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A REVISED STRUCTURE FOR CLAVICIPITIC ACID

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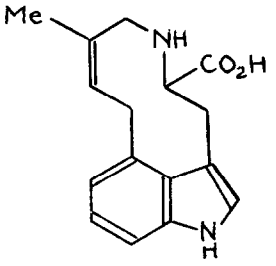
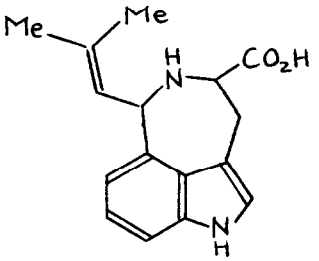
Clavicipitic acid was obtained by Robbers and Floss[1] from submerged cultures of Claviceps strain SD-58, to which DL-ethionine had been added to inhibit N-methylation in clavine alkaloid biosynthesis. On mass spectral, n.m.r., and biosynthetic evidence they proposed the structure (I).



(I)



(II)

We had concurrently obtained the same compound (M^+ $C_{16}H_{18}N_2O_2$, λ_{max} 285nm) from submerged culture, with or without DL-ethionine, of Claviceps fusiformis strain 139/2/1G.[2] Clavine alkaloids (mainly agroclavine) were removed from culture filtrate at pH 10.5 with n-butanol. A further extract with n-butanol at pH 3.5 was evaporated to small volume at reduced pressure, mixed with water (pH 5.0, 10 vols.), filtered and applied to a Zeocarb 225

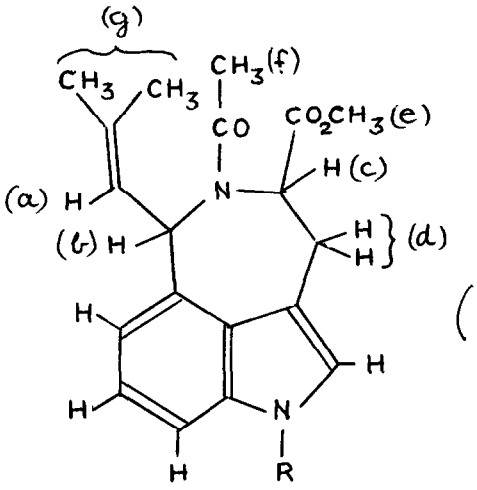
(H^+) column. Amphoterics were eluted with 2N-ammonium hydroxide, taken to dryness and chromatographed on silica gel GF_{254} using chloroform/methanol/benzene (2:1:1). One principal substance (Rf 0.38) showed a mass spectral fragmentation pattern and chromatographic behaviour identical with that of clavicipitic acid[3], for which we propose the revised structure (II).[4]

Isolation from extracts of larger volumes of culture filtrate showed decomposition losses of amphoterics on the strongly acidic ion-exchange resin. Thus ion-exchange was replaced by chromatography on a Sephadex G10 column to which the n-butanol extract was applied in, and subsequently eluted with, 10% methanol/water. Amphoterics, eluted slowly after rapid removal of high molecular weight material, were then separated on thin-layer chromatograms thus yielding clavicipitic acid. Use of chloroform/methanol/0.880 ammonia (75:25:1) further resolved the acid into two fractions (Rf 0.24 and 0.29), both of which had ultraviolet and mass spectra identical with those of the original material. Circular dichroism measurements[5] (in methanol) established that the isomer of Rf 0.29 had $\Delta\varepsilon_{max}$ - 2.95 at 289nm and that of Rf 0.24 had $\Delta\varepsilon_{max}$ -1.09 at 286nm. These supposed diastereoisomers could not be interconverted by the action of heat or 0.880 ammonia.

Attempts to prepare the chloroform-soluble trimethyl derivative[1] by the action of diazomethane on clavicipitic acid were unsuccessful, mixtures of mono- and di-methyl derivatives being obtained. However reaction of the acid (mixed diastereoisomers) with acetic anhydride in methanol[6] at room temperature resulted in esterification as well as N-acetylation giving in nearly quantitative yield the compound (III, R=H), m.p. 107-109° (M^+ 326, λ_{max} 285nm) which was easily soluble in chloroform. Treatment of the acetylated product with methyl iodide/methylsulphinyl carbanion in dimethylsulphoxide[6] resulted in methylation of the indolic nitrogen giving III (R = Me, M^+ $C_{20}H_{24}N_2O_3$) thus proving the absence of a primary amino-function in the acid.

The structure (III, R=H) of the acetyl methyl ester follows from the 100MHz p.m.r. spectrum (in $CDCl_3$).[7] The signals at (τ) 1.8 (1H, broad singlet), 2.8 - 3.2 (3H, multiplet) and 3.3 (1H, doublet) are due to the

indole protons. The one-proton doublet (J=7 Hz) at 4.26 is coupled to another one-proton doublet (J=7 Hz) at 4.86; the former is due to the vinylic proton (a) and the latter the benzylic proton (b), the downfield shift from its position in, for example, agroclavine resulting from the effect of the amido-substituent. The one-proton multiplet at 5.70 is coupled to the two-proton multiplet at 6.3 - 6.8, and these represent protons (c) and (d) respectively. The three-proton singlets at 6.36 and 7.94 are due respectively to the ester (e) and acetyl (f) methyl groups, while the three-proton singlets at 8.18 and 8.32 represent two olefinic methyl groups (g, h) in somewhat different environments. It should be noted the stereochemical purity (relating to (b)) is not known.



(III)

Clavicipitic acid may, therefore, be a novel type of microbial metabolite, provided that it is not a product of the extraction process. However the only features of our process in common with that of Robbers and Floss are an exposure to a pH range 3.5 - 10.5 in air, and contact with one or more common organic solvents.

REFERENCES

1. J.E. Robbers and H.G. Floss, Tetrahedron Letters, 1857 (1969).
2. C.A. Szczyrbak, Ph.D. Thesis, University of London, (1972).
3. We are indebted to Dr. H.G. Floss for a sample of clavicipitic acid and a copy of its mass spectral element map.
4. This possibility (among others) was originally suggested by Dr. K.D. Barrow.
5. We thank Dr. P.M. Scopes for these determinations.
6. H. Morris, D.H. Williams and R.P. Ambler, Biochem. J., 125, 189 (1971).
7. We thank Mr. S. Roberts for these measurements. The mass spectral fragmentation is also consistent with the proposed structure.