature of the cyclodextrins and guests. In the case of aromatic guest molecules with polar substituents, this may be understood in terms of a model in which the aromatic moiety enters the cyclodextrin annulus and one or more polar substituents of the guest interact with the hydroxy or other polar groups of the cyclodextrin with varying degrees of intensity.[1–5,8–11] Thus, *R*- and *S*-guests experience different geometric and electrostatic interactions with the cyclodextrin which may generate differing stabilities in the two diastereomeric host–guest complexes. In this study we are particularly interested in the combined effects of the coordinating ability of the metal centres and the chirality of the modified cyclodextrins or metallocyclodextrins on the enantioselectivity between guest enantiomers in host–guest complexes.[12–14] In a preliminary report[14] we showed that 6^A-(3-aminopropylamino)-6^A-deoxy-β-cyclodextrin (βCDpn) formed a nickel(II) metallocyclodextrin ($[Ni(\beta CDpn)]^{2+}$), which was enantioselective for the (*S*)-tryptophan anion [(*S*)-Trp^-)] in a ratio of 10:1 over (*R*)-tryptophan anion [(*R*)-Trp^-] in forming the nickel(II)-6^A-(3-aminopropylamino)-6^A-deoxy-β-cyclodextrin–tryptophan anion host–guest complex, $[Ni(\beta CDpn)Trp]^+$, in aqueous solution. This appears to be the greatest degree of enantioselectivity between chiral guests so far reported for a metallocyclodextrin, and we now examine the effects of the variation of the nature of the metal ion on this enantioselectivity, and make comparisons with related systems.

Experimental

Preparation of Materials

6^A-(3-Aminopropylamino)-6^A-deoxy-β-cyclodextrin prepared as in the literature,[14] and (*R*)-, (*S*)- and (*RS*)-tryptophan‡ (Sigma) were dried to constant weight and stored in the dark over P_2O_5 in a vacuum desiccator prior to use. The enantiomeric purities of (*R*)- and (*S*)-Trp were determined to be >99% by HPLC analysis [Pirkle covalent (*S*)-phenylglycine column] of the respective esters formed with thionyl chloride pretreated methanol at 348 K. These purity limits were used in calculations of error limits of the stability constants characterizing the complexation of these enantiomers. Metal perchlorates (Fluka) were twice recrystallized from water, and were dried and stored over P_2O_5 under vacuum. (Caution: Anhydrous perchlorate salts are potentially powerful oxidants and should be handled with care.) All solutions were prepared using deionized water purified with a MilliQ-reagent system to produce water with a specific resistance of >15 MΩ cm, which was then boiled to remove CO_2.

Equilibrium Studies

Titrations were performed using a Metrohm Dosimat E665 titrimator, an Orion SA 720 potentiometer, and an Orion 8103 Ross combination pH electrode which was filled with 0.10 mol dm^{-3} $NaClO_4$. Throughout a titration a stream of fine nitrogen bubbles (previously passed through aqueous 0.10 mol dm^{-3} $NaClO_4$) was passed through the titration solution which was magnetically stirred and thermostatted at 298.2 ± 0.1 K in a water-jacketed 20 cm^3 titration vessel which was closed to the atmosphere with the exception of a small exit for the nitrogen stream.

The 0.100 mol dm^{-3} $Ni(ClO_4)_2$, $Cu(ClO_4)_2$ and $Zn(ClO_4)_2$ stock solutions were standardized by EDTA titration in the presence of Murexide indicator in the first two cases and Eriochrome Black T in the case of $Zn(ClO_4)_2$.[15] Ion exchange of Co^{2+} on an Amberlite HRC-120 cation-exchange column in the acid form followed by back titration of the liberated acid was used as the standardization method for the 0.100 mol dm^{-3} $Co(ClO_4)_2$ stock solution.

In all titrations, standardized 0.100 mol dm^{-3} NaOH was titrated against the species of interest in solutions 0.010 mol dm^{-3} in $HClO_4$ and 0.090 mol dm^{-3} in $NaClO_4$. Thus the pK_a values of $\beta CDpnH_2^{2+}$ and $TrpH^+$ were determined from titrations of 10.00 cm^3 aliquots of their 0.001 mol dm^{-3} solutions. The stability constants for the formation of the $\beta CDpn \cdot (R)\text{-}Trp^-$ and $\beta CDpn \cdot (S)\text{-}Trp^-$ complexes were

† β-Cyclodextrin = cycloheptamaltaose.

‡ Protonated tryptophan, tryptophan zwitterion and tryptophan anion are denoted as $TrpH^+$, Trp and Trp^-, respectively, prefixed by (*R*)- or (*S*)- as appropriate.

determined by titration of 5.00 cm^3 each of 0.001 mol dm^{-3} solutions of either (*R*)-$TrpH^+$ or (*S*)-$TrpH^+$ and $\beta CDpnH_2^{2+}$, and the stability constants for the formation of the $[M(Trp)]^+$, $[M(\beta CDpn)]^{2+}$ and related complexes were determined by titration of 10.00 cm^3 aliquots of 0.001 mol dm^{-3} solutions of either $TrpH^+$ or $\beta CDpnH_2^{2+}$ with either 0.095 cm^3 or 0.045 cm^3 of $M(ClO_4)_2$ solution added. The stability constants for the formation of $[M(\beta CDpn)(R)\text{-}Trp]^+$ and $[M(\beta CDpn)(S)\text{-}Trp]^+$ and related complexes were determined by titration of 5.00 cm^3 each of 0.001 mol dm^{-3} solutions of either (*R*)-$TrpH^+$ or (*S*)-$TrpH^+$ and $\beta CDpnH_2^{2+}$ with 0.045 cm^3 of $M(ClO_4)_2$ solution added. E_0 and pK_w values were determined by titration of 0.010 mol dm^{-3} $HClO_4$ (0.090 mol dm^{-3} in $NaClO_4$) against 0.100 mol dm^{-3} NaOH. Derivations of the stability constants were carried out using the program SUPERQUAD.[16] At least three runs were performed for each system, and at least two of these runs were averaged; the criterion for selection for this averaging being that X^2 for each run was <12.6 at the 95% confidence level.[16]

Results and Discussion

General Aspects

Several complexes formed in aqueous solutions of βCDpn, M^{2+} and tryptophan in the 2.5–11.5 pH range of this study. Their stabilities were determined from the differences between the pH profiles arising from titration against NaOH of solutions containing different combinations of the complexing species using the program SUPERQUAD. The sequence of these titrations was: (i) pK_a determinations of $\beta CDpnH_2^{2+}$ and $TrpH^+$, followed by determination of the stability constants of complexes in solutions of (ii) M^{2+} and $TrpH^+$, (iii) M^{2+} and $\beta CDpnH_2^{2+}$, (iv) $\beta CDpnH_2^{2+}$ and either (*R*)-$TrpH^+$ or (*S*)-$TrpH^+$, and (v) M^{2+}, $\beta CDpnH_2^{2+}$ and either (*R*)-$TrpH^+$ or (*S*)-$TrpH^+$. The pK_as determined in (i) were employed as known values in the determination of stability constants in (ii)–(v), and the stability constants determined in (ii)–(iv) were employed as known values in the determination of stability constants in (v). The titration data were fitted to equilibria containing the minimum number of species required for a good fit, and any newly determined species found to be $<5\%$ of the total βCDpn or tryptophan concentrations were considered to be insignificant. Two such pH titration profiles are shown in Fig. 1. Plots of the major species present in the Cu^{2+}–βCDpn–(*S*)-tryptophan system are shown in Fig. 2 and 3. The stability constants of the major M^{2+} complexes appear in Table 1, and those for other

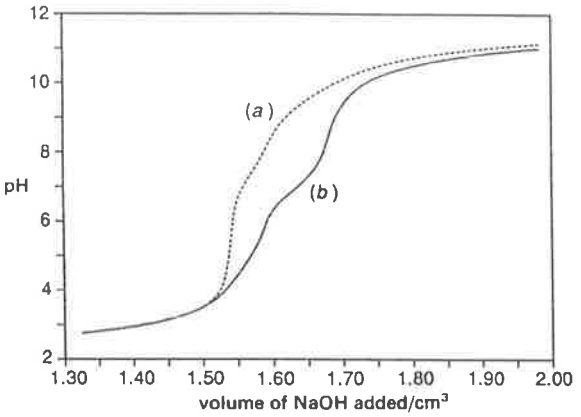

Fig. 1 Titration profiles for (*a*) $\beta CDpnH_2^{2+}$ (5.04×10^{-4} mol dm^{-3}) and (*R*)-$TrpH^+$ (5.08×10^{-4} mol dm^{-3}), and (*b*) $\beta CDpnH_2^{2+}$ (5.02×10^{-4} mol dm^{-3}), (*R*)-$TrpH^+$ (5.05×10^{-4} mol dm^{-3}) and $Cu(ClO_4)_2$ (4.50×10^{-4} mol dm^{-3}), each in aqueous 0.010 mol dm^{-3} $HClO_4$ and 0.090 mol dm^{-3} $NaClO_4$, against 0.101 mol dm^{-3} NaOH.

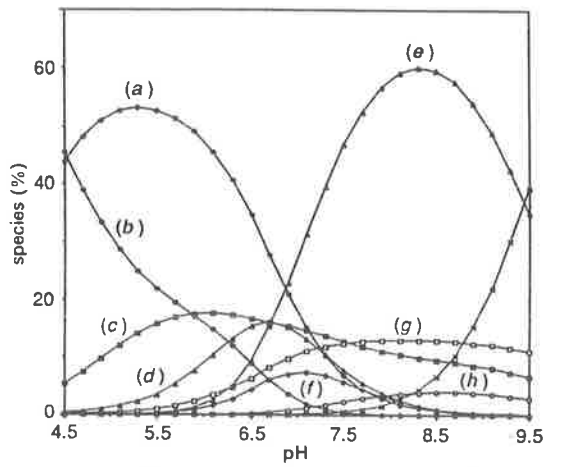

Fig. 2 Plot of Cu^{2+} species in a solution 0.000 95, 0.001 and 0.001 mol dm^{-3} in total Cu^{2+}, βCDpn and (*S*)-tryptophan concentrations, respectively, plotted relative to total [βCDpn] = total [(*S*)-Tryptophan] = 100%. (*a*) $[Cu((S)\text{-}Trp)]^+$, (*b*) Cu^{2+}, (*c*) $[Cu((S)\text{-}Trp)_2]$, (*d*) $[Cu(\beta CDpn)(S)\text{-}Trp]^{2+}$, (*e*) $[Cu(\beta CDpn)(S)\text{-}Trp]^+$, (*f*) $[Cu(\beta CDpn)]^{2+}$, (*g*) [Cu((*S*)-Trp)OH], (*h*) $[Cu(\beta CDpn)OH]^+$ and (*i*) $[Cu(\beta CDpn)(S)\text{-}Trp)OH]$.

Table 1 Stability constants[a] characterizing metallo-6^A-(3-aminopropylamino)-6^A-deoxy-β-cyclodextrins and related complexes in aqueous solution at 298.2 K and $I = 0.10$ ($NaClO_4$)

	M^{2+}			
	Co^{2+}	Ni^{2+} [b]	Cu^{2+}	Zn^{2+}
$\log(K_2/dm^3\ mol^{-1})$	4.22 ± 0.02	5.2 ± 0.1	7.35 ± 0.04	4.96 ± 0.08
$\log(K_3/dm^3\ mol^{-1})$	2.5 ± 0.2	3.1 ± 0.1	3.09 ± 0.04	3.0 ± 0.1
$\log(K_4/dm^3\ mol^{-1})$	4.41 ± 0.05	5.42 ± 0.03	8.11 ± 0.03	4.90 ± 0.04
$\log(K_5/dm^3\ mol^{-1})$	4.01 ± 0.08	4.67 ± 0.03	7.20 ± 0.07	
$\log(K_{6R}/dm^3\ mol^{-1})$	4.04 ± 0.03 (0.1)	4.1 ± 0.2 (0.2)	7.85 ± 0.07 (0.07)	5.3 ± 0.1 (0.1)
$\log(K_{6S}/dm^3\ mol^{-1})$	4.32 ± 0.05 (0.09)	5.1 ± 0.2 (0.2)	8.09 ± 0.05 (0.06)	5.3 ± 0.1 (0.1)
$\log(K_{7R}/dm^3\ mol^{-1})$			5.29 ± 0.05 (0.1)	
$\log(K_{7S}/dm^3\ mol^{-1})$			5.4 ± 0.1 (0.2)	

[a] Errors quoted for K (the mean of N runs) represent the standard deviations, $\sigma = \sqrt{[\Sigma(K_i - K)^2]/(N-1)}$ where K_i is a value from a single run for the best fit of the variation of pH with added volume of NaOH titrant obtained through SUPERQUAD, and $i = 1, 2, \ldots, N$. When a K derived in this way was employed as a constant in the subsequent derivation of another K, the error associated with the first K was propagated in the derivation of the second K. For the diastereomers, the first and second errors quoted are calculated assuming 100 and 99% enantiomeric purity of tryptophan, respectively. [b] Ref. 14.

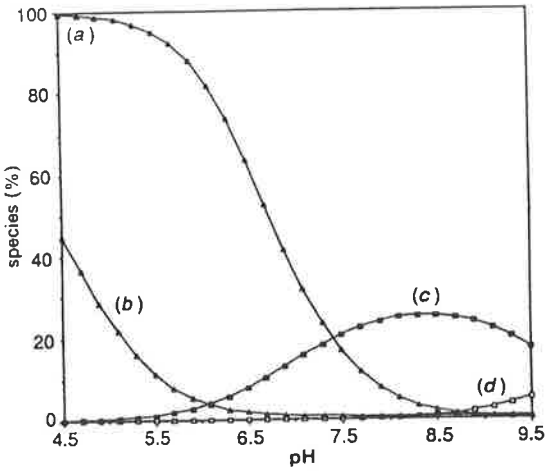

Fig. 3 Plot of non-Cu^{2+} species in a solution 0.000 95, 0.001 and 0.001 mol dm^{-3} in total Cu^{2+}, βCDpn and (*S*)-tryptophan concentrations, respectively, plotted relative to total [βCDpn] = total [(*S*)-tryptophan] = 100%. (*a*) $\beta CDpnH_2^{2+}$, (*b*) (*S*)-Trp, (*c*) $\beta CDpnH^+$ and (*d*) βCDpn.

species appear below. A detailed discussion of the various equilibria now follows.

Cyclodextrin Equilibria

The acid dissociations of the diprotonated aminopropylamino substituent of $\beta CDpnH_2^{2+}$:

$$\beta CDpnH_2^{2+} \overset{K_{a1}}{\rightleftharpoons} \beta CDpnH^+ + H^+ \quad (1)$$

$$\beta CDpnH^+ \overset{K_{a2}}{\rightleftharpoons} \beta CDpn + H^+ \quad (2)$$

derived from data in the pH range 6.0–11.5 are characterized by $pK_{a1} = 7.39 \pm 0.04$ and $pK_{a2} = 9.9 \pm 0.1$. The acid dissociations of $TrpH^+$ characterized by pK_as of 2.40 ± 0.02 and 9.28 ± 0.01, and derived from data obtained in the pH ranges 2.0–3.0 and 8.0–10.5, respectively, are similar to those in the literature.[17]

For complexations of either (*R*)-Trp^- or (*S*)-Trp^- by βCDpn:

$$\beta CDpn + (R)\text{-}Trp^- \overset{K_{1R}}{\rightleftharpoons} \beta CDpn \cdot (R)\text{-}Trp^- \quad (3)$$

$$\beta CDpn + (S)\text{-}Trp^- \overset{K_{1S}}{\rightleftharpoons} \beta CDpn \cdot (S)\text{-}Trp^- \quad (4)$$

$\log(K_{1R}/dm^3\ mol^{-1}) = 3.41 \pm 0.02$ (0.05) and $\log(K_{1S}/dm^3\ mol^{-1}) = 3.40 \pm 0.07$ (0.1) were derived from data in the pH range 8.5–11.5, where the first and second errors are calculated on the basis of tryptophan being 100 and 99% pure, respectively.

Complexation of 6^A-(3-Aminopropylamino)-6^A-deoxy-β-cyclodextrin and the Tryptophan Anion by Divalent Metal Ions

The stability of the metallocyclodextrin formed by βCDpn:

$$M^{2+} + \beta CDpn \overset{K_2}{\rightleftharpoons} [M(\beta CDpn)]^{2+} \quad (5)$$

varies with the nature of M^{2+} as shown by the variation of the magnitude of K_2 in the sequence $Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$ (Table 1) as anticipated from the Irving–Williams series.[18] In the cases of $[Ni(\beta CDpn)]^{2+}$ and $[Cu(\beta CDpn)]^{2+}$, pK_as of 9.20 ± 0.04 and 7.84 ± 0.03, respectively, were determined and are thought to correspond to the deprotonation of aqua ligands bound to the metal centres. Similar deprotonations were not reliably detected for the Co^{2+} and Zn^{2+} analogues, because the precipitation of a metal hydroxide species above pH 8.5 and 7.5, respectively, obscured the formation of $[Co(\beta CDpn)OH]^+$ and $[Zn(\beta CDpn)OH]^+$ and rendered titrations above these pHs impractical.

The formation of $[M(\beta CDpnH)]^{3+}$:

$$M^{2+} + \beta CDpnH^+ \overset{K_3}{\rightleftharpoons} [M(\beta CDpnH)]^{3+} \quad (6)$$

is less favoured (Table 1) as anticipated from the charge repulsion between M^{2+} and $\beta CDpnH^+$ and the monodentate nature of $\beta CDpnH^+$. The pK_a of $[M(\beta CDpnH)]^{3+}$ is 8.3 ± 0.1, 7.83 ± 0.02, 5.74 ± 0.05 and 8.1 ± 0.1 when $M^{2+} = Co^{2+}$, Ni^{2+}, Cu^{2+} and Zn^{2+}, respectively. These values probably characterize the deprotonation of the monoprotonated aminopropylamino substituents of $\beta CDpnH^+$ in the metallocyclodextrins. The stability constants K_2 and K_3 and the corresponding pK_as were derived from data obtained in the pH ranges 6.0–8.5, 5.5–8.5, 5.5–9.0 and 5.5–7.5, when $M^{2+} = Co^{2+}$, Ni^{2+}, Cu^{2+} and Zn^{2+}, respectively.

The formation of $[M(Trp)]^+$ and $[M(Trp)_2]$ also occurs:

$$M^{2+} + Trp^- \overset{K_4}{\rightleftharpoons} [M(Trp)]^+ \quad (7)$$

$$[M(Trp)]^+ + Trp^- \overset{K_5}{\rightleftharpoons} [M(Trp)_2] \quad (8)$$

The stability constants, K_4 and K_5, determined in this study (Table 1) are in reasonable agreement with those in the literature,[17] and also exhibit variations anticipated from the Irving–Williams series.[18] For $[Ni(Trp)]^+$ and $[Cu(Trp)]^+$, pK_as of 9.1 ± 0.1 and 7.28 ± 0.07, respectively, were determined, which probably correspond to the deprotonation of aqua ligands bound to the metal centres. Similar deprotonations were not reliably detected for the Co^{2+} and Zn^{2+} analogues, because the precipitation of a metal hydroxide species above pH 8.5 and 7.5, respectively, interfered with the titrations. The stability constants K_4 and K_5 and the corresponding pK_as were derived from data obtained in the pH ranges 6.5–8.5, 5.0–9.0, 3.0–6.5 and 5.5–7.0, when $M^{2+} = Co^{2+}$, Ni^{2+}, Cu^{2+} and Zn^{2+}, respectively.

Enantioselectivity in the Complexation of (*R*)- and (*S*)-Tryptophan Anion by Divalent Metal Complexes of 6^A-(3-Aminopropylamino)-6^A-deoxy-β-cyclodextrin

The stability of the complexes formed by $[M(\beta CDpn)]^{2+}$ with (*R*)-Trp^- and (*S*)-Trp^-:

$$[M(\beta CDpn)]^{2+} + (R)\text{-}Trp^- \overset{K_{6R}}{\rightleftharpoons} [M(\beta CDpn)(R)\text{-}Trp]^+ \quad (9)$$

$$[M(\beta CDpn)]^{2+} + (S)\text{-}Trp^- \overset{K_{6S}}{\rightleftharpoons} [M(\beta CDpn)(S)\text{-}Trp]^+ \quad (10)$$

also varies with the nature of M^{2+} as shown by the variation of the magnitude of K_{6R} and K_{6S} in the sequence $Co^{2+} \leqslant Ni^{2+} < Cu^{2+} > Zn^{2+}$ (Table 1). In addition, there is a ten-fold enantioselectivity for (*S*)-Trp^- when $M^{2+} = Ni^{2+}$, as a comparison of K_{6R} with K_{6S} shows. When $M^{2+} = Co^{2+}$ and Cu^{2+}, there is a moderate enantioselectivity for (*S*)-Trp^-, but when $M^{2+} = Zn^{2+}$, no enantioselectivity is observed. The effect of enantioselectivity on the concentrations of several species in the Ni^{2+} system is shown in Fig. 4.

The lower stabilities of βCDpn·(*R*)-Trp^- and βCDpn·(*S*)-Trp^- by comparison with those of $[M(\beta CDpn)(R)\text{-}Trp]^+$ and $[M(\beta CDpn)(S)\text{-}Trp]^+$, demonstrate that M^{2+} strengthens the complexation of Trp^-. However, as $[M(\beta CDpn)(R)\text{-}Trp]^+$ and $[M(\beta CDpn)(S)\text{-}Trp]^+$ (K_{6R} and K_{6S}) are less stable than

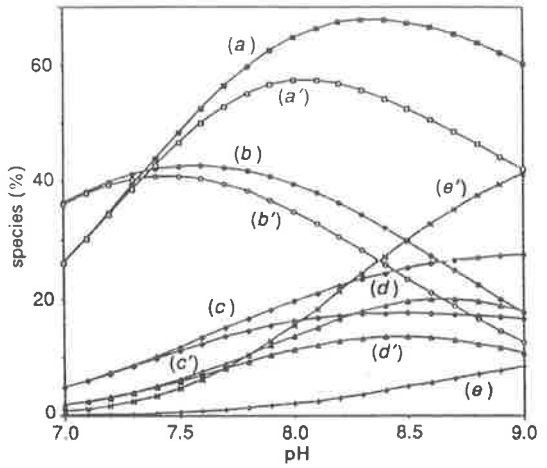

Fig. 4 Plot of selected species in a solution 0.000 95, 0.001 and 0.001 mol dm^{-3} in total Ni^{2+}, βCDpn and either (*R*)-tryptophan or (*S*)-tryptophan (indicated by primed letters) concentrations, respectively, plotted relative to total [βCDpn] = either total [(*R*)-tryptophan] or total [(*S*)-tryptophan] = 100%. (*a*) $\beta CDpnH^+$, (*a'*) $\beta CDpnH^+$, (*b*) $[Ni((R)\text{-}Trp)]^+$, (*b'*) $[Ni((S)\text{-}Trp)]^+$, (*c*) $[Ni((R)\text{-}Trp)_2]$, (*c'*) $[Ni((S)\text{-}Trp)_2]$, (*d*) $[Ni(\beta CDpn)]^{2+}$, (*d'*) $[Ni(\beta CDpn)]^{2+}$, (*e*) $[Ni(\beta CDpn)(R)\text{-}Trp]^+$ and (*e'*) $[Ni(\beta CDpn)(S)\text{-}Trp]^+$.

$[M(Trp)]^+$ (K_4) when $M^{2+} = Co^{2+}$, Ni^{2+} and Cu^{2+}, it appears that the factors stabilizing complexation of (*R*)-Trp^- and (*S*)-Trp^- by βCDpn and M^{2+} in $[M(\beta CDpn)(R)\text{-}Trp]^+$ and $[M(\beta CDpn)(S)\text{-}Trp]^+$ do not reinforce each other. In contrast, $[Zn(\beta CDpn)(R)\text{-}Trp]^+$ and $[Zn(\beta CDpn)(S)\text{-}Trp]^+$ are more stable than $[Zn(Trp)]^+$, consistent with mutual reinforcement of the complexation of (*R*)-Trp^- and (*S*)-Trp^- by βCDpn and Zn^{2+}, but there is no enantioselection between (*R*)-Trp^- and (*S*)-Trp^-.

The structure envisaged for $[M(\beta CDpn)(R)\text{-}Trp]^+$ and $[M(\beta CDpn)(S)\text{-}Trp]^+$ has the indole moiety of Trp^- inside the cyclodextrin annulus with the Trp^- chiral centre in the vicinity of the primary hydroxy groups of the cyclodextrin, and the Trp^- amine and carboxylate groups coordinated to M^{2+}, as shown in Fig. 5. It has been argued that the indole moiety is only inside the cyclodextrin annulus for the diastereomer with the higher stability in the Cu^{2+} complexes of 6^A-[4-(2-aminoethyl)imidazoyl]- 6^A-deoxy-β-cyclodextrin and 6^A-deoxy-6^A-[2-(4-imidazoyl)ethylamino]- β-cyclodextrin, which preferentially complex (*S*)-Trp^- and (*R*)-Trp^-, respectively.[12,13] We have no evidence for such major structural differences in the complexes studied here.

The influence of the nature of M^{2+} on the stabilities of $[M(\beta CDpn)(R)\text{-}Trp]^+$ and $[M(\beta CDpn)(S)\text{-}Trp]^+$ reflects the

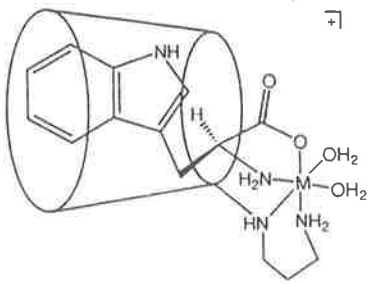

Fig. 5 A possible structure for $[M(\beta CDpn)(S)\text{-}Trp]^+$, where the cyclodextrin annulus is shown as a truncated cone with the narrow and wide ends representing the circles delineated by primary and secondary hydroxy groups, respectively.

variation in the ionic radii of six-coordinate Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}, which are 0.745, 0.69, 0.73 and 0.74 Å,[19] respectively, and the geometric constraints arising from ligand-field effects in Co^{2+}, Ni^{2+} and Cu^{2+}.[20] It is particularly interesting that $[Zn(\beta CDpn)(R)\text{-}Trp]^+$ and $[Zn(\beta CDpn)(S)\text{-}Trp]^+$ are of the same stability, whereas the analogous diastereomers for the other three metals are of different stability. This suggests that the absence of ligand-field-generated geometric constraints on d^{10} Zn^{2+} allows more flexibility in the structures of $[Zn(\beta CDpn)(R)\text{-}Trp]^+$ and $[Zn(\beta CDpn)(S)\text{-}Trp]^+$ and as a result enantioselectivity is decreased. In contrast, the d^9 electronic configuration for the similar sized Cu^{2+} imposes a tetragonally distorted octahedral geometry which may place greater constraints on the interaction of the chiral centres of (*R*)-Trp^- and (*S*)-Trp^- with the βCDpn moiety and decrease the stability of $[Cu(\beta CDpn)(R)\text{-}Trp]^+$ by comparison with that of $[Cu(\beta CDpn)(S)\text{-}Trp]^+$. Similar arguments may be applied in the cases of d^7 Co^{2+} and d^8 Ni^{2+} whose six-coordinate geometries more closely approach regular octahedrons. The greater enantioselectivity caused by Ni^{2+} indicates that the size of the metal centre is important, and that a difference of 0.04 Å can result in a substantial change in the degree of enantioselectivity.

The stabilities of $[Cu(\beta CDpn)(R)\text{-}Trp]^{2+}$ and $[Cu(\beta CDpn)(S)\text{-}Trp]^{2+}$:

$$[Cu(\beta CDpn)]^{2+} + (R)\text{-}Trp \overset{K_{7R}}{\rightleftharpoons} [Cu(\beta CDpn)(R)\text{-}Trp]^{2+} \quad (11)$$

$$[Cu(\beta CDpn)]^{2+} + (S)\text{-}Trp \overset{K_{7S}}{\rightleftharpoons} [Cu(\beta CDpn)(S)\text{-}Trp]^{2+} \quad (12)$$

are lower than those of $[Cu(\beta CDpn)(R)\text{-}Trp]^+$ and $[Cu(\beta CDpn)(S)\text{-}Trp]^+$ (Table 1) and there is no significant enantioselectivity, probably because (*R*)-Trp and (*S*)-Trp act as monodentate ligands and are less sterically constrained than bidentate (*R*)-Trp^- and (*S*)-Trp^-. The deprotonations of $[Cu(\beta CDpn)(R)\text{-}Trp]^{2+}$, $[Cu(\beta CDpn)(S)\text{-}Trp]^{2+}$, $[Cu(\beta CDpn)(R)\text{-}Trp]^+$ and $[Cu(\beta CDpn)(S)\text{-}Trp]^+$ are characterized by pK_as of 6.72 ± 0.08 (0.1), 6.6 ± 0.1 (0.2), 9.48 ± 0.07 (0.09) and 9.37 ± 0.04 (0.05), respectively. The pK_as for the first pair may characterize the deprotonation of either the $TrpH^+$ or the $\beta CDpnH^+$ ligand, but an unambiguous assignment is not possible. The pK_as for the second pair probably characterize the deprotonation of an aqua ligand. These reactions were not detected when $M^{2+} = Co^{2+}$, Ni^{2+} and Zn^{2+}.

The stability constants for the complexations shown in eqn. (9)–(12) were derived from data obtained in the pH ranges 7.5–8.7, 7.0–9.2, 4.5–9.5 and 6.5–8.0, when $M^{2+} = Co^{2+}$, Ni^{2+}, Cu^{2+} and Zn^{2+}, respectively.

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References

1 N. J. Smith, T. M. Spotswood and S. F. Lincoln, *Carbohydrate Res.*, 1989, **192**, 9.
2 S. F. Brown, J. H. Coates, S. F. Lincoln, D. R. Coghlan and C. J. Easton, *J. Chem. Soc., Faraday Trans.*, 1991, **87**, 2699.
3 S. E. Brown, J. H. Coates, P. A. Duckworth, S. F. Lincoln, C. J. Easton and B. L. May, *J. Chem. Soc., Faraday Trans.*, 1993, **89**, 1035.

4 I. Tabushi, Y. Kuroda and T. Mizutani, *J. Am. Chem. Soc.*, 1986, **108**, 4514.
5 I. Tabushi, Y. Kuroda, M. Yamada and T. Sera, *J. Incl. Phenom.*, 1988, **6**, 599.
6 K. Harata, *Bull. Chem. Soc. Jpn.*, 1987, **60**, 2763.
7 D. Greatbanks and R. Pickford, *Mag. Reson. Chem.*, 1987, **25**, 208.
8 R. J. Clarke, J. H. Coates and S. F. Lincoln, *Adv. Carbohyd. Chem. Biochem.*, 1989, **46**, 205.
9 J. Szejtli, *Cyclodextrin Technology*, Kluwer, Dordrecht, 1988.
10 J. F. Stoddard, *Cyclodextrins*, Royal Society of Chemistry, Cambridge, 1990.
11 W. Saenger, *Incl. Comp.*, 1984, **2**, 231.
12 G. Impellizzeri, G. Maccarrone, E. Rizzarelli, G. Vecchio, R. Corradini and R. Marchelli, *Angew. Chem., Int. Ed. Engl.*, 1991, **30**, 1348.
13 V. Cucinotta, F. D'Alessandro, G. Impellizzeri, G. Maccarrone and G. Vecchio, *J. Chem. Soc., Chem. Commun.*, 1992, 1743.
14 S. E. Brown, J. H. Coates, C. J. Easton, S. J. van Eyk, S. F. Lincoln, B. L. May, M. A. Stile, C. B. Whalland and M. L. Williams, *J. Chem. Soc., Chem. Commun.*, 1994, 47.
15 A. I. Vogel, *Quantitative Inorganic Analysis*, Longmans, London, 3rd edn., 1961.
16 P. Gans, A. Sabatini and A. Vacca, *J. Chem. Soc., Dalton Trans.*, 1985, 1195.
17 *Critical Stability Constants*, ed., R. M. Smith and A. E. Martell, Plenum Press, New York, 1975, vol. 1.
18 H. Irving and R. J. P. Willliams, *J. Chem. Soc.*, 1953, 3192.
19 R. D. Shannon, *Acta Crystallogr., Sect. A*, 1976, **32**, 751.
20 A. Cotton and G. Wilkinson, *Advanced Inorganic Chemistry*, New York, 4th edn., 1980.

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