The Biosynthesis of the Aflatoxins

By G. C. ELSWORTHY, J. S. E. HOLKER,* (MRS.) J. M. MCKEOWN, J. B. ROBINSON, and (in part) L. J. MULHEIRN
(*Robert Robinson Laboratories, University of Liverpool, P.O. Box* 147, *Liverpool* L69 3BX)

Summary A new metabolite of *Aspergillus versicolor*, demethylsterigmatocystin (II) has been converted into 5-hydroxydihydrosterigmatocystin (IV) specifically labelled with ^{14}C in the *O*-methyl group—this compound is shown to be a biogenetic precursor of aflatoxins B_2 and G_2, (IX) and (XI), respectively, in *A. parasiticus*: a second new metabolite of *A. versicolor* has been shown to be 6-deoxyversicolorin-A (XII).

RECENT publications[1,2] have reported the incorporation of labelled acetate into sterigmatocystin (I) and aflatoxin B_1 (VIII) by the organisms *Aspergillus versicolor* and *A. flavus*, respectively. The observed distributions of label in these metabolites are compatible with the suggestion of Thomas[3] that the aflatoxins could be derived from a precursor of the sterigmatocystin type by oxidative cleavage of ring A [dotted line in (I)].

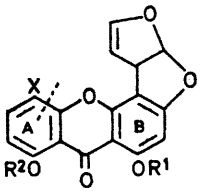
In early studies[4] we reported that labelled sterigmatocystin is not incorporated into the aflatoxins produced by either *A. flavus* or *parasiticus*. We now show that 5-hydroxydihydrosterigmatocystin (IV) is incorporated by *A. parasiticus* into aflatoxins B_2 and G_2, (IX) and (XI), respectively.

5-Hydroxydihydrosterigmatocystin (IV), specifically labelled with ^{14}C in the *O*-methyl group was prepared from a new metabolite demethylsterigmatocystin (II), isolated from a strain of *A. versicolor* (C.M.I. 49124) which had been repeatedly sub-cultured. This metabolite, m.p. 253—255°,

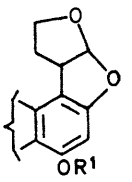
$[\alpha]_D^{24}$ −483° (*c* 0·20, $CHCl_3$) is a dihydric phenol and gave a dimethyl ether identical with 8-*O*-methylsterigmatocystin (III).[5]

Hydrogenation of demethylsterigmatocystin gave a dihydro-derivative (V), m.p. 202—204°, $[\alpha]_D^{24}$ −378° (*c* 0·07, $CHCl_3$) which on partial methylation with 1·2 molar equiv. of [^{14}C]methyl iodide and silver oxide in chloroform gave a mixture of two isomeric monomethyl ethers (VI) and (VII), separated from unchanged starting material by chromatography on silica gel. Oxidation of this mixture by the Elb's persulphate method gave, *inter alia*, 5-hydroxydihydrosterigmatocystin with ^{14}C in the *O*-methyl group (IV), separated by chromatography and identical with the compound previously prepared by similar oxidation of dihydrosterigmatocystin (VI).[6]

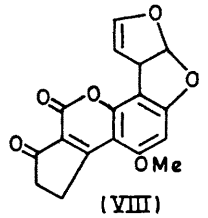
The labelled compound (IV) (28·8 μCi) was fed in acetone solution to a shake culture of *A. parasiticus* and, after 1 week at 25°, the aflatoxins were isolated, diluted with authentic samples and separated by t.l.c. on silica gel. The individual aflatoxins were then further diluted with pure samples and each one crystallised to constant activity. The total incorporations were as follows: aflatoxin B_1 (0·06%), B_2 (1·94%), G_1 (0·06%), and G_2 (0·41%). The retention of the label in the *O*-methyl groups of B_2 and G_2 was demonstrated by Zeisel degradation and isolation of the resultant methyl iodide as methyltriethylammonium iodide.



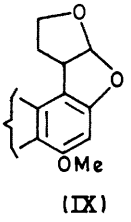
(I) R^1 = Me, R^2 = X = H
(II) R^1 = R^2 = X = H
(III) R^1 = R^2 = Me, X = H



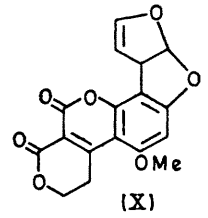
(IV) R^1 = Me, R^2 = H, X = OH
(V) R^1 = R^2 = X = H
(VI) R^1 = Me, R^2 = X = H
(VII) R^1 = X = H, R^2 = Me



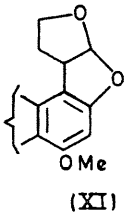
(VIII)



(IX)



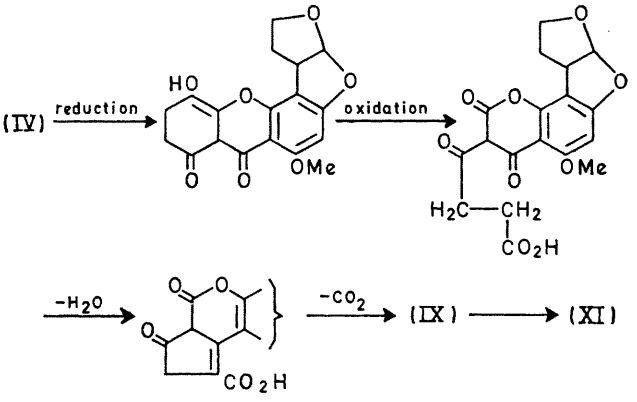
(X)



(XI)

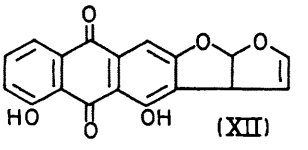
97% and 95% of the total activities of B_2 and G_2 respectively were found in the quaternary salts. The low total incorporations into B_1 and G_1 precluded similar estimations in these cases.

Aflatoxins B_2 (IX) and G_2 (XI) contain the same saturated four-carbon side-chain as in 5-hydroxydihydrosterigmatocystin (IV) whereas aflatoxins B_1 (VIII) and G_1 (X) contain the unsaturated side-chain. Compound (IV) would therefore be expected to be a direct precursor of aflatoxins B_2 and G_2 but not of B_1 and G_1. The relatively high total incorporations into B_2 and G_2 compared with the low incorporations into B_1 and G_1 support this thesis. The probable reaction sequence involved in the transformation is illustrated in the Scheme.



SCHEME

During the isolation of demethylsterigmatocystin from *A. versicolor* chromatographic fractions containing this compound were found to be contaminated by minor amounts of an orange impurity. This minor component was obtained pure by further extensive chromatography and was found to form prisms from acetone, m.p. 245—246°, $[\alpha]_D$ − 439° (*c* 0·9, dioxan). The compound, $C_{18}H_{10}O_6$, gave a dimethyl ether, m.p. 220—221° and a diacetyl derivative, m.p. 220—225°. Detailed spectral analyses on the parent and its dimethyl ether, which will be reported fully elsewhere, clearly established that the metabolite was 6-deoxyversicolorin-A (XII). Experiments are in hand to test the possibility that this anthraquinone is the biological precursor of sterigmatocystin.



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[6] J. E. Davies, D. Kirkaldy, and J. C. Roberts, *J. Chem. Soc.*, 1960, 2169.