Metabolites from a *Scytalidium* Species

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The structure **1**, 3,6,8-trihydroxytetralone, proposed (1) earlier for scytalone is corroborated by synthesis of an aromatization product 1,3,8-trimethoxynaphthalene. A new minor metabolite of the same *Scytalidium* species is shown to be 4,8-dihydroxytetralone (**6**).

La structure **1**, trihydroxy-3,6,8 tétralone, qui a été proposée auparavant (1) pour la scytalone est corroborée par la synthèse d'un produit d'aromatisation soit le triméthoxy-1,3,8 naphtalène. On démontre qu'un nouveau métabolite mineur de la même espèce *Scytalidium* est la dihydroxy-4,8 tétralone, **6**. [Traduit par le journal]

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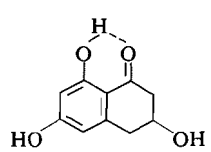
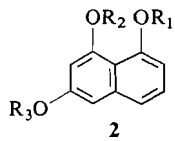
Recently we proposed (1) the structure **1**, 3,6,8-trihydroxytetralone, for a new metabolite isolated from culture filtrates which had supported growth of a species of *Scytalidium*. The juxtaposition of the oxygen substituents in **1** was deduced, in part, from its transformation into tri-oxygen substituted naphthalene derivatives whose substitution patterns were determined to be 1,3,8 by analysis of n.m.r. data. One of these derivatives, namely the triacetate **2***a* (m.p. 120–123°), has been reported by Stetter and Heidel (2) to have a melting point of 111°.[1] These authors prepared their material by acetylation of the product of dehydrogenation of 1,3,8-decalintrione. This discrepancy in physical characteristic prompted us to seek synthetic corroboration of our conclusions about the oxygen substitution pattern. Accordingly we have now transformed scytalone into 1,3,8-trimethoxynaphthalene in 76% yield by treatment with dimethylsulfate and potassium carbonate in anhydrous acetone and confirmed the structure of this aromatization product by independent synthesis.

Treatment of 3-bromopropionic acid with triphenylphosphine in boiling benzene provided the corresponding triphenylphosphonium salt from which the ylid **3** was generated and condensed with commercially available 3,5-dimethoxybenzaldehyde (**4**) to furnish 4-(3′,5′-dimethoxyphenyl)-3-butenoic acid (**5**) in 60% yield. Attempts to cyclize **5** to 8-hydroxy-1,3-dimethoxynaphthalene (**2***c*) under a variety of conditions gave poor yields and it was tentatively concluded that product **5** must be largely the *trans* isomer. Since in the n.m.r. spectrum of **5** the signals for the olefinic protons are superimposed on those arising from the aromatic protons it was not possible to make any firm conclusions about *cis–trans* isomer ratios. Conversion of the cyclization product **2***c* to 1,3,8-trimethoxynaphthalene was accomplished using the same reagents employed for the trimethylation of scytalone. The product **2***b* proved to be identical in all respects with the scytalone aromatization product.

It should be noted that attempts to bring

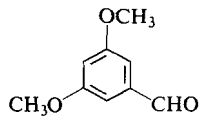


1



2

a $R_1, R_2, R_3 = COCH_3$
b $R_1, R_2, R_3 = CH_3$
c $R_1 = H; R_2, R_3 = CH_3$



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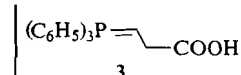

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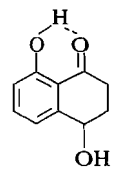

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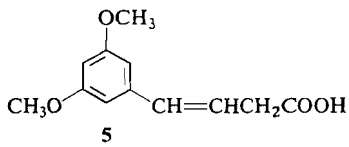

5

[1]A sample (m.p. 111°) supplied later by Dr. H. Stetter proved to be non-identical with our material and appeared to be 6,8-diacetoxytetralone from spectral data.

about a substantial degree of isomerization or possible migration of the double bond in **5** by treatment with strong base were ineffective.

From the same culture filtrates which afforded scytalone we have now isolated a minor metabolite whose structure **6**, 4,8-dihydroxytetralone, was tentatively assigned on the basis of spectroscopic data and confirmed by synthesis of its racemate from juglone. The mass spectrum of this new metabolite shows a molecular ion *m/e* 178 ($C_{10}H_{10}O_3$) and a base peak at *m/e* 160 ($M^+ - H_2O$). As in the case of scytalone the presence of an orthohydroxyphenone system is indicated by the u.v. spectrum chromophore (3) and supported by i.r. data. The n.m.r. spectrum ($CDCl_3$) shows a one-proton singlet at δ 12.4 p.p.m. typical of a bonded hydroxyl proton of such orthohydroxyphenone systems (1) while the presence of a 1,2,3-trisubstituted aromatic ring is apparent from the three single proton signals centered at δ 6.9, 7.0, and 7.5 p.p.m. whose mutual couplings (*vide infra*) can only be interpreted in terms of an aromatic AMX system (4). A one-proton triplet at δ 5.9 (J = 5 Hz) is assigned to the C-4 hydrogen of **6** while a pair of two-proton multiplets centered at δ 2.2 and 2.7 p.p.m. are accounted for by the C-3 and -2 methylenes, respectively. The remaining hydroxyl proton gives rise to a broad signal centered at δ 1.9 p.p.m. Thus the features of the n.m.r. spectrum are adequately explained in terms of structure **6**.

To confirm this conclusion racemic 4,8-dihydroxytetralone was prepared by reduction of β-hydrojuglone obtained from juglone (5). The n.m.r., u.v., and solution ($CHCl_3$) i.r. spectra are identical with those recorded from the new metabolite. Minor differences in the solid state (KBr) i.r. spectra and melting point characteristics indicate that the material from the natural source occurs in optically active form. Insufficient material was available to make optical rotation measurements.

In contrast to syctalidin (6) neither scytalone nor 4,8-dihydroxytetralone proved to have significant antifungal activity against selected test organisms.

Experimental

The i.r. spectra were recorded on a Perkin-Elmer model 237B instrument. Mass spectra were obtained with an Hitachi Perkin-Elmer RMU-6D spectrometer. The u.v. spectra were determined on a Perkin-Elmer model 467 spectrophotometer. N.m.r. spectra were recorded with a Varian T-60 instrument employing tetramethylsilane as internal standard. A Kofler hot stage apparatus was employed to determine melting points which are uncorrected.

Methylation of Scytalone (1)

A mixture of scytalone (**1**) (27 mg), dimethylsulfate (0.5 ml), and anhydrous potassium carbonate (0.96 g) in dry acetone (8 ml) was refluxed for 36 h. Evaporation of acetone, dilution with water, and extraction with chloroform afforded a brown oil which was purified by preparative t.l.c. to furnish a yellowish crystalline product (20 mg, 76%): m.p. 74–76°; ν_{max} (KBr) 2930, 2830, 1620, 1580, 1450, 1410, 1385, 1350, 1290, 1250, 1215, 1200, 1155, 1125, 1100, 1040, 995, 940, 960, 845, 795, 745, 725 cm^{-1}; δ ($CDCl_3$) 3.9 (3H, s), 3.95 (6H, s), 6.4–6.8 (4H, complex multiplet), 7.2–7.4 (1H, m); λ_{max} (95% C_2H_5OH) 235, 285, 296, 320, 335 (log ε 4.69, 3.73, 3.73, 3.4, 3.6) nm; *m/e* 218 (M^+, $C_{13}H_{14}O_3$, 100%), 203 ($M - CH_3$), 189, 175, 160, 145, 130, 115.

3-Triphenylphosphonium Propanoic Acid Bromide

A mixture of triphenylphosphine (28.8 g), 3-bromopropanoic acid (15.3 g), and dry benzene (50 ml) was refluxed for 18 h. The benzene layer was decanted and the yellow oily residue was triturated with acetone affording 3-triphenylphosphonium propanoic acid bromide (33.1 g, 80%) as white crystals: m.p. 196–197°; ν_{max} ($CHCl_3$) 3100 (broad), 2940, 1740, 1590, 1480, 1440, 1385, 1320, 1110, 1000 cm^{-1}; δ ($CDCl_3$) 2.96 (2H, m), 3.8 (2H, m), 7.8 (15 H, bd) p.p.m.

4-(3′,5′-Dimethoxyphenyl)-3-butenoic Acid (5)

A mixture of 3,5-dimethoxybenzaldehyde (8.3 g) and 3-triphenylphosphonium propanoic acid bromide (20.8 g) in dimethylsulfoxide and tetrahydrofuran (50 ml each) was dropped slowly into a flask containing sodium hydride (2.4 g) at 0° under nitrogen. The mixture was stirred overnight at room temperature and then water was added and the solution washed three times with ether. The aqueous layer was acidified with dilute hydrochloric acid and extracted with three portions of ether. After washing with water and drying ($MgSO_4$) the ether was evaporated yielding a brown viscous oil (11.9 g) which was chromatographed on a silica gel column. From ether eluates the white solid (6.7 g, 60%) was recovered and recrystallized from benzene–*n*-hexane yielding compound **5**: m.p. 56–64°; ν_{max} ($CHCl_3$) 3060 (broad), 2940, 2820, 1720, 1590, 1460, 1280, 1150 cm^{-1}; δ ($CDCl_3$) 3.25 (2H, d, J = 5 Hz), 3.75 (6H, s), 6.5 (5H, m) and 11.4 (1H, s) p.p.m.; λ_{max} (95% C_2H_5OH) 227, 260, 298 (log ε 4.58, 4.35, 3.78) nm; M (mass spectrometry) 222.

6,8-Dimethoxy-1-naphthol (2c)

A mixture of 4-(3′,5′-dimethoxyphenyl)-3-butenoic acid (**5**) (0.44 g) and polyphosphoric acid (22 g) was stirred at 90–100° for 4 h. The resulting black viscous liquid was dissolved in ice cold water and extracted with chloroform. Evaporation of the chloroform layer afforded a brown paste (0.38 g) which was subjected to preparative t.l.c. White crystalline solid **5** (0.028 g, 7%) m.p. 57–63° was thus recovered: ν_{max} (KBr) 3360 (broad), 2920, 1610, 1590, 1450, 1430, 1405, 1370, 1320, 1240, 1195, 1160, 1125, 1070, 1060, 1025, 980, 935, 830, 815, 750, 710 cm^{-1}; δ ($CDCl_3$) 3.9 (3H, s), 4.0 (3H, s), 6.45 (1H, d, J = 2 Hz),

6.6–6.8 (2H, m), 7.2–7.5 (2H, m), 9.1 (1H, s) p.p.m.; λ_{max} (95% C_2H_5OH) 232, 288, 300, 323, 337.5 (log ε 4.38, 3.55, 3.55, 3.36, 3.38); *m/e* 204 ($C_{12}H_{12}O_3^+$, 100%), 189 ($M^+ - CH_3$), 175, 161.

1,3,8-Trimethoxynaphthalene (2b) from 6,8-Dimethoxy-1-naphthol (2c)

A mixture of 6,8-dimethoxy-1-naphthol (**2***c*) (30 mg), dimethylsulfate (0.5 ml), and anhydrous potassium carbonate (0.96 g) in dry acetone (8 ml) was refluxed for 16 h. Evaporation of acetone, dilution with water, and chloroform extraction afforded a brown oil (0.45 mg) which was purified by preparative t.l.c. giving crystalline 1,3,8-trimethoxynaphthalene (**2***b*) (28 mg, 92%), m.p. 74–76° identical in spectroscopic characteristics with material obtained from scytalone (**1**) and giving no melting point depression on admixture.

Isolation of 4,8-Dihydroxytetralone from Scytalidium FY Strain Culture Medium

The crude chloroform extract (0.55 g) from the culture filtrate medium which had supported growth of *Scytalidium* FY strain (2) was chromatographed on a silica gel column. The ether–benzene (1.4) eluates were combined, evaporated, and rechromatographed on a preparative t.l.c. silica gel plate developed with acetone–benzene 1:4. The metabolite (R_f 0.46) showed green phosphorescence under long u.v. light and was recovered as a yellowish white solid (12 mg) and recrystallized from *n*-hexane to furnish crystals: m.p. 70–72°; ν_{max} ($CHCl_3$) 3600, 2925, 1640, 1580, 1450, 1365, 1335, 1260, 1100, 985, 930, 890 cm^{-1}; ν_{max} (KBr) 3320, 2920, 2850, 1630, 1615, 1570, 1450, 1405, 1365, 1336, 1325, 1308, 1260, 1216, 1200, 1160, 1100, 1075, 1065, 1046, 1030, 973, 890, 845, 820, 806, 770, 746, 703, 615 cm^{-1}; λ_{max} (95% C_2H_5OH) 220, 260, 332 (log ε 4.1, 3.8, 3.5); λ_{max} (95% C_2H_5OH + OH^-) 223, 230, 263, 372 (log ε 4.2, 4.1, 3.7, 3.7); *m/e* 178 (M^+, $C_{10}H_{10}O_3$), 160 ($M - H_2O$), 150 (100%, $M - C_2H_4$); δ ($CDCl_3$) 1.9 (1H, broad, exchanges with D_2O), 2.2 (2H, m), 2.75 (2H, m), 5.9 (1H, t, J = 5 Hz), 6.9 (1H, dd, J = 8 Hz, J = 1 Hz), 7.0 (1H, bd, J = 8 Hz), 7.5 (1H, t, J = 8 Hz), 12.4 (1H, s, exchanges with D_2O) p.p.m.

Reduction of β-Hydrojuglone to 4,8-Dihydroxytetralone

A solution of β-hydrojuglone (5) (0.55 g) in dry tetrahydrofuran (10 ml) was dropped slowly into a flask containing lithium aluminum hydride (38 mg) in dry tetrahydrofuran (10 ml) immersed in a Dry Ice – acetone bath. The mixture was stirred for 7 h at −40°. Water was added and the aqueous suspension treated with dilute hydrochloric acid and extracted with ether until the aqueous layer remained clear. The green ethereal extract was washed with water, dried ($MgSO_4$), and evaporated. Purification of the crude product by preparative t.l.c. gave yellowish white crystalline **6** (0.25 g, 38%): m.p. 99–100°; ν_{max} (KBr) 3270, 2980, 2870, 1630, 1610, 1570, 1450, 1410, 1360, 1345, 1330, 1310, 1290, 1250, 1215, 1200, 1160, 1095, 1075, 1045, 1030, 982, 925, 895, 845, 815, 780, 746, 705, 610 cm^{-1}. Other spectral data are identical with those of material isolated from the natural source (*vide supra*).

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