Pentaketide Metabolites of the Fungus *Phialophora lagerbergii*

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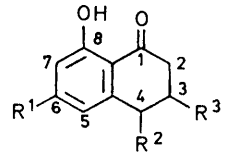
Phialophora lagerbergii produces scytalone [3,4-dihydro-3,6,8-trihydroxynaphthalen-2(1*H*)-one] (1) and flaviolin (2,5,7-trihydroxy-1,4-naphthoquinone) (6). The incorporation of [1-^{13}C]acetate into scytalone confirms its pentaketide origin.

In the course of screening fungi for biological activity we found that *Phialophora lagerbergii* produced a new crystalline metabolite, $C_{10}H_{10}O_4$, in high yield. Spectroscopic evidence suggested structure (1) for the compound, and this was supported by its ready dehydration to naphthalene-1,3,8-triol under acidic or basic conditions. Compound (1) has recently been isolated from a *Scytalidium* sp.[1] and named scytalone, and we therefore use this trivial name for the *P. lagerbergii* metabolite. The identity of the material from the two sources was confirmed by direct comparison of our metabolite with a sample of the *Scytalidium* metabolite kindly provided by Dr. J. A. Findlay.

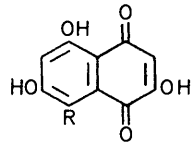
Because fungal metabolites can be produced in (+)- and (−)-forms by different organisms we have compared the optical rotations of scytalone produced by *P. lagerbergii* and by the *Scytalidium* sp. Both samples had $[\alpha]_D$ 0, but showed the same, very weak, negative c.d. curve with a trough at 220 nm. We therefore believe that both samples of scytalone are optically active and have the same absolute configuration. That natural scytalone is optically active is supported by the fact that its m.p. and solid state i.r. spectrum differ from those of racemic synthetic material.[2]

The structure of scytalone suggested that it is a member of a group of fungal metabolites[3] which are hydroxylated naphthalenes or naphthoquinones, *e.g.* flaviolin (6), a co-metabolite of scytalone in *P. lagerbergii*, and mompain (7). Although the oxygenation patterns of the compounds strongly suggest a pentaketide origin, as in (8) though other foldings of the chain are possible, this has never been confirmed experimentally. Indeed, degradation of mompain (7) produced in the presence of [2-^{14}C]acetate or [2-^{14}C]malonate gave results inconsistent with a pentaketide biosynthesis,[4] and structurally related compounds from higher plants are derived from shikimate.[5] It therefore seemed worthwhile to investigate the biosynthesis of scytalone and we chose to do this using [1-^{13}C]acetate and ^{13}C n.m.r. spectroscopy.

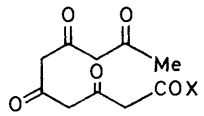
The ^{13}C n.m.r. spectrum of scytalone shows ten well-resolved signals which can be assigned as shown in the Table. In the spectrum of scytalone produced in the presence of sodium [1-^{13}C]acetate the signals due to C-1, C-3, C-4a, C-6, and C-8 are enhanced to about twice their natural intensity thus confirming the pentaketide origin of scytalone and, by inference, its co-metabolite flaviolin. It seems likely that other related compounds produced by fungi are also formed by the polyketide route.



	R^1	R^2	R^3
(1)	OH	H	OH
(2)	H	OH	OH
(3)	OH	OH	OH
(4)	OH	OH	H
(5)	H	OH	OH



(6) R = H
(7) R = OH



(8)

^{13}C N.m.r. spectrum of scytalone

Assignment:	C-1	C-6 [a]	C-8 [a]	C-4a	C-8a	C-5	C-7	C-3	C-2 [a]	C-4 [a]
δ_{obs}	202·6	166·1	160·9	146·3	111·6	109·6	101·6	66·2	48·7	39·3
$\delta_{calc.}$ [c]		160·9	156·8	140·3	118·3	109·2	100·2			

[a] Assignment of the signals due to these pairs of atoms cannot be made with confidence. [b] In p.p.m. from tetramethylsilane (low field positive). [c] Calculated as described by G. C. Levy and G. L. Nelson ('Carbon-13 Nuclear Magnetic Resonance for Organic Chemists,' Wiley-Interscience, New York, 1972, p. 81).

Other recently reported fungal hydroxytetralones are the 3,4,8-trihydroxy- (2), 3,4,6,8-tetrahydroxy- (3), and 4,6,8-trihydroxy- (4) derivatives, all from *Pyricularia oryzae*,[6,7] and the 4,8-dihydroxy-derivative (5), a minor co-metabolite of scytalone in *Scytalidium*.[8]

EXPERIMENTAL

Isolation of the Metabolites.—(a) *Scytalone. Phialophora lagerbergii* (C.M.I. 96,745, No. 5347 in our collection) was grown in Thompson vessels each containing 1 l of Raulin–Thom medium with 0·1% yeast extract (Oxoid) and 5% sugar. After 19 days the filtrate from 90 bottles (52 l; pH 3·9) was acidified to pH 2 and extracted with ethyl

[1] J. A. Findlay and D. Kwan, *Canad. J. Chem.*, 1973, **51**, 1617.
[2] B. W. Bycroft, M. M. Cashyap, and T. K. Leung, unpublished results.
[3] W. B. Turner, 'Fungal Metabolites,' Academic Press, London, 1971, p. 130.
[4] S. Natori, Y. Inoye, and H. Nishikawa, *Chem. and Pharm. Bull. (Japan)*, 1967, **15**, 380.
[5] E. Leistner and N. H. Zenk, *Z. Naturforsch.*, 1968, **23b**, 259.
[6] S. Iwasaki, H. Muro, S. Nozoe, and S. Okuda, *Tetrahedron Letters*, 1972, 13.
[7] S. Iwasaki, H. Muro, S. Sasaki, S. Nozoe, and S. Okuda, *Tetrahedron Letters*, 1973, 3537.
[8] J. A. Findlay and D. Kwan, *Canad. J. Chem.*, 1973, **51**, 3299.

acetate (2 × 10 l) to give a brown gummy solid (74·9 g). A preliminary crystallisation from aqueous ethanol gave a product (62·65 g) which was almost pure although still heavily coloured. This material was dissolved in acetone–chloroform (1 : 1; 300 ml) and filtered through a column of silica gel (500 g). The filtrate was evaporated to dryness and the residue was dissolved in acetone. The solution was treated with charcoal, filtered, concentrated to a small volume, and diluted with ether (1500 ml) to give prisms of pure scytalone (23·5 g). The mother liquor was evaporated and the residue recrystallised from ethyl acetate–light petroleum (b.p. 60—80°) to give further pure scytalone (17·8 g). Scytalone [3,4-dihydro-3,6,8-trihydroxynaphthalen-2(1*H*)-one] crystallises from organic solvents as prisms, m.p. 167—168° (lit.,[1] 160—168°), $[\alpha]_D^{25.5}$ 0° (*c* 1·68 in MeOH) (Found: C, 61·8; H, 5·4%; *m/e* 194·0566. Calc. for $C_{10}H_{10}O_4$: C, 61·85; H, 5·2%; *M*, 194·0579); $\lambda_{max.}$ (MeOH) 232infl, 283, and 317infl nm (ε 8170, 12,640, and 4850); $\nu_{max.}$ 3400, 3140, 1637, and 1590 cm^{-1}; τ [$(CD_3)_2CO$] −2·74 (1H, s, bonded OH), 3·75 (1H, m, ArH), 3·86 (1H, d, *J* 2 Hz, ArH), 5·70 (1H, m, *J* 7, 7, 4, and 4 Hz, $CH_2{\cdot}CH{\cdot}CH_2$), 6·93 (1H, dd, *J* 16 and 4 Hz) and 7·12 (1H, dd, *J* 16 and 7 Hz, $ArCH_2{\cdot}CHOH$), and 7·18 (1H, dd, *J* 17 and 4 Hz) and 7·35 (1H, dd, *J* 17 and 7 Hz, $CO{\cdot}CH_2{\cdot}CHOH$).

Crystallisation of scytalone from water gives needles, m.p. 172—174°; $\nu_{max.}$ 3170, 1638, and 1588 cm^{-1}.

(b) [^{13}C]*Scytalone*. *P. lagerbergii* (5347) was grown in two 500 ml conical flasks each containiug 200 ml of Czapek–Dox medium with 0·1% yeast extract (Oxoid) and 5% sugar, shaken at 250 rev. min^{-1} with a 2 in throw. Sodium [1-^{13}C]acetate (0·1 g) in water (5 ml) was added to each flask at inoculation and again after 5 days. After 9 days the combined filtrate (300 ml; pH 6·1) was acidified to pH 2 and extracted with ethyl acetate (4 × 80 ml). The recovered brown gummy product (1·18 g) was adsorbed from solution in acetone onto silica gel (10 ml) and placed at the top of a column of silica gel (25 ml) made up in toluene. Elution with toluene–chloroform (1 : 1; 1 l) and chloroform (100 ml) gave an orange solid (21·6 mg) containing flaviolin. Further elution with chloroform (2·3 l) gave a pale brown gum (716 mg) which crystallised from water to give scytalone (396 mg), further purified by recrystallisation from water (charcoal).

(c) *Flaviolin*. The extract (3·7 g) from several trial experiments as described in (b) was adsorbed from solution in acetone onto silica gel (20 ml) and placed on a column of silical gel (55 ml) made up in toluene. Elution with toluene–chloroform (1 : 1) gave fractions as follows: (i) (3 l) 342·0 mg red solid/gum; (ii) (2 l) 359·1 mg yellow solid, (iii) (5 l) 1·819 g brown solid. Fraction (iii) was shown by t.l.c. to be mainly scytalone and (ii) to be a mixture of scytalone and a red compound which was the major component of (i). Fraction (i) was recrystallised twice from dioxan–benzene giving red prisms of flaviolin (22 mg) containing 1 mol. equiv. of dioxan of crystallisation [Found: *m/e* 206·0194 (base peak). Calc. for $C_{10}H_6O_5$: *M*, 206·0215], τ [$(CD_3)_2SO$] −2·47 (1H, s, bonded OH), 3·07 (1H, d, *J* 2·5 Hz, ArH), 3·50 (1H, d, *J* 2·5 Hz, ArH), 4·01 (1H, s, *CH*=COH, exchangeable with D_2O), and 6·45 (8H, s, dioxan of crystallisation). The i.r. spectrum of a solution in dioxan was identical with that of an authentic sample, kindly provided by Dr. B. W. Bycroft.

Dehydration of Scytalone.—(a) *With alkali*. A solution of scytalone (500 mg) in N-sodium hydroxide (25 ml) was heated under nitrogen on a steam-bath for 1 h, cooled, acidified, and extracted with ethyl acetate. The recovered product was filtered through a short silica gel column in chloroform–acetone (10 : 1). A solution of the product in acetone was treated with charcoal and filtered, and evaporated. Recrystallisation of the residue from acetone–chloroform gave *naphthalene*-1,3,8-*triol* as prisms, m.p. 197—199° (Found: *m/e* 176·0483. $C_{10}H_8O_3$ requires *M*, 176·0473); $\nu_{max.}$ 3100—2600br, 1645w, 1630w, and 1595 cm^{-1}; τ [$(CD_3)_2SO$] 2·92 (1H, dd, *J* 8 and 7 Hz), 3·01 (1H, dd, *J* 8 and 2 Hz), 3·46 (1H, d, *J* 2 Hz), 3·54 (1H, dd, *J* 7 and 2 Hz), and 3·65 (1H, d, *J* 2 Hz). The estimated carbon content was consistently low, probably owing to the presence of water of crystallisation.

(b) *With acid*. A solution of scytalone (100 mg) in trifluoroacetic acid (0·5 ml) was heated to 60° for 1·5 h, cooled, and evaporated. The solid product was shown by t.l.c. to be a *ca.* 2 : 1 mixture of two components. Crystallisation from acetone–chloroform gave the major component, naphthalene-1,3,8-triol.

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