Stereochemical Course of the α,β-Desaturation of L-Tryptophan in the Biosynthesis of Cryptoechinuline A in *Aspergillus Amstelodami*

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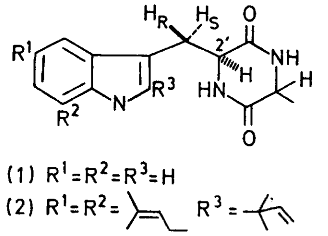
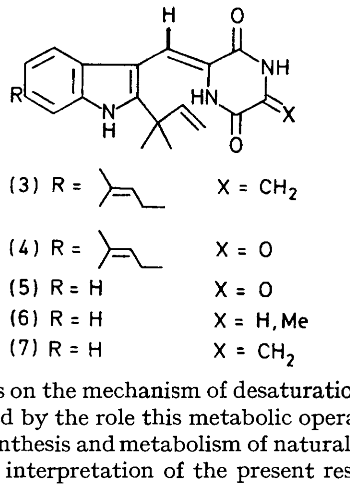
Summary Incorporation of stereospecifically labelled L-tryptophan into cryptoechinuline A (**3**), which has been assigned the (*Z*)-configuration from examination of its natural abundance and ^{13}C-enriched ^{13}C n.m.r. spectra, proceeds with removal of a *pro-S* hydrogen atom from the β-methylene position; a new metabolite, cryptoechinuline C (**5**) has also been isolated.

Among the isoprenylated piperazinedione derivatives containing a dehydrotryptophan grouping, recently isolated from *Aspergillus* spp. cultures,[1] cryptoechinuline A (**3**) may be biosynthetically useful because of its possible intermediacy in the conversion of *cyclo*-alanyl-tryptophanyl (**1**) into neoechinuline (**4**).[2]

Analysis of the biosynthetic pathway is being carried out by Dr. Marchelli, whilst we are interested in the metabolic operations of the oxidation process. We now report studies designed to establish the steric course of the formal dehydrogenation involved in the incorporation of L-tryptophan into cryptoechinuline A (**3**).

Samples of L-tryptophan stereospecifically labelled with tritium in the β-methylene position were synthetised from enantiomeric forms of DL-serine, stereospecifically labelled with tritium at position 3, and indole, using fibre-entrapped tryptophan synthetase (EC 4.2.1.20) from *E. coli*.[3] Incorporation of (3′*R*)[3′-^{3}H; 3′-^{14}C]L-tryptophan into echinuline (**2**) and cryptoechinuline A (**3**) took place with *ca.* 95% and 98% tritium retention, respectively, whereas the (3′*S*)-isomer of doubly labelled tryptophan was incorporated into (**2**) and (**3**) with *ca.* 96% and 5% tritium retention. The incorporation ranged from 0·8—6%. A stereospecific removal of a *pro-S* hydrogen atom from the β-methylene position thus occurs in the formal α,β-desaturation of L-tryptophan in the biosynthesis of cryptoechinuline A (**3**). The same stereochemical course has been observed during incorporation of L-tyrosine into mycelianamide.[4]





Investigations on the mechanism of desaturation of amino acids are justified by the role this metabolic operation could play in the biosynthesis and metabolism of natural products.[5] However, a full interpretation of the present result can be drawn from the knowledge of the geometry of cryptoechinuline A (**3**). This has been tentatively assigned as follows. The ^{13}C n.m.r. spectrum of cryptoechinuline A (**3**) shows signals, arising from the carbonyl carbon atoms, at δ 157·1 (triplet, with splitting *ca.* 3 Hz) and 155·7 (complex multiplet). The former has been assigned to the carbonyl group present in the dehydrotryptophan grouping of (**3**) because the ^{13}C n.m.r. spectrum of (**3**) biosynthetised in *Aspergillus amstelodami* from 90% enriched [1-^{13}C] glycine, a known precursor of the carbon atoms at position 1′ and 2′ of the tryptophan grouping in this and other classes of secondary metabolites,[1b,6] showed a 100% intensity enhancement of this signal in comparison with the remaining spectrum. Its triplet structure can be attributed to the coupling of the carbonyl carbon atom with amidic and vinylic hydrogen atoms. The lack of a coupling larger than 3 Hz would rule out a *trans* relationship between the carbonyl carbon in the dehydrotryptophan grouping and the vinylic hydrogen, according to the known stereospecificity of the coupling.[7] These observations suggest a (*Z*)-geometry for cryptoechinuline A (**3**) and a formal *cis* loss of hydrogen atoms from the side chain of L-tryptophan during its biosynthesis.

The possible relevance of α,β-desaturation to the metabolic operations of isoprenylated cyclic dipeptides in *Aspergillus* spp. is further supported by the presence in *A. amstelodami* of a substance, cryptoechinuline C, which has been assigned formula (**5**) on the basis of spectral data and from the observation that 2(1,1-dimethylallyl) 3-formylindole accompanies compound (**6**) in *A. glaucus*.[1a] Cryptoechinuline C (**5**) and the isoprenylated aldehyde might arise from neoechinuline B (**7**) by selective cleavage of the dehydroalanine and of the dehydrotryptophan groupings, respectively. Experiments to test this hypothesis are in progress.

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