

THE CONFIGURATION OF ECHINULIN PART III*

THE ABSOLUTE CONFIGURATION OF ECHINULIN

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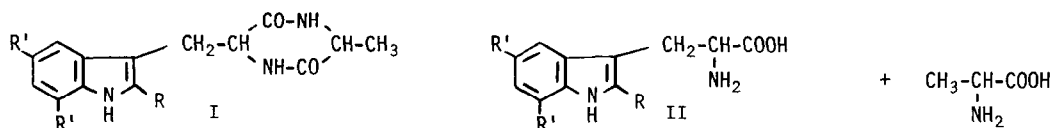
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Echinulin (Ia) was shown by Quillico and co-workers¹⁾ to be a cyclic dipeptide incorporating alanine and 2'-(1,1-dimethylprop-2-enyl)-5',7'-di-(3-methylbut-2-enyl)-tryptophan (IIIa). L-alanine was obtained by acid hydrolysis of echinulin (Ia) and hexahydroechinulin (Ib) thus



- a) $\text{R} = -\text{C}(\text{CH}_3)_2-\text{CH}=\text{CH}_2$, $\text{R}' = -\text{CH}_2-\text{CH}=\text{C}(\text{CH}_3)_2$
 b) $\text{R} = -\text{C}(\text{CH}_3)_2-\text{CH}_2-\text{CH}_3$, $\text{R}' = -\text{CH}_2-\text{CH}_2-\text{CH}(\text{CH}_3)_2$
 c) $\text{R} = \text{R}' = -\text{H}$

establishing the configuration of one asymmetric center in the piperazine-2,5-dione ring²⁾. Hydrolysis of echinulin with HBr does not yield echinin (IIa) but gives an intractable gum²⁾; hydroechinulin (Ib) gives a mixture of two separable products²⁾ but in our hands gave an intractable gum. Accordingly, the configuration of echinin (IIa) and hydroechinin (IIb) were established by comparing the ORD spectra of echinulin (Ia), hydroechinulin (Ib) and the four diastereoisomers of cyclo-alanyltryptophans (Ic). This established³⁾ that the alanyl and tryptophyl moieties belonged to the L-amino acid series, a result which has been confirmed by t.l.c.⁴⁾.

Further confirmation of this result has now been obtained by ozonolysis of echinulin (100 mg) in formic acid-water (10:1.5 ml) at room temperature, followed by decomposition of the ozonide by warming with hydrogen peroxide (1 ml, 3%) on the steam bath for 30 min and hydrolysis by boiling with 6N-HCl (10 ml) for 2 hr.⁵⁾ The presence of alanine and aspartic acid in the hydrolysate was established by paper chromatography⁶⁾.

Table 1

Paper chromatography	
	Rf-value (phenol-water (4:1 w/w))
Ala	5.7
Asp	2.5

Amino acid analysis (Automatic Amino Acids Analyser LC-5S, Yanagimoto Co. Ltd.) and microbiology assay⁷⁾ using the Leuconostoc mesenteroides P-60 showed that 94% of the aspartic acid was in the L-form (Table 2) confirming that the echinin moiety (Ia) of echinulin has the L-amino acid configuration.

Table 2

Analysis of amino acids in hydrolysate from ozonolysis of echinulin		
	μg/ml (amino acid analyser)	μg/ml (microbioassay)
Asp	547	537
Ala	667	640
L-Asp	-	499 (94%)
D-Asp	-	38 (6%)

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