Journal of Natural Products
Vol. 56, No. 10, pp. 1835-1838, October 1993

DEOXYSCYTALIDIN AND LIGNICOL: NEW METABOLITES FROM *SCYTALIDIUM* SPECIES

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ABSTRACT.—As part of a chemosystematic study of the genus *Scytalidium*, several strains belonging to the closely related species *Scytalidium album, Scytalidium lignicola, Scytalidium aurantiacum,* and *Scytalidium circinatum* have been grown in liquid shake culture. The various strains examined did not show a consistent pattern of metabolites. Scytalidin [**1**], the major metabolite of *Sc. album*, was produced by two of three tested strains but not demonstrated by any strain of *Sc. lignicola, Sc. aurantiacum,* or *Sc. circinatum*. The scytalidin-producing strains also produced a closely related but undescribed metabolite, deoxyscytalidin [**2**]. One strain of *Sc. lignicola* produced a new compound which we call lignicol [**3**]. Other strains of *Sc. lignicola* produced small amounts of one or more other metabolites including scytalone (3,6,8-trihydroxytetralone). *Sc. circinatum* produced only 4,8-dihydroxytetralone.

Scytalidium lignicola Pesante (Class Hyphomycetes) and its close relatives *Scytalidium album* Beyer & Klingström and *Scytalidium aurantiacum* Klingström & Beyer are known to occur on wood of conifers and hardwood where they cause stain and rot. A recent study (1) showed that *Sc. lignicola* was one of the commonest microfungi causing soft rot in preservative-treated Douglas fir and southern pine utility poles. Originally, the species *Sc. album* and *Sc. aurantiacum* were distinguished from *Sc. lignicola* by slight differences in colonial morphology and by the inability of isolates to grow at 35° (2). Isolates of *Sc. album* and *Sc. aurantiacum* were also found to be more vigorous in antagonism to some wood decay fungi (3), leading to suggestions that these species might be useful in biological control programs. Antagonism appeared to be due to a diffusible substance (3). One of the compounds considered responsible for the antagonism was the antibiotic scytalidin [**1**] (4–6) produced by an isolate of *Scytalidium* sp. designated the FY strain (UAMH 3620) (7). This strain was later identified as *Sc. album* (8). Other studied metabolites from the FY strain include scytalone (3,6,8-trihydroxytetralone) and a minor compound 6,4,8-dihydroxytetralone (9,10) and two hexenophenones (11), produced when the fungus was grown on agar. The agar culture did not produce scytalidin (11).

Although isolates of the three species vary in their colonial morphologies, the uniformity in their microscopic appearance has led to the suggestion that both *Sc. album* and *Sc. aurantiacum* should be considered synonyms of *Sc. lignicola* (12). To confirm that suggestion, we began a chemosystematic study of these species and another related species from utility poles, *Scytalidium circinatum* Sigler & Wang (12), to determine whether similar metabolites might be produced by different colonial variants. We also reexamined the metabolites produced in liquid shake culture (malt extract broth) by the FY strain.

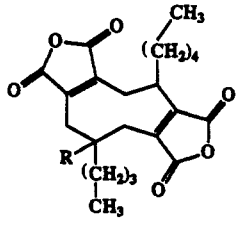
The metabolites were separated by a combination of flash chromatography, preparative tlc, and fractional crystallization. The major metabolite, scytalidin [**1**] was produced only in extracts from two strains of *Sc. album* (UAMH 3620=FY strain=ATCC 16675 and UAMH 3611=ex-type=ATCC 22476). Both strains produced a closely related,

but previously unreported minor compound, deoxyscytalidin [**2**]. A third strain of *Sc. album* (UAMH 6085 = MUCL 6857) failed to produce either compound. Similarly, no tested strain of *Sc. lignicola* (UAMH 1502 ex-type = IMI 62532, UAMH 5101, UAMH 6291, UAMH 6293), *Sc. aurantiacum* (UAMH 3612 ex-type), or *Sc. circinatum* Sigler and Wang (UAMH 6830) (12) produced either scytalidin [**1**] or deoxyscytalidin. *Sc. circinatum* did produce 4,8-dihydroxytetralone (10), and *Sc. lignicola* (UAMH 6291) gave small amounts of scytalone (3,6,8-trihydroxy-1-tetralone), (9).

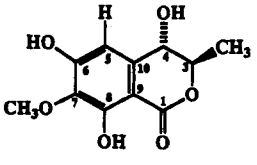
The identity of scytalidin [**1**] ($C_{22}H_{28}O_7$) was established by comparison of physical properties (mp, ir, nmr, ms) with those reported (4–6). The minor component **2** had the same unsaturation number as scytalidin but possessed one less oxygen. Both compounds show similar anhydride absorption (**1**, 1849, 1827, 1770 cm^{-1}; **2**, 1848, 1825, 1772 cm^{-1}) in the ir. However, **1** shows hydroxyl absorption (3570 cm^{-1}) whereas **2** does not. The ^{1}H-nmr spectra of **1** and **2** are very similar, but the ^{13}C-nmr spectrum of **2** clearly shows the presence of two sp^3 methine carbons whereas that of **1** shows only one. This indicates that deoxyscytalidin possesses structure **2**.

Of the several strains of *Sc. lignicola* examined, only UAMH 6291 produced scytalone. Strains 1502 and 6293 produced very small amounts of metabolites, and these consisted mainly of glycerides and fatty acids. In one case (UAMH 6293) fumaric acid was also identified. *Sc. lignicola* (UAMH 5101), however, produced a compound $C_{11}H_{12}O_6$ which appears to be new. This compound, for which we suggest the name lignicol, possesses structure **3**.

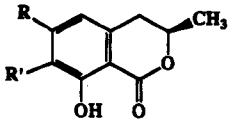
Lignicol [**3**] is optically active. The molecular formula was determined by hrms. The ir spectrum shows hydroxyl (3290 cm^{-1}), carbonyl (1660 cm^{-1}), and benzenoid (1625, 1589, 1511 cm^{-1}), carbonyl (1660 cm^{-1}), and benzenoid (1625, 1589, 1511 cm^{-1}), absorption. The arrangement of the substituents on the benzenoid ring is based on the nOe difference spectrum. Irradiation of the aromatic hydrogen (δ 6.67) causes enhancement of the signal of the unchelated phenolic hydroxyl (δ 6.4) and H-4 (δ 4.50). The reverse enhancements were also observed. In addition to enhancing the H-5 signal, irradiation of H-4 also caused enhancement (6.3%) of the signal for the H's of the C-3 methyl group. The second phenolic hydroxyl is chelated to the lactone carbonyl and appears at δ 11.36. The chelation accounts for the low frequency carbonyl absorption (1660 cm^{-1}) of the lactone carbonyl. This leaves C-7 as the only possible location of the aromatic methoxyl (δ 4.03). The trans stereochemistry of the C-3 and C-4 substituents is based on the nOe enhancement mentioned above and on the 2D *J*-resolved spectrum which shows the H-3, H-4 coupling is 8 Hz. Lignicol [**3**] is thus *trans*-4,6,8-trihydroxy-7-methoxy-3-methyldihydroisocoumarin. The absolute stereochemistry of lignicol is assigned as 3*R*, 4*S* on the basis of the cd spectrum



1 R=OH
2 R=H



3



4 R=R'=H
5 R=OH, R'=OMe

which shows a negative Cotton effect at 269 nm, similar to that of *R*-mellein [**4**] (13). Lignicol [**3**] is related in structure to reticulol [**5**], a phosphodiesterase inhibitor isolated from *Streptomyces mobaraensis* (14).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—High resolution mass spectra were recorded on an A.E.I. MS-50 mass spectrometer coupled to a DS 50 computer. Ir spectra were recorded on a Nicolet 7199 FT spectrometer. High field nmr spectra were recorded on Bruker WH-300 and WH-360 spectrometers coupled to an Aspect 3000 computer system and on a Varian Unity 500 spectrometer with Sun computer system. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter. Melting points were determined on a Leitz-Wetzlar melting point apparatus and are uncorrected. E. Merck Si gel (230–400 mesh) was used for flash chromatography. Analytical tlc utilized Merck precoated plates of Si gel 60F-254 on aluminum foil with visualization by uv light or 5% phosphomolybdic acid in 5% H_2SO_4, followed by careful charring on a hot plate. Preparative tlc was carried out on Merck precoated plates of Si gel 60F-254 on glass. All solvents were distilled prior to use. Skellysolve B refers to Skelly Oil Company petroleum ether, bp 62–70°.

FUNGAL CULTURES.—The *Scytalidium* strains used in this study are maintained in The University of Alberta Microfungus Collection and Herbarium (UAMH). They were grown on either 2% malt extract agar or cereal agar plates. After 5–7 days at 17° the mycelium from one agar plate was blended with approximately 100 ml of sterile H_2O, and 20 ml aliquots were used to inoculate 5×1 liter 2% Difco malt extract broth in 2-liter Erlenmeyer flasks. After inoculation, the shake cultures were maintained at room temperature for 15 days. The culture broth was filtered from the mycelium, concentrated in vacuo to about 500 ml, and extracted by stirring with an equal volume of CH_2Cl_2 for 12 h. The extraction procedure was repeated three times. The combined CH_2Cl_2 extracts were dried and concentrated to give crude broth extract. The mycelium was air-dried, pulverized, and subjected to Soxhlet extraction with CH_2Cl_2. The CH_2Cl_2 extract was dried and concentrated to give crude mycelial extract.

ISOLATION OF SCYTALIDIN [**1**] AND DEOXYSCYTALIDIN [**2**].—*From* Sc. album *UAMH 3620.*—Five liters of culture afforded dry mycelium (25.3 g), CH_2Cl_2 broth extract (125.5 mg), and CH_2Cl_2 mycelial extract (2.03461 g). The crude broth extract was submitted to flash chromatography on Si gel in Skellysolve B-EtOAc (2:1). The fraction containing the two uv active spots was rechromatographed using preparative tlc in Skellysolve B-EtOAc (2:1) to afford scytalidin [**1**] (19.9 mg) and deoxyscytalidin [**2**] (1.4 mg). A portion (436 mg) of the mycelium extract was submitted to flash chromatography in the same solvent system to give triglycerides of unsaturated fatty acids (6 mg), scytalidin [**1**] (60.6 mg), and deoxyscytalidin [**2**] (16.3 mg): mp 88–89° (from Skellysolve B/Et_2O); $[\alpha]_D$ −82.2° ($CHCl_3$, c=0.135); ^{1}H nmr (360 MHz, $CDCl_3$) δ 3.31 (m, 1H), 2.90–2.52 (4H), 2.24 (m, 2H), 1.92 (m, 1H), 1.62–1.18 (14H), 0.86 (t, 3H, J=Hz), 0.80 (t, 3H, J=7Hz); ^{13}C nmr (125 MHz, $CDCl_3$) δ 165.54 (s), 165.34 (s), 165.30 (s), 164.91 (s), 144.24 (s), 143.47 (s), 143.27 (s), 143.25 (s), 31.46 (t), 29.11 (t), 28.13 (t), 28.08 (t), 27.14 (t), 27.08 (t)., 22.52 (t), 22.41 (t), 49.99 (d), 34.98 (d), 13.99 (q), 13.97 (q); ir ($CHCl_3$ cast) ν max cm^{-1} 1848, 1825, 1772; hreims *m/z* (%) $[M]^+$ 388.1883 (calcd for $C_{22}H_{28}O_6$, 388.1886) 8.51 $[M-H_2O-CO]^+$ 342 (22.8), $[M-CO-CO_2]^+$ 316 (64.1), $[M-C_6H_4O_3]^+$ 264 (100.0).

From Sc. album *UAMH 3611.*—A 5 liter batch of the fungal culture gave dry mycelium, (21.4 g), CH_2Cl_2 broth extract, (452.6 mg), and CH_2Cl_2 mycelial extract (1.214 Hg). Fractional crystallization of the crude broth extract from CH_2Cl_2/Et_2O afforded 59.9 mg of pure **1** and 25.3 mg of liquors. From these, 2.2 mg of **2** was obtained using preparative tlc as in the case of *Sc. album* UAMH 3620. The mycelial extract, upon crystallization, gave 104.9 mg of **1** and 89.1 mg of a mixture containing **1** and **2**.

ISOLATION OF LIGNICOL [**3**].—*Sc. lignicola* UAMH 5101 was grown in shake culture on 2% malt extract broth (6 liters) for 3 weeks. The mycelium was separated from the broth, and the broth was concentrated to 1 liter and extracted with EtOAc. The crude extract (0.22 g) was subjected to flash chromatography over Si gel. Elution with Skellysolve B-EtOAc (2:1) gave lignicol (3.5 mg): mp 180–181° after crystallization from CH_2Cl_2 containing 1% MeOH; $[\alpha]_D$ +2.7° (MeOH); ir ($CHCl_3$) 3290, 1660, 1625, 1589, 1511 cm^{-1}; uv (MeOH) λ max 216 (15,400), 272 (9700), 304 nm (sh, 3800); CD (MeOH, c=0.13) 269 nm (Δε −23.3); ^{1}H nmr ($CDCl_3$) δ 11.36 (1 H, s, 8-OH), 6.67 (1 H, s, H-5), 6.42 (1 H, s, 6-OH), 4.50 (2 H, m. H-3 and H-4), 4.03 (3 H, s, OMe), 2.06 (1 H, d, J=6 Hz, 4-OH, exchangeable), 1.50 (3 H, d, J=6 Hz, Me); ^{13}C nmr (CD_3OD) δ 170.5 (C-1) 158.8 (C-6 or C-8), 157.4 (C-8 or C-6), 140.2 (C-10), 135.7 (C-7), 106.9 (C-5), 100.9 (C-9), 81.4 (C-3), 69.5 (C-4), 60.9 (OCH_3), 18.1 (CH_3); ms $[M]^+$ 240.0633 ($C_{11}H_{12}O_6$) (100%), $[M-H_2O]^+$ 222 (14%), $[M-CO_2]^+$ 196 (37%), $[M-H_2O-CO_2]^+$ 178 (20%), $[C_8H_8O_4]^+$ 168 (51%).

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Received 16 March 1993