1232. *The Production of Trypacidin and Monomethylsulochrin by* Aspergillus fumigatus

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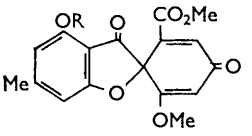
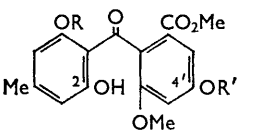
In the course of an examination of the metabolites of *Aspergillus fumigatus* we have isolated from the mycelium two related compounds, $C_{18}H_{16}O_7$, m. p. 230—238°, and $C_{18}H_{18}O_7$, m. p. 198—199°. The former is identical with the recently isolated trypanocidal antibiotic, trypacidin,[1] to which structure (I; R = Me) has now been assigned;[2] the work described here provides further evidence for this structure. The properties of the latter compound suggested that it might be a dihydro-derivative of the former, and this was confirmed by its formation on hydrogenation of trypacidin.



(I)



(II)

The nuclear magnetic resonance spectra of the two compounds are very similar; in particular, both show sixteen protons on carbon. The extra protons in the dihydro-compound must therefore be on oxygen, suggesting that trypacidin is a spirocoumaranone (cf. geodin and erdin [3]), a formulation consistent with its spectroscopic properties. Methylation of the dihydro-compound gave a monomethyl derivative (II; R = R′ = Me) identical with the dimethyl ether of the mould metabolite sulochrin (II; R = R′ = H). The dihydro-compound is therefore a monomethylsulochrin and, in view of its formation by reduction of trypacidin, must have the structure (II; R = Me, R′ = H), so that trypacidin has structure (I; R = Me). Trypacidin is thus the methyl ether of (−)-bisdechlorogeodin (I; R = H) and this is confirmed by the identity of its infrared spectrum with that of the methyl ether of (+)-bisdechlorogeodin, kindly provided by Dr. C. E. Stickings.

It is of interest that trypacidin is obtained when *A. fumigatus* is grown on Czapek–Dox medium while monomethylsulochrin is obtained on Raulin–Thom medium, the principal difference between the media being that the nitrogen is present as nitrate in the former and as ammonia in the latter.

Experimental.—Isolation of metabolites. (a) *Aspergillus fumigatus* (CMI 45,338, number 1813 in our collection) was grown at 25° in Glaxo vessels each containing 250 ml. of Czapek–Dox medium. The mycelium from 50 flasks was harvested 13 days after inoculation, washed with water, and macerated twice with chloroform (1 l.). The combined chloroform extracts were dried and evaporated, to give a mixture (3·5 g.) of solid and oil which was extracted at room temperature with light petroleum, leaving a residue (1·95 g.) which was chromatographed on silica gel (64 g.). Elution with benzene–chloroform (4 : 1) gave a solid (462 mg.) which was recrystallised from ethyl acetate to give trypacidin (323 mg.) as prisms, m. p. 230—238°, $[\alpha]_D^{26}$ −166° (*c* 0·1 in MeOH) (Found: C, 62·7; H, 4·8. Calc. for $C_{18}H_{16}O_7$: C, 62·8; H, 4·7%), $\nu_{max.}$ 1730, 1712, 1663, 1620, 1606 cm.$^{-1}$, $\lambda_{max.}$ 286 (ε 23,400), λ_{infl} 325 mμ (ε 6900). The infrared spectrum was identical with that of a sample of trypacidin kindly provided by Dr. J. Balan.

(b) *A. fumigatus* was grown as described above, but with Raulin–Thom medium in place of the Czapek–Dox medium. The fermentation was harvested as before to give a crude extract

[1] J. Balan, L. Ebringer, P. Nemec, S. Kovac, and J. Dobias, *J. Antibiotics*, 1963, **16***A*, 157.
[2] J. Balan, A. Kjaer, S. Kovac, and R. H. Shapiro, *Acta Chem. Scand.*, 1965, **19**, 528.
[3] D. H. R. Barton and A. I. Scott, *J.*, 1958, 1767.

(8·08 g.) which was extracted with light petroleum, leaving an insoluble residue (1·93 g.) which was chromatographed on silica gel as above, to give a solid (220 mg.) which was recrystallised from ethyl acetate–light petroleum, to give *monomethylsulochrin* (II; R = Me, R′ = H) as elongated plates, m. p. 198—199° (Found: C, 62·5; H, 5·3. $C_{18}H_{18}O_7$ requires C, 62·4; H, 5·2%), $\nu_{max.}$ 3386, 1694, 1603 cm.$^{-1}$, $\lambda_{max.}$ 285 (ε 14,700), λ_{infl} 325 mμ (ε 7350), τ 2·95 (1) (doublet, J = 2·5 c./sec.), 3·32 (1) (doublet, J = 2·5 c./sec.), 3·52 (1), 3·85 (1) 6·97 (3), 6·30 (3), 6·61 (3), 7·69 (3). The methyl ether gave no colour with ferric chloride.

Methylation of monomethylsulochrin with methyl iodide in acetone in the presence of potassium carbonate gave dimethyl sulochrin (II; R = R′ = Me) as prisms, m. p. 155—156° (Found: C, 62·1; H, 5·6. Calc. for $C_{19}H_{20}O_7$: C, 63·3; H, 5·6%), identical with a sample prepared by methylation of sulochrin under the same conditions.

Reduction of trypacidin. A solution of trypacidin (9·6 mg.) in ethyl acetate (4 ml.) was shaken with hydrogen in the presence of palladised charcoal (5%; 10·8 mg.) for 2 hr. The reaction mixture was worked up in the usual way, to give a gum which was dissolved in ethyl acetate and filtered through a small column of silica gel, to give a solid which was recrystallised from ethyl acetate–light petroleum, to give monomethylsulochrin (II; R = Me, R′ = H), m. p. 195—199°; its infrared spectrum was identical with that of the naturally occurring compound.

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