New Metabolites of *Aspergillus terreus*: 3-Hydroxy-2,5-bis-(*p*-hydroxyphenyl)penta-2,4-dien-4-olide and Derivatives

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We have found a strain of *Aspergillus terreus* which produces a series of metabolites of novel structural type for this organism.[1] We describe here the isolation, identification, and synthesis of the parent member of this series, aspergillide B1 (1a), which is structurally related to the derivatives of pulvic acid (2) commonly encountered in lichens.[2]

A mixture of aspergillides was obtained by acidification of the fermentation broth of our *Aspergillus terreus* strain grown on Czapek-Dox medium. The components could be partially separated on the basis of their differing acidities. Aspergillide B1 is soluble in aqueous sodium hydrogen carbonate and this allowed its separation (along with aspergillides B2 and B3) from other components. These aspergillides were then *O*-methylated with diazomethane, and the products separated chromatographically.

The ^{1}H n.m.r. spectrum of tri-*O*-methylaspergillide B1 shows two overlapping AA'BB' systems (8H) in the aromatic region and sharp signals at δ 6.23 (1 H, =CH) and 3.83 (9 H, 3 × OMe). Its i.r. spectrum shows an intense band at 1780 cm^{-1} but lacks hydroxy-absorptions. Hence, two structures, (1b) and (3a), are possible. Structure (3a) was initially favoured after examination of the products from alkaline degradation, and was synthesised by base-catalysed condensation of 1,3-bis-(*p*-methoxyphenyl)propan-2-one with diethyl oxalate in benzene (*cf.* refs. 3 and 4) [to yield compound (3b)] followed by methylation with diazomethane. However, the resulting tri-*O*-methyl ether (3a) was spectroscopically different (except for mass spectra) from tri-*O*-methylaspergillide B1. Although this synthetic ether and tri-*O*-methyl aspergillide B1 could have been geometrical isomers of structure (3a), this possibility was eventually discounted after further consideration of the products from alkaline degradation of the naturally derived ether. Under basic conditions, compounds (1b) and (3a) are probably both in equilibrium with the cyclopentenedione (4a) (*cf.* analogous reactions in ref. 5), and so degradation products obtained under these conditions are not indicative of a unique structure. Tri-*O*-methylaspergillide B1 was then shown to have structure (1b) by comparison with a synthetic sample (see below). Since a ^{1}H n.m.r. spectrum of a crude mixture of aspergillides shows no resonances attributable to methoxy-groups, structure (1a) is thereby established for aspergillide B1. However, from the evidence described and the synthetic work (see below), the configuration of the C(4)–C(5) bond in (1a and b) remains uncertain.‡ Preliminary structural evidence concerning other aspergillides is given in refs. 7*a* and *b*.

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‡ The calculated [6] chemical shift of the vinylic proton in the *Z*-isomer of structure (1b) is δ 6.01, whereas that for the *E*-isomer is δ 6.23, favouring the *E*-configuration for compound (1b) (δ 6.23).

[1] Previously studied metabolites from *A. terreus* are listed in M. W. Miller, 'The Pfizer Handbook of Microbial Metabolites,' McGraw-Hill, New York, 1961; T. Korzybski and W. Kurylowicz, 'Antibiotica,' Gustav Fischer Verlag, Jena, 1961; S. Shibata, S. Natori, and S. Udagawa, 'List of Fungal Products,' University of Tokyo Press, Tokyo, 1964.

[2] *Cf.* C. F. Culbertson, 'Chemical and Botanical Guide to Lichen Products,' University of North Carolina Press, Chapel Hill, North Carolina, 1969; R. L. Edwards and M. Gill, *J.C.S. Perkin I*, 1973, 1529, 1538 (and references cited therein). Pulvic acid (2) and derivatives have recently been isolated from higher fungi (see *e.g.* A. Bresinsky, H. Besl, and W. Steglich, *Phytochemistry*, 1974, **13**, 271; M. C. Gaylord and R. L. Brady, *J. Pharm. Sci.*, 1971, **60**, 1503).

[3] W. E. Bachmann, G. I. Fujimoto, and L. B. Wick, *J. Amer. Chem. Soc.*, 1950, **72**, 1995.

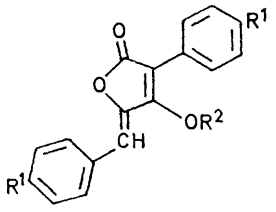
[4] E. Wenkert, S. K. Bhattacharya, and E. M. Wilson, *J. Chem. Soc.*, 1964, 5617.

[5] F. Kögl, H. Becker, G. de Voss, and E. Wirth, *Annalen*, 1928, **465**, 243.

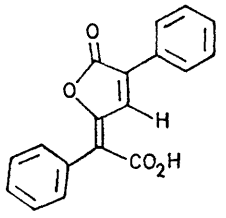
[6] U. E. Matter, C. Pascual, E. Pretsch, A. Pross, W. Simon, and S. Sternhell, *Tetrahedron*, 1969, **25**, 691.

[7] (*a*) B. T. Golding, Ph.D. Thesis, Manchester, 1965; (*b*) D G. Manwaring, M.Sc. Thesis, Manchester, 1966; Ph.D. Thesis, A.N.U., Canberra, 1969. Other aspergillides are derivatives of (1a) with an extra OH and/or C_5 unit (*e.g.* 3,3-dimethylallyl).

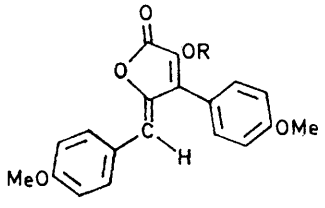
Structure (1b) was initially synthesised by using a rearrangement discovered by Claisen [8] [(4c) ⇌ (1c) at 200 °C· see refs. 7*a*, 9, and 10 for discussion of its mechanism]. Thus, condensation of 1,3-bis-(*p*-methoxyphenyl)-propan-2-one with diethyl oxalate in hot ethanolic sodium ethoxide gave a high yield of 4-hydroxy-2,5-bis-(*p*-methoxyphenyl)cyclopent-4-ene-1,3-dione (4b). On



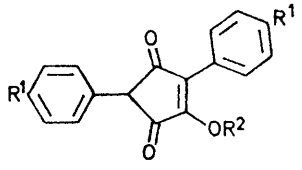
(1) a; R^1 = OH, R^2 = H
b; R^1 = OMe, R^2 = Me
c; R^1 = R^2 = H
d; R^1 = OMe, R^2 = H



(2)



(3) a; R = Me
b; R = H



(4) a; R^1 = OMe, R^2 = Me
b; R^1 = OMe R^2 = H
c; R^1 = R^2 = H

heating compound (4b) at 250 °C it was partially converted into two other compounds. Fractionation of this mixture with alkali gave compound (1d) which, on methylation with diazomethane, afforded a product identical (i.r. and u.v. spectra and t.l.c.) with tri-*O*-methylaspergillide B1.

A preparatively significant synthesis of tri-*O*-methylaspergillide B1 was achieved by condensing 1,4-bis-(*p*-methoxyphenyl)butane-2,3-dione with methyl chloroformate to give the lactone (1d), followed by methylation with diazomethane. The product (1b) was also identical (m.p. and mixed m.p., t.l.c., and i.r. and u.v. spectra) with tri-*O*-methylaspergillide B1.

[8] L. Claisen and T. Ewan, *Annalen*, 1895, **284**, 245.
[9] A. Schönberg and A. Sina, *J. Chem. Soc.*, 1946, 601.
[10] H. W. Moore and W. Weyler, *J. Amer. Chem. Soc.*, 1971, **93**, 2812.

EXPERIMENTAL

1,3-Bis-(*p*-methoxyphenyl)propan-2-one [11] and 1,4-bis-(*p*-methoxyphenyl)butane-2,3-dione [12] were prepared as described.

M.p.s were determined on a microscope hot-stage and are corrected. Silica gel for column chromatography was Whatman SG31 grade. KH refers to Kieselgel H for t.l.c. U.v. spectra were determined for solutions in clinical ethanol. ^{1}H N.m.r. spectra (60 MHz) were measured on *ca.* 10% solutions in $CDCl_3$ (except where stated otherwise). All solvents were AnalaR grade or redistilled laboratory reagents.

Isolation of Aspergillides.—Strain 86 of *Aspergillus terreus* was grown on Czapek-Dox medium by the procedure of Raistrick and Smith.[13] Yellow pigments were obtained as follows: (i) from an ethereal extract of the mycelium; (ii) as a precipitate from acidifying the fermentation broth (this fraction contained most product), further fractionated by extraction with chloroform, in which only part dissolved; and (iii) from an ethereal extract of the filtrate from (ii). At the end of a subsequent fermentation, which had been performed in darkness, the pigments were isolated by freeze-drying the fermentation broth to give a residue which was taken up in water, acidified with aqueous acetic acid, and extracted with ether. This milder procedure was carried out in order to minimise the possibility of chemical change during isolation. Comparison of the products of each fermentation by t.l.c. (KH; acetone–chloroform, 3 : 2) showed them to be similar.

The attempted isolation of pure aspergillides (B1—B3 and C1—C3) from these products [especially the chloroform-soluble part of fraction (ii)] and their eventual separation as methyl ethers are described in detail in ref. 7*a*.

3-Methoxy-2,5-bis-(p-*methoxyphenyl)penta-2,4-dien-4-olide* (1b) (*Tri-O-methylaspergillide B*1).—An ethereal solution of *ca.* 12 g of the chloroform-soluble part of fraction (ii) (see above) was extracted with ice-cold aqueous 5% sodium hydrogen carbonate, and the extract was rapidly made weakly acidic with ice-cold N-sulphuric acid. Filtration removed a yellow precipitate, and the filtrate was extracted with ether and ethyl acetate. The combined extracts were dried and evaporated to give a yellow oil (600 mg). This was treated in methanol with an excess of ethereal diazomethane for 2 h at room temperature. Evaporation left a yellow oil (630 mg) which was separated into *O*-methylaspergillides B1, B2, and B3 by repeated column chromatography (silica gel and neutral alumina). The combined fractions of tri-*O*-methylaspergillide B1 were recrystallised from ether–hexane giving greenish yellow *needles* (25 mg), m.p. 143—144°, $\lambda_{max.}$ 237 (log ε 4.3) and 353 nm (4.5), $\nu_{max.}$ (CCl_4) 1 780 and 1 760sh cm^{-1}, δ 3.83 (9 H, s, 3 × OCH_3), 6.23 (1 H, s, H-5), and 6.90, 6.93, 7.48, and 7.76 (each 2 H, d, *J* 9—10 Hz, 2 × AA′BB′ for aromatic protons), R_F 0.3 (KH; 1% methanol in chloroform) (Found M^+, 338.114 79. $C_{20}H_{18}O_5$ requires *M*, 338.115 41).

4-Hydroxy-2,5-bis-(p-*methoxyphenyl)cyclopent-4-ene-1,3-dione* (4b) (cf. *Ref.* 8).—To ethanolic sodium ethoxide (4.56 ml, 4 mmol) were added 1,3-bis-(*p*-methoxyphenyl)-propan-2-one (540 mg, 2 mmol) and diethyl oxalate (0.27

[11] S. B. Coan, D. E. Trucker, and E. I. Becker, *J. Amer. Chem. Soc.*, 1955, **77**, 60.
[12] I. Hagedorn, U. Eholzer, and A. Luttringhaus, *Chem. Ber.*, 1960, **93**, 1584.
[13] H. Raistrick and G. Smith, *Biochem. J.*, 1936, **30**, 1315.

ml, 2 mmol). After refluxing for 20 min the solution had become dark green and was cooled, poured onto ice–water, and acidified with ice-cold N-sulphuric acid, giving a yellow precipitate which was extracted with ether. The extract was shaken with aqueous 5% sodium carbonate and the combined alkaline extracts were acidified, giving a yellow precipitate which was filtered off [430 mg, 66% of fairly pure (4b)]. Three recrystallisations from benzene gave a *sample* of m.p.s 170—202 and 205—235° (due to thermal rearrangement—see text), $\lambda_{max.}$ 228 (log ε 4.21), 253 (4.28), and 352 nm (4.17), shifting on addition of alkali to 273 (4.30) and 373 nm (4.03), $\nu_{max.}$ ($CHCl_3$) 3 420, 1 730, and 1 685 cm^{-1}, δ [$(CD_3)_2CO$] 3.81 (3 H, s, OCH_3), 3.90 (3 H, s, OCH_3), 4.24 (1 H, s, H-2), *ca.* 5br (1 H, s, OH, disappears on addition of D_2O), 7.1 (6 H, m, aromatic H), and 8.36 (2 H, d, *J* 9—10 Hz, aromatic H) (Found: C, 70.3; H, 5.05. $C_{19}H_{16}O_5$ requires C, 70.35; H, 5.0%).

4-*Methoxy*-2,5-*bis*-(p-*methoxyphenyl*)*cyclopent*-4-*ene*-1,3-*dione* (4a).—Compound (4b) was treated with a slight excess of ethereal diazomethane to give (4a) (75%; recrystallised from ether). A further recrystallisation (ether) gave a sample of m.p. 150—150.5° (lit.,[5] 147°), $\lambda_{max.}$ 238 (log ε 4.23), 252 (4.30), and 353 nm (4.19), $\nu_{max.}$ (CCl_4), 1 690 cm^{-1}, δ 4.43 (3 H, s, OCH_3), 3.88 (3 H, s, OCH_3), 3.81 (3 H, s, OCH_3), 4.05 (1 H, s, H-2), 7.1 (6 H, m, aromatic H), and 8.13 (2 H, d, *J* 9—10 Hz, aromatic H).

2-*Methoxy*-3,5-*bis*-(p-*methoxyphenyl*)*penta*-2,4-*dien*-4-*olide* (3a) (cf. *Refs.* 3 *and* 4).—Benzene (10 ml) containing sodium methoxide (108 mg, 2 mmol) and diethyl oxalate (0.27 ml, 2 mmol) was refluxed for 5 min and cooled. To the resulting mixture was added a solution of 1,3-bis-(*p*-methoxyphenyl)propan-2-one (540 mg, 2 mmol) in benzene (10 ml). Stirring for 16 h at room temperature gave a deep red-brown solution which was diluted with ether and extracted with ice-cold aqueous 1% sodium carbonate. The combined alkaline extracts were acidified and the precipitate was extracted with ether to give crude product as a yellow solid. Recrystallisation (benzene–hexane) gave (3b) (100 mg, 15%) as straw-coloured needles, m.p. 205—223°. Two further recrystallisations (methanol) gave material homogeneous by t.l.c., m.p. 210—228° (decomp.). Compound (3b) was treated with an excess of diazomethane to give the *ether* (3a) as pale green needles, m.p. 170—172° (from ether). Two further recrystallisations (ether) gave a sample of m.p. 172.5—173.5°, $\lambda_{max.}$ 234 (log ε 4.16), 246 (4.16), and 353 nm (4.41), $\nu_{max.}$ (CCl_4) 1 770 cm^{-1}, δ 4.04 (3 H, s, OCH_3), 3.93 (3 H, s, OCH_3), 3.88 (3 H, s, OCH_3), 5.92 (1 H, s, H-5), and 6.96, 7.09, 7.53, and 7.77 (each 2 H, d, *J* 9—10 Hz, 2 × AA'BB' for aromatic protons) (Found: C, 71.2; H, 5.55. $C_{20}H_{18}O_5$ requires C, 71.0; H, 5.35%).

Synthesis of the Lactone (1b).—(i) Compound (4b) (5 mg) was heated at 245 °C under nitrogen for 50 min; t.l.c. (KH; methanol–chloroform, 1 : 4) then showed three spots: a red spot at high R_F, a spot corresponding to (4b) (R_F 0.3), and a greenish-yellow spot at R_F 0.2. The products in ether were extracted with aqueous 5% sodium acetate, which removed (4b). Extraction with aqueous 5% sodium hydrogen carbonate followed by acidification and extraction with ether gave a greenish yellow solid (0.5 mg), which was methylated with diazomethane. The resulting methyl ether was identical [t.l.c. (KH; 2% methanol in chloroform) and i.r. and u.v. spectra] with tri-*O*-methylaspergillide B1.

(ii) To a stirred suspension of anhydrous potassium carbonate (1.50 g, 11 mmol) in dry acetone (200 ml) were added 1,4-bis-(*p*-methoxyphenyl)butane-2,3-dione (1.50 g, 5.0 mmol) and methyl chloroformate (0.33 ml, 4.3 mmol) and the mixture was refluxed for 5 h. After cooling, acetone was removed *in vacuo*. The residue was shaken with ether and water (a crystalline precipitate may separate here, but dissolves on addition of more water). The aqueous layer was separated and the ethereal layer was further extracted with aqueous 1% sodium carbonate until the alkaline extracts were nearly colourless. The alkaline extracts were acidified, giving a yellow precipitate which was extracted with ether and ethyl acetate. After drying and removal of solvents, a yellow crystalline product (520 mg) was obtained, which was recrystallised from ethyl acetate to give (1d) (220 mg, 16%) as glistening greenish yellow needles, m.p. 230—250° (decomp). A further recrystallisation from benzene–acetone gave material homogeneous by t.l.c., $\lambda_{max.}$ 234 (log ε 4.24), 248sh (4.19), 322sh (4.34), 332 (4.35), and 370 nm (4.35), shifting in alkali to 231 (4.34), 252 (4.30), 316 (4.48), 325sh (4.46), and 377 nm (4.16).

Compound (1e) (160 mg) in methanol (40 ml) and ether (100 ml) was treated with an excess of ethereal diazomethane. After 30 min at room temperature excess of reagent and solvents were removed to give a crystalline residue. This was chromatographed on silica gel (elution with 1% methanol in chloroform, increasing to 4% methanol in chloroform) to give the product (164 mg). Recrystallisation from ether–hexane and then ethyl acetate–hexane gave the lactone (1b) (80 mg), m.p. 143—144°, identical with (1b) from *A. terreus* (t.l.c., mixed m.p., and i.r. and u.v. spectra).

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