The structure of trifolitoxin.
A bacteriocin from *Rhizobium leguminosarum* biovar *trifolii* strain T24.

by

Benjamin James Lethbridge
B.Ag.Sc. (Hons.) Adelaide

Department of Agricultural Biochemistry
Waite Agricultural Research Institiute
University of Adelaide
South Australia

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SUMMARY

This study investigates the biochemical and chemical characteristics of a bacteriocin produced by *Rhizobium leguminosarum* biovar *trifolii* Strain T24 (trifolitoxin).

A stable biologically active derivative of trifolitoxin has been purified by reverse phase chromatography, gel filtration and high voltage paper electrophoresis.

Utilizing ultraviolet (UV), infrared (IR), nuclear magnetic resonance (NMR), fast atom bombardment mass spectroscopy (FAB MS), high voltage paper electrophoresis (HVPE), enzymatic, Edman sequencing, amino acid analysis and hydrolyses techniques, trifolitoxin has been shown to consist of a linear peptide (MW=1037 (FAB MS)) (asp-ile-gly-gly-ser-(arg-X-gly)-cys-val-ala (Edman/partial hydrolysis)). It contains two UV absorbing chromophores. One is an acid-labile thiazoline, the other chromophore,X, (pKa=5.1, λ^{max} pH 7.0, 239 nm, 302nm,$\varepsilon^{239\ nm} = 9700$, $\varepsilon^{302nm} = 5000$) has been characterized but its structure has not been determined . This blue fluorescent chromophore is linked to glycine and a modified arginine , which yields a D-L mixture of arginine after complete acid hydrolysis .

Hydrolysis of the thiazoline ring system reduces toxicity (98%) and leads to a multiplicity of active forms due to the oxidation of the free thiol.

Analysis of proteolytic fragments or acetylated derivatives of trifolitoxin and trifolitoxin sulphonic acid indicate that an N terminal amino group is required for toxicity. Cleavage of C terminal amino acids or reduction of the blue fluorescent chromophore completely eliminates activity.

The significance of the biological activity is discussed in the light of the current structure which is shown opposite.