Tetrahedron Letters No. 48, pp 4269 - 4270, 1975. Pergamon Press. Printed in Great Britain.

ERGOT ALKALOIDS III[1] THE ISOLATION OF N-METHYL-4-DIMETHYLALLYLTRYPTOPHAN FROM CLAVICEPS FUSIFORMIS.

by

Kevin D. Barrow*† and Francoise R. Quigley

(Biochemistry Department, Imperial College of Science and Technology, South Kensington, London S.W.7)

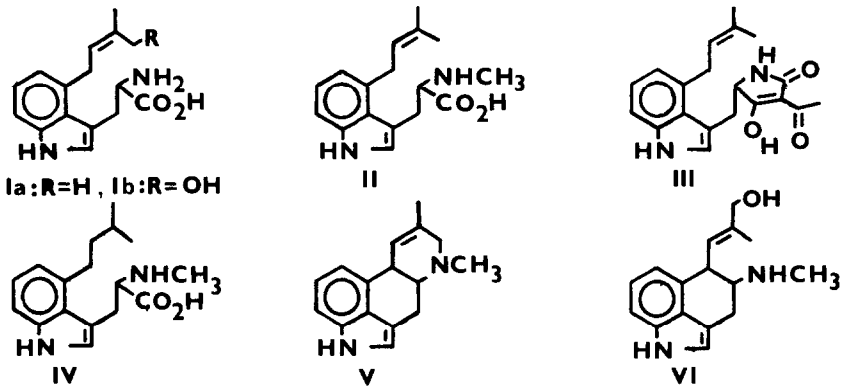
(Received in UK 13 October 1975; accepted for publication 20 October 1975)

The biosynthesis of ergot alkaloids commences with the alkylation of tryptophan by dimethylallylpyrophosphate to give 4-dimethyl-allyltryptophan[2] (Ia). The next step is probably a hydroxylation to give 4[Z-4-hydroxy-3-methyl-Δ^2-butenyl]-tryptophan (Ib) and this compound of undefined stereochemistry has been isolated from Claviceps purpurea cultures[3]

We report the isolation of N-methyl-4-dimethylallyltryptophan (II) from cultures of Claviceps fusiformis deprived of oxygen. Claviceps fusiformis was grown aerobically in submerged cultures in both shaken flasks and stirred fermenters. When alkaloid production began anaerobic conditions were imposed and the cultures stood for a further three days. Clavine alkaloids were extracted with chloroform at alkaline pH and then the amphoteric metabolites with n-butanol at neutral pH. The butanol extract, which contained considerable quantities of chanoclavines and other oxygenated clavine alkaloids, was chromatographed on silica with chloroform/methanol/ammonia as the eluant. We obtained small amounts of the isomers of clavicipitic acid[4,6](m/e 270, 215, 182, 169, 154), a new metabolite $C_{17}H_{22}N_2O_2$ identified as N-methyl-4-dimethylallyltryptophan (II) and 4-dimethylallyltryptophan (Ia)[5] (λ_{max} 275, 280 and 293 nm, ν_{max} 3240 (broad), 1590, 1500, 1410 cm^{-1}, m/e 272, 198, 156, 155)

N-methyl-4-dimethylallyltryptophan crystallized from methanol as needles m.p. 232°C, λ_{max} 274, 280 and 295 nm, ν_{max} 3580, 3250 (broad) 1640, 1400, 770 cm^{-1}, n.m r. (CD_3COOD) inter alia τ 8.64 (s,6H), 7.64 (S,3H) 5.06 (t, 1H, J 7.0Hz) and τ 6.3 - 7 (complex, 4H); m/e 286, 198 (100%) 156, 155, 154. The chemical shifts of the two allylic methyl groups of (II) are identical, as in bissecodehydrocyclopiazonic acid[7] (III). The fragmentation of (II) under electron impact is also very similar to (III) with allylic cleavage of the amino acid side chain giving the ion of m/e 198, followed by cyclisation to a series of tricyclic ions m/e

† Present address. School of Biochemistry, University of New South Wales, P.O. Box 1, KENSINGTON. N.S.W. 2033. AUSTRALIA



156, 155, 154 with elimination of a C-3 unit. Cyclisation of this type is only possible if the two side chains are located in the peri-positions of the indole nucleus, i.e. at positions 3 and 4

Further support for the structure (II) comes from the mass spectrum of the hydrogenation product (IV) which shows m/e 288, 200 and 144 (100%). A metastable peak at m/e 103.6 indicates that the base peak m/e 144 arises from the ion of mass 200 by the loss of the fragment $(CH_3)_2$ CH = CH_2. This is again analogous to the cyclopiazonic acid series[7]

Feeding experiments with [^{14}C-methyl]-methionine showed that (II) was the only labelled amphoteric tryptophan metabolite produced and the incorporation was 4-8%. Refeeding labelled (II) from the [^{14}C-methyl]-methionine feeding gave labelled agroclavine (V) with 1.4% incorporation. Other clavine alkaloids were also labelled but were not purified.

Whether N-methyl-4-dimethylallyltryptophan is an obligatory precursor of the ergot-alkaloids or merely a product that accumulates under conditions of oxygen deprivation has not been established. We are unable to detect (II) in normal aerobic cultures of Claviceps fusiformis. However, the facile production of the compound (II) would suggest that the N-methylation step, whether it precedes or follows the hydroxylation of the 2-methyl group of the 4-dimethylallyl substituent, occurs before the decarboxylation/cyclisation step(s) that give the tricyclic chanoclavines (VI).

References

1. K.D. Barrow and F R. Quigley, J. Chromat., 1975, 105, 393.
2. J.E. Robbers and H.G. Floss, Arch. Biochem. Biophys., 1968, 126, 967.
3. J.A. Anderson and M.S. Saini, Tetrahedron Letters, 1974, 2107.
4. J.E. Robbers and H.G. Floss, Tetrahedron Letters, 1969, 1857.
5. S. Agurell and J.E. Lindgren, Tetrahedron Letters, 1968, 5127
6. G.S. King, P.G. Mantle, C.A. Szczyrbak and E.S. Waight. Tetrahedron Letters, 1973, 215.
7. C.W. Holzapfel, R.D. Hutchinson and D C. Wilkins, Tetrahedron 1970, 26, 5239.