Studies in Mycological Chemistry. Part XXII.[1] Total Synthesis of (±)-Aflatoxin-B2

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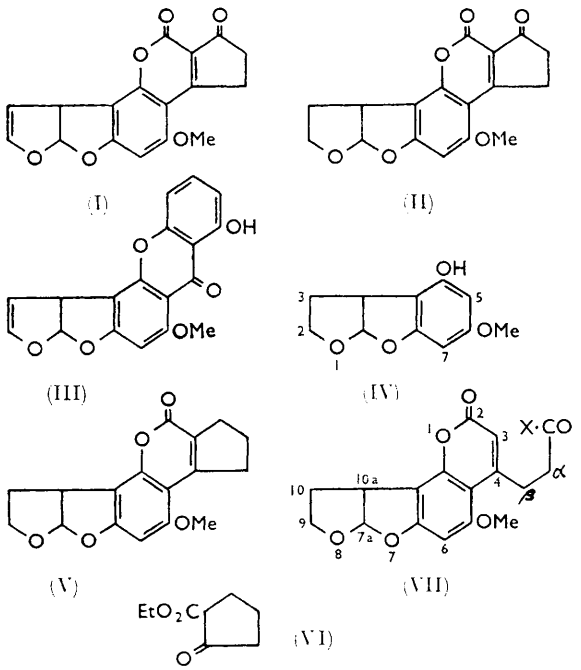
Total syntheses of (±)-tetrahydrodeoxoaflatoxin-B1 and of (±)-aflatoxin-B2 from a common intermediate, (±)-tetrahydro-4-hydroxy-6-methoxyfuro[2,3-*b*]benzofuran, are described.

THE aflatoxins are a group of highly toxic mould metabolites (produced by certain strains of *Aspergillus flavus* [2] and *A. parasiticus* [3]) which have been recognised as the causative agents of Turkey 'X-Disease,' [4] and which are now known [5] to be powerful carcinogens. Natural aflatoxin appears always to contain a variable proportion of at least four closely-related metabolites [6] which are termed aflatoxin-B1, -B2, -G1, and -G2. The structures of these four compounds have been elucidated [7] and a preliminary account of the synthesis of (±)-aflatoxin-B1 (I) has been published.[8] We now report a synthesis of (±)-aflatoxin-B2 (II).



In the course of work directed towards the confirmation of the structure of a related mould metabolite, sterigmatocystin (III), we had developed [9] a synthesis of (±)-tetrahydro-4-hydroxy-6-methoxyfuro[2,3-*b*]-benzofuran (IV) and it occurred to us that this compound might act as a useful intermediate for a synthesis of (±)-aflatoxin-B2 (II). As a preliminary to our main objective, we attempted the easier task of converting this intermediate (IV) into (±)-tetrahydrodeoxoaflatoxin-B1 (V), a racemic form of the laevorotatory hydrogenation product [7] of aflatoxin-B1 (I). To this end, the intermediate (IV) was condensed (in ethanolic hydrogen chloride) with ethyl cyclopentanone-2-carboxylate (VI). A product was obtained (in 35% yield) which was proved (see Experimental section) to be the desired (±)-tetrahydrodeoxoaflatoxin-B1 (V). (In a preliminary Communication [10] we recorded the isolation of this compound in a yield of 3% but, in this instance, we had used hydrogen chloride in acetic acid as the condensing agent.) We then turned our attention to the synthesis of a racemate corresponding to the naturally occurring metabolite (−)-aflatoxin-B2 (II).

Pechmann condensation of the intermediate (IV) with diethyl β-oxoadipate in ethanolic hydrogen chloride gave the coumarin-ester (VII; X = EtO) which was readily hydrolysed to the acid (VII; X = HO). Considerable difficulty was experienced in cyclising this acid in order to obtain the desired product (II). Most of the methods attempted were either too mild to cause ring-closure or else were too drastic and resulted in the destruction of the sensitive tetrahydrofurofuran system. Cyclisation was finally achieved in the following way (cf. ref. 8). The acid (VII; X = HO) was converted, by means of oxalyl chloride, into the corresponding acid chloride (VII; X = Cl) (not isolated) which, under mild Friedel–Crafts conditions, resulted in a mixture from which, by preparative thin-layer and column chromatography, there was separated (in 33% yield) a single product. This product crystallised from chloroform–methanol as colourless needles, m. p. 303—306° (decomp.) with the correct molecular formula (mass spectrum) for a compound of structure (II). That this synthetic product was (±)-aflatoxin-B2 was proved by the demonstration that its spectral and chromatographic properties (see below) were virtually identical with those of a sample of (−)-aflatoxin-B2 which we had isolated, by preparative thin-layer chromatography, from a crude mixture of natural aflatoxins (*ex. A. parasiticus*). The nature of our synthetic product was further confirmed through

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[3] C. W. Hesseltine, O. L. Shotwell, J. J. Ellis, and R. D. Stubblefield, *Bact. Rev.*, 1966, **30**, 795.
[4] P. C. Spensley, *Endeavour*, 1963, **22**, 75.
[5] F. Dickens and H. E. H. Jones, *Brit. J. Cancer*, 1965, **19**, 392.
[6] R. D. Hartley, B. F. Nesbitt, and J. O'Kelly, *Nature*, 1963, **198**, 1056.
[7] T. Asao, G. Büchi, M. M. Abdel-Kader, S. B. Chang, E. L. Wick, and G. N. Wogan, *J. Amer. Chem. Soc.*, 1965, **87**, 882.
[8] G. Büchi, D. M. Foulkes, M. Kurono, and G. F. Mitchell, *J. Amer. Chem. Soc.*, 1966, **88**, 4534.
[9] J. A. Knight, J. C. Roberts, and P. Roffey, *J. Chem. Soc.* (*C*), 1966, 1308.
[10] J. A. Knight, J. C. Roberts, P. Roffey, and A. H. Sheppard, *Chem. Comm.*, 1966, 706.

the observation that it was convertible, by catalytic hydrogenation, into a compound which was identical in all respects with (±)-tetrahydrodeoxoaflatoxin-B1 (V) which we had previously synthesised by an alternative route (see above).

The structure of aflatoxin-B2 is therefore confirmed.

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus. Infrared spectra were measured (unless otherwise stated) on compounds in chloroform solution with a Perkin-Elmer spectrophotometer (model 237). Ultraviolet spectra were recorded (unless otherwise indicated) on compounds in ethanolic solution with a Unicam spectrophotometer (SP 700). Proton magnetic resonance spectra were determined on compounds in deuteriochloroform solution with a Perkin-Elmer spectrometer (R10, 60 Mc./sec.), tetramethylsilane being used as an internal reference; in the sequel, figures in parentheses, following the statement of the nature of the signal, indicate intensities.

The silica used for thin-layer chromatography was 'Kieselgel G nach Stahl' (Merck). 'Alumina' was aluminium oxide (Spence, Type H) which had been 'acid-washed.'

(±)-*Tetrahydrodeoxoaflatoxin-B1* (V).—A solution of (±)-tetrahydro-4-hydroxy-6-methoxyfuro[2,3-*b*]benzofuran [9] (100 mg.) and ethyl cyclopentanone-2-carboxylate [11] (90 mg.) in absolute ethanol (15 ml.) was saturated (at 0°) with dry hydrogen chloride. The solution, having been kept at room temperature for 8 hr., was poured into ice–water. The resulting precipitate was collected and was crystallised from methanol to give (±)-*tetrahydrodeoxoaflatoxin-B1* (50 mg.) as needles, m. p. 209—210° [Found (on a sublimed, 150°/0·1 mm., and recrystallised sample): C, 67·8; H, 5·4%; *M* (mass spectrum), 300·102. $C_{17}H_{16}O_5$ requires C, 68·0; H, 5·4%; *M*, 300·100], $\lambda_{max.}$ 214 (infl.), 254, 264, and 332 mμ (10^{-3} ε 28·1, 7·7, 8·4, and 13·5, respectively); $\nu_{max.}$ 1713, 1625, 1608, and 1582 cm.$^{-1}$. The ^{1}H n.m.r. spectrum showed (i) a doublet (1) at τ 3·5 (J = 6 c./sec.) (–O–C*H*–O–), (ii) a singlet (1) at τ 3·63 (Ar-*H*) (iii) a singlet (3) at τ 6·10 (–O·CH_3), and (iv) complex signals (corresponding to *ca.* 11 aliphatic protons) at τ 5·65—8·20.

(–)-Tetrahydrodeoxoaflatoxin-B1 [obtained by hydrogenation [12] of (–)-aflatoxin-B1 (*ex A. flavus*)] formed needles, m. p. 247—248°, $\lambda_{max.}$ 215(infl.), 254, 264, and 332 mμ (10^{-3} ε 24·4, 6·8, 7·5, and 12·0, respectively); $\nu_{max.}$ 1713, 1625, 1607, and 1582 cm.$^{-1}$. This 'natural product' and the synthetic product had the same blue fluorescence in u.v. light and, on t.l.c. on silica plates, both showed the following R_F values in the four stated solvent systems: (i) chloroform–methanol (99 : 1 v/v.), 0·53; (ii) benzene–ethyl acetate (2 : 1), 0·67; (iii) ethyl acetate, 0·80; (iv) chloroform–acetone (9 : 1), 0·87.

Ethyl (±)-β-{7a,9,10,10a-*Tetrahydro*-5-*methoxy*-2-*oxo*-2H-*furo*[3′,2′:4,5]*furo*[2,3-h][1]*benzopyran*-4-*yl*}*propionate* (VII; X = EtO).—A solution of (±)-tetrahydro-4-hydroxy-6-methoxyfuro[2,3-*b*]benzofuran [9] (1·0 g.) and diethyl β-oxoadipate [13] (1·1 g.) in absolute ethanol (40 ml.) was saturated at 0° with dry hydrogen chloride. The solution, having been kept at room temperature overnight, was poured into ice–water. The resulting precipitate was collected and was crystallised twice from ethanol to give the *ester* (330 mg.) as needles, m. p. 131—131·5° [Found: C, 63·3; H, 5·6%; *M* (mass spectrum), 360. $C_{19}H_{20}O_7$ requires C, 63·3; H, 5·6%; *M*, 360], $\lambda_{max.}$ 213 (infl.), 254, 262, and 330 mμ (10^{-3} ε 30·8, 8·1, 9·4, and 13·3); $\nu_{max.}$ 1740 (sh.), 1725, 1630, and 1608 cm.$^{-1}$. The ^{1}H spectrum showed (i) a doublet (1) at τ 3·55 (J = 6 c./sec.) (–O·C*H*–O–); (ii) a singlet (1) at τ 3·65 (Ar-*H*); (iii) a singlet (1) at τ 4·0 (coumarin-*H*); (iv) a quartet (2) at τ 5·8 (–O·CH_2·CH_3); (v) a singlet (3) at τ 6·1 (–O·CH_3); (vi) complex signals (*ca.* 9) at τ 6·6—7·6 [–CH_2– (other than those in the ethyl group) and >C*H*–]; and (vii) a triplet (3) at τ 8·7 (–CH_2·CH_3).

(±)-β-{7a,9,10,10a-*Tetrahydro*-5-*methoxy*-2-*oxo*-2H-*furo*-[3′,2′:4,5]*furo*[2,3-h][1]*benzopyran*-4-*yl*}*propionic Acid* (VII; X = HO).—A solution of the foregoing ester (300 mg.) in ethanol (10 ml.) together with 10% aqueous potassium hydroxide (10 ml.) was heated under reflux for 2 hr. The cooled solution was extracted with ether and the aqueous portion was then rendered strongly acid (conc. hydrochloric acid). The resulting precipitate was collected and was washed with water. A solution of this material in 10% aqueous sodium hydrogen carbonate was warmed (charcoal) for 10 min. The mixture was filtered and the filtrate was acidified (conc. hydrochloric acid). The precipitate was collected and was crystallised from ethanol to give the *acid* (208 mg.) as needles, m. p. 252—253° [Found: C, 61·4; H, 4·9%; *M* (mass spectrum), 332. $C_{17}H_{16}O_7$ requires C, 61·4; H, 4·9%; *M*, 332], $\lambda_{max.}$ 214 (infl.) 255, 262, and 329 mμ (10^{-3} ε 28·9, 8·1, 9·3, and 13·0); $\nu_{max.}$ (KBr disc, Unicam SP 200 spectrophotometer) 3500, 2950 (broad), 1710, 1625, and 1600 cm.$^{-1}$.

(±)-*Aflatoxin-B2* (II).—Oxalyl chloride (0·5 ml.) was allowed to react for 2 hr. at room temperature and under nitrogen with a suspension of the foregoing acid (80 mg.) in methylene dichloride (3 ml.) which had previously been distilled from phosphoric oxide. The solvent was removed *in vacuo* and a further 0·5 ml. of oxalyl chloride in methylene dichloride was added. These operations were repeated until a total of 2 ml. of oxalyl chloride had been used. A stirred solution of the resulting acid chloride in methylene dichloride (7·5 ml.) under nitrogen was then treated with freshly sublimed aluminium chloride (40 mg.) at less than –5° for 3 hr. After the addition of excess of 2N-hydrochloric acid, the organic layer was separated. The aqueous residue was extracted twice with methylene dichloride and the combined organic layers were washed with 5% aqueous sodium hydrogen carbonate (2 × 3 ml.) and then with water (2 × 3 ml.). The solution was dried ($MgSO_4$) and filtered and the solvent was removed. (The ensuing operations were then carried out, as far as practicable, in the dark since the aflatoxins are light sensitive.) Preparative t.l.c. of the residue on silica plates, using chloroform–acetone (9 : 1 v/v.) as the developing solvent, produced a band, R_F 0·45—0·50, which had an intense blue fluorescence in u.v. light. The material in this band was extracted into chloroform–methanol (3 : 1). The solvents were removed and a chloroform solution of the residue was allowed to percolate through a small column of alumina (5 g.). Evaporation of the percolate gave a residue (35 mg.) which crystallised from chloroform–methanol to give (±)-*aflatoxin-B2* as fine needles (27 mg.), m. p. 303—306° (decomp.) [Found (on a sample recrystallised from chloroform–methanol/charcoal):

[11] *Org. Synth.*, Coll. Vol., II, 1943, 116.

[12] D. A. van Dorp, A. S. M. van der Zijden, R. K. Beerthuis, S. Sparreboom, W. O. Ord, K. de Jong, and R. Keuning, *Rec. Trav. chim.*, 1963, **82**, 587.

[13] B. Riegel and W. M. Lilienfeld, *J. Amer. Chem. Soc.*, 1945, **67**, 1273.

M, 314·0790. $C_{17}H_{14}O_6$ requires *M*, 314·0790], $\lambda_{max.}$ (MeOH) 220, 240 (infl.), 266, and 363 mμ (10^{-3} ε 17·9, 12·8, 12·0, and 23·4, respectively); $\nu_{max.}$ 1765, 1695, 1635, 1600, and 1565 $cm.^{-1}$. These crystals tenaciously retained a small quantity of chloroform (detectable in the mass spectrum) which was not removed at 80°/10 mm. (Found: C, 64·1, 63·9; H, 4·5, 4·4. $C_{17}H_{14}O_6$ requires C, 65·0; H, 4·5. $C_{17}H_{14}O_6$, 1/20 $CHCl_3$ requires C, 64·0; H, 4·4%). The product could not be sublimed without some decomposition. Similar difficulties over analytical results, due to clathrated solvents, have previously been encountered in the case of the structurally related compound, sterigmatocystin (III).[14]

Both the synthetic product and (−)-aflatoxin-B2 (see below) showed the following R_F values in the four stated solvent systems: (i) chloroform–acetone (9 : 1 v/v.), 0·33; (ii) ethyl acetate–methanol (9 : 1), 0·53; (iii) acetone, 0·79; (iv) chloroform–methanol (4 : 1), 0·90.

(−)-*Aflatoxin*-B2.—A mixture (500 mg.) of aflatoxins (*ex. A. parasiticus*) was subjected to preparative t.l.c. on silica. Chloroform–acetone (9 : 1) produced bands (fluorescing under u.v. light) at R_F 0·05, 0·12, 0·36, 0·43, 0·48, and 0·55. The material in the bands at R_F 0·48 was extracted into chloroform–methanol (3 : 1). The solvent was evaporated and the chromatographic separation was repeated four times. A chloroform solution of the purified material was allowed to percolate through a small column of alumina (2 g.). Evaporation of the percolate gave a residue which crystallised from chloroform–methanol to yield (−)-aflatoxin-B2 (2·5 mg.) as very pale yellow prisms, m. p. >310° (decomp.), $\lambda_{max.}$ (MeOH) 223, 240 (infl.), 266, and 363 mμ (10^{-3} ε 17·8, 12·4, 13·3, and 26·4). The i.r. spectrum was identical with that of the synthetic material (see above).

Hydrogenation of (±)-*Aflatoxin-B2*.—A solution of (±)-aflatoxin-B2 (5 mg.) in chloroform (3 ml.) with Adams catalyst (5 mg.) was shaken in an atmosphere of hydrogen for 2 hr. Removal of the catalyst and evaporation of the solvent gave a colourless oil which soon crystallised. Recrystallisation from methanol yielded (±)-tetradehydrodeoxoaflatoxin-B1 (2 mg.)as needles, m. p. 207—209° (not depressed by admixture with a sample prepared as above). The spectral (u.v. and i.r.) and chromatographic (t.l.c. on silica) properties of this compound were identical with those of a sample which we had previously synthesised in an alternative way (see above).

We are grateful to Dr. D. A. van Dorp for a specimen of (−)-tetrahydrodeoxoaflatoxin-B1 [prepared from (−)-aflatoxin-B1 (*ex. A. flavus*)] and to Professor W. D. Ollis for a gift of a mixture of aflatoxins (*ex. A. parasiticus*). We also thank the S.R.C. for the award of maintenance grants (to A. H. S. and P. R.).

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