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MOLECULAR STRUCTURE OF CRYPTOECHINULINE A, A NEW METABOLITE OF *ASPERGILLUS AMSTELODAMI*, ISOLATED DURING INVESTIGATIONS ON ECHINULINE BIOSYNTHESIS

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In a study designed to establish the stereochemical course of the biological synthesis of tryptophan from serine and indole or indole-containing derivatives we planned feeding experiments of serine samples [1] stereospecifically labelled with tritium at position 3 to Aspergillus amstelodami cultures producing echinuline (3)[2] and neoechinuline (1)[3], which contain a tryptophan and a dehydrotryptophan moiety, respectively. Indeed, it seemed possible to determinate the stereochemical course of the tryptophan biosynthesis measuring the tritium retentions into (3) and (1), biosynthetised from the enantiomeric forms of the asymmetrically labelled serine, once the stereochemistry of the dehydrogenation of tryptophan during its incorporation into neoechinuline (1) had been determined in feeding experiments of available [4] samples of tryptophan stereospecifically labelled with tritium in position 3'.

Preliminary experiments with randomly labelled tryptophan have shown that all the tritium present at position 3' in the precursor is retained into echinuline (3), whereas ca. 50% is lost in the conversion into neoechinuline (1).[5]

However, since experiments with [3-^{3}H;3-^{14}C]serine indicated a 6% incorporation into echinuline (3), accompanied by ca. 30% tritium loss, it was necessary to establish the manner of incorporation of serine and of compounds metabolically related to it [6] into the two metabolites (1) and (3), using ^{13}C-labelled precursors owing to the lack of easy degradation procedures for the two abovementioned compounds (1) and (3). Furthermore, we expected in this way to obtain information on the origin of the oxalamide moiety present in the dioxopiperazine ring of (1).

From cultures of Aspergillus amstelodami, grown for feeding purposes on synthetic medium, neoechinuline (1) was not isolated, a new metabolite, cryptoechinuline A, being isolated instead in small amount, close to a large quantity of echinuline (3).

Cryptoechinuline A, $C_{24}H_{27}N_3O_2$, optically inactive, m.p. 190-2°, forms yellowish crystals (from benzene). Its UV spectrum [λ_{max} (EtOH) 231; 273; 283(s), and 380 nm (ε 37.000; 24.100;

21.400, and 14.000)] resembles that of neoechinuline (1).

Comparison of the ^{13}C-n.m.r. spectrum of cryptoechinuline A with that of neoechinuline (1) (Table) showed a close similarity of chemical shifts in the region of both saturated and unsaturated carbon atoms. Moreover, the absence of one carbonyl signal in the spectrum of the former compound, together with the appearance of two more signals in the olefinic region (at δ 102.8 and δ 134.8) pointed to structure (2) for cryptoechinuline A.

Further support in favour of this structural assignment arose from the following evidence. In the mass spectrum the more relevant peaks are 389 (100%), 346 (6%), 320 (50%), 251 (11%), 250 (6%), 182 (15%), 69 (44%), and 41 (25%). High resolution mass spectrometry showed molecular ion of exact mass 389.2111 (± 0.004), corresponding to $C_{24}H_{27}N_3O_2$, and the following major fragments of exact mass 321.1489 (± 0.004) [$C_{19}H_{19}N_3O_2$, $M - C_5H_8$], 251.1660 (± 0.002) [$C_{18}H_{21}N$, $M - C_6H_6N_2O_2$] and 250.1589 (± 0.002) [$C_{18}H_{20}N$, $M - C_6H_7N_2O_2$], respectively.

Permanganate oxidation of cryptoechinuline A (2) led to a complex mixture of acidic compounds which, once methylated, was submitted to g.l.c.-mass spectrometric analysis. The presence of the dimethylester of amino teraphtalic acid was shown by comparison with an authentic sample, thus supporting the indicated substitution pattern in the benzene moiety of (2). Furthermore, the IR and the ^{1}H-n.m.r. spectra are in agreement with the proposed structure.

The initial aim, the determination of the origin of the dehydroalanine moiety of cryptoechinuline A and the search for a possible biosynthetic relationship between cryptoechinuline A and neoechinuline (1), require that conditions are available for incorporation into these metabolites of ^{13}C-labelled precursors, whose use is especially required at the light of the Birch's observation[7] of the large randomization of the labelling in echinuline (3) biosynthetised from ^{14}C-labelled alanine.

With these purposes in mind we preliminarly examined the natural abundance ^{13}C-n.m.r. spectrum of echinuline (3). The saturated carbon atoms region consists of 10 signals which were staightforwardly assgned to the 13 carbon atoms, the peaks due to methyl groups C_{2a}, C_{7d} and C_{7e} having double intensity because of the accidental degenerancy of their chemical shifts.

Assignments, based both on signals multiplicity and chemical shift criteria, are as follows. (in δ values from internal $(Me)_4Si$, 0.2 M in $CHCl_3$) C_{3b} 54.8; C_{3d} 50; C_{2a} 39.1; C_{5a} 34.6; C_{7a} 31.4; C_{3a} 29.6; C_{2d} 27.3; $C_{5,7e}$ 25.7; C_{3e} 19.9; $C_{5,7d}$ 17.9. The unsaturated carbon atoms region was not fully assigned because not relevant to the present investigation (see below). Finally, in the carbonyls region two signals were measured at 167.9 and 168.7, which at this stage could not be assigned.

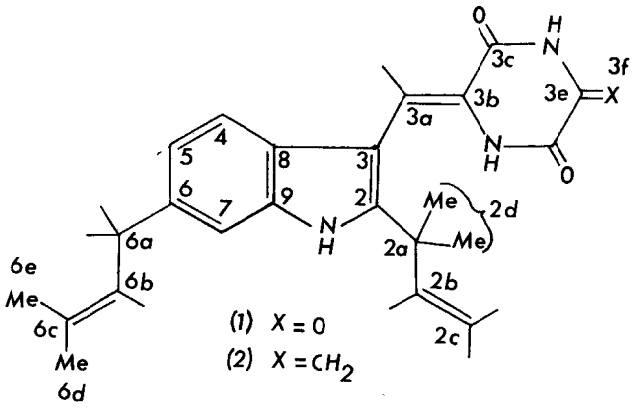
The ^{13}C-n.m.r. spectrum of echinuline (3) biosynthetised from 90% enriched [2-^{13}C]glycine, a known precursor[7] of echinuline (typically, 100 mg fed to 1 litre sugar beet molasses medium - 20 flasks - producing ca. 1.2 g of (3) and 10-20 mg of (1)) showed a 120% intensity enhancement of the signal due to C_{3b}, whereas the sample of (3) derived from 90% enriched [1-^{13}C]glycine indicated a similar enhancement of the carbonyl signal at δ 169.9. This

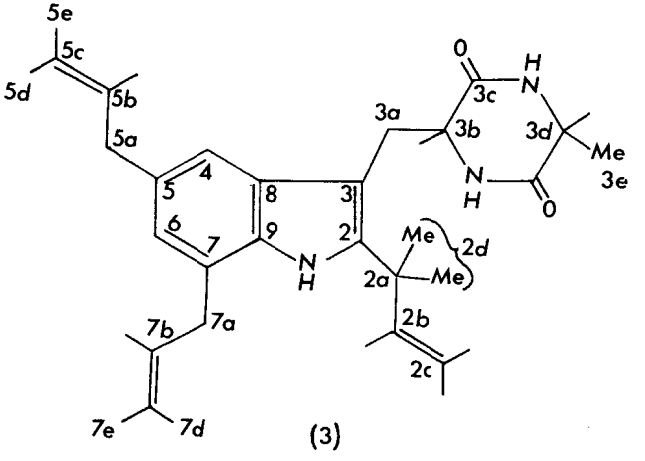
Table

^{13}C chemical shifts (in δ from internal Me_4Si, ca. 0.1 soln. in d_6-DMSO)

	C_{6e}	C_{6d}	C_{2d}	C_{6a}	C_{2a}	C_{3f}	C_3	C_7	C_{2c}	C_4	C_5	C_{3a}	C_8	C_6	C_{6b}	C_2	C_9	C_{3e}	C_{6c}	C_{2b}	C_{3b}
(1)	17.6	25.4	27.7	33.9	38.9	--	103.4	110.8	111.9	116.3	119.2	120.9	123.1	124.0	124.0	131.1	134.6	---	135.7	145.1	145.4
(2)	17.6	25.4	27.5	33.9	38.6	100.	102.8	110.9	111.7	112.	118.5	120.7	124.0	124.0	124.0	131.1	134.4	134.8	135.6	144.1	142.2

	carbonyls		
(1)	152.0	157.0	160.2
(2)		155.7	157.1





The stereochemistry around C_{3a} and C_{3b} is unsettled.

result allows assignment of the above signal to C_{3c}, in so far as the assumption of an intact incorporation of glycine into the C-1' and C-2' carbon atoms of the tryptophan moiety of (3) can be accepted. Unfortunately, neoechinuline (1) obtained from these feeding experiments could not be purified in the manner required for the spectral investigations. Therefore, the evidence only indicates the specific incorporation of glycine into the carbon atoms at positions 1' and 2' of the tryptophan moiety of echinuline (3). Experiments under different growing conditions and with other precursors are in progress.

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