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METABOLITES OF *LEPTOGRAPHIUM WAGENERI*, THE CAUSATIVE AGENT OF BLACK STAIN ROOT DISEASE OF CONIFERS

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ABSTRACT.—The metabolites produced by *Leptographium wageneri* var. *pseudotsugae*, the causative agent of black root disease of Douglas fir, when grown on solid (rye) and liquid media have been examined. Two new xanthones, vertixanthone [**2**] and hydroxyvertixanthone [**5**], and two new α-pyrones, vertipyronol [**15**] and vertipyronediol [**17**], have been identified. 1-Hydroxy-8-methoxyanthraquinone [**9**], not previously reported as a natural product, 1,8-dimethoxynaphthalene [**1**], β-sitosterol, β-sitosteryl palmitate, isoevernin aldehyde [**13**], 1,3,6,8-tetrahydroxyanthraquinone [**8**], 3,4-dihydro-3,4,8-trihydroxy-1(2*H*)-naphthalenone [**14**], and mycoxanthone [**7**] were also obtained. The structures of the new compounds were assigned on the basis of spectroscopic (^{1}H and ^{13}C nmr, ir, uv, ms) data and by correlation with compounds of known structure. The metabolites are compared to those previously reported from a *Leptographium* sp. also isolated from Douglas fir. Compounds **2**, **5**, and **7** cause inhibition of water transport in pine seedlings.

Black stain root disease, characterized by longitudinal dark brown to black stains in the roots and lower bole of conifers, is caused by the fungus *Leptographium wageneri* (Kendr.) Wingf. (= *Verticicladiella wageneri* Kendrick) (1–3). The disease, which was first reported in 1938 on ponderosa pine in California (4), is debilitating to the tree and frequently results in mortality. In western Canada it is particularly troublesome on Douglas fir and lodgepole pine (1). Three varieties of *L. wageneri* are known; these are host-specific for pinyon pines, Douglas fir, and hard pines (5). The taxonomy of this genus has recently been updated (2). We have previously reported on the metabolites produced when a *Leptographium* sp. (= *Verticicladiella* sp.) from Douglas fir was grown on a V-8 juice-glucose medium (6). We now report on the metabolites of *L. wageneri* var. *pseudotsugae* Harrington et Cobb (3) when grown on liquid and on solid (rye) media and also on the metabolites of the previously studied isolate when grown on solid medium.

RESULTS AND DISCUSSION

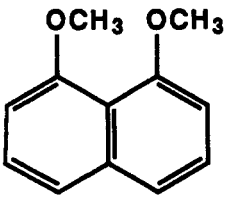
L. wageneri was cultured on moist, sterile winter rye for 6 weeks. It was also grown in liquid culture on a medium of 10% filtered V-8 juice containing 1% added glucose. The solid culture was extracted successively with Skellysolve B (light petroleum, bp 64–70°), Et_2O, CH_2Cl_2, and EtOAc. A similar quantity of uninfected rye was extracted in the same way for comparison purposes.

The Skellysolve B extract contained two components (tlc) that were separated by flash chromatography over Si gel. The major component proved to be 1,8-dimethoxynaphthalene [**1**], the structure of which was established by spectroscopic methods and confirmed by comparison with an authentic sample prepared by methylation of 1,8-dihydroxynaphthalene. 1,8-Dimethoxynaphthalene [**1**] has previously been reported as a metabolite of the fungus *Daldinia concentrica* (7). The minor component of the Skellysolve B extract proved to be β-sitosterol.

The Et_2O extract was separated by flash chromatography using gradient elution with Skellysolve B/EtOAc. Skellysolve B alone eluted 1,8-dimethoxynaphthalene [**1**] and β-sitosteryl palmitate. The latter was identified by nmr and ms and by hydrolysis to β-sitosterol and palmitic acid. β-Sitosteryl palmitate has previously been isolated from wheat and rye (8). Elution with Skellysolve B-EtOAc (9:1) gave a yellow crystalline compound $C_{15}H_{10}O_5$ [**2**] that appears not to have been described before and for

which we suggest the name vertixanthone. The uv spectrum of **2** is characteristic of a hydroxyxanthone (9), and the ir spectrum shows broad, H-bonded hydroxyl absorption (2800–3100 cm^{-1}) along with carbonyl absorption at 1730 and 1640 cm^{-1}. In acetylvertixanthone [**3**], the ketonic carbonyl absorption is shifted to 1660 cm^{-1}, the normal xanthone position (10), indicating the H-bonded nature of the ketonic carbonyl in vertixanthone itself. The ^{1}H-nmr spectrum of **2** shows a downfield, D_2O exchangeable signal (δ 12.24) attributed to the H-bonded hydroxyl hydrogen, an ester methoxyl (δ 4.05), and six aromatic hydrogens as two AMX systems which were identified by spin decoupling experiments. Because each AMX system shows only ortho (8 Hz) and meta (1 Hz) couplings, both aromatic rings are 1,2,3-trisubstituted. From these data it was apparent that vertixanthone was a xanthone bearing an H-bonded hydroxyl and a methoxycarbonyl located on different aromatic rings. Only structures **2** and **4** accommodate these characteristics. The chemical shift of a hydrogen peri to the carbonyl in a xanthone is usually observed below δ 8 (11). The lowest field aromatic signal in vertixanthone appears at δ 7.65, indicating that it possesses structure **2**, which is also favored on biogenetic grounds (12).

The fraction which elutes with Skellysolve B-EtOAc (3:2) was composed of two



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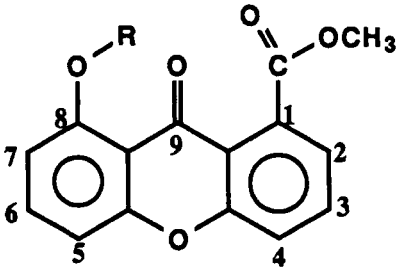
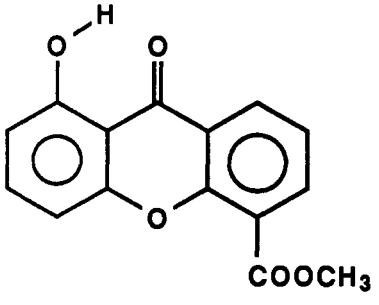


2 R=H
3 R=Ac



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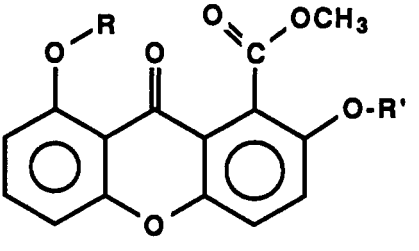
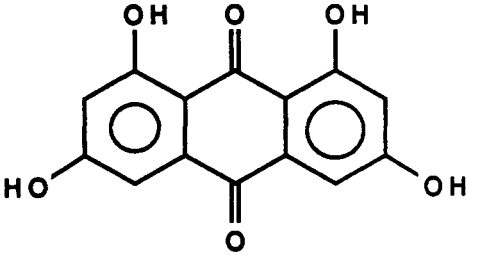


5 R=R'=H
6 R=R'=Ac
7 R=H, R'=Me



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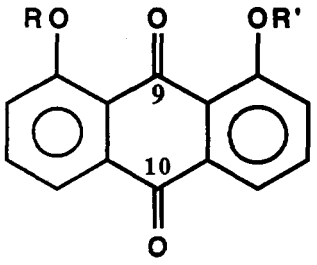
components, an orange-red compound and a yellow compound, which were separated by fractional crystallization. The yellow pigment, which we call hydroxyvertixanthone, is assigned structure **5**. It possesses one more oxygen than vertixanthone [**2**] and shows similar ir and uv characteristics. Treatment with Ac_2O/pyridine gives the diacetyl derivative **6**, indicating the presence of two hydroxyl groups in **5**. The ^{1}H-nmr spectra of **5** and **6** show the presence of five aromatic protons made up of an AB and an AMX spin system. The AB spin system shows ortho coupling constants ($J = 8.5$ Hz) while the AMX spin system shows ortho and meta couplings ($J = 8.0$ and 0.8 Hz) suggesting the presence of one 1,2,3,4-tetrasubstituted and one 1,2,3-trisubstituted aromatic ring. The fact that the hydrogens of the AB system are downfield (δ 7.65 and 7.49) from the hydrogens of both H_m (δ 7.07) and H_x (δ 6.80) of the AMX system indicates that the 1,2,3,4-tetrasubstituted aromatic ring carries the carbomethoxyl group. Comparison of the ir spectra of **5** and **6** reveals that the xanthone carbonyl absorption shifts from 1640 cm^{-1} to 1658 cm^{-1} and the ester from 1706 cm^{-1} to 1734 cm^{-1} on acetylation indicating that both carbonyls are H-bonded in hydroxyvertixanthone [**5**]. Hydrolysis (K_2CO_3, aqueous MeOH) and decarboxylation (quinoline, 240°) of **6** gave the known 1,7-dihydroxyxanthone (euxanthone) (13), confirming structure **5** for hydroxyvertixanthone.

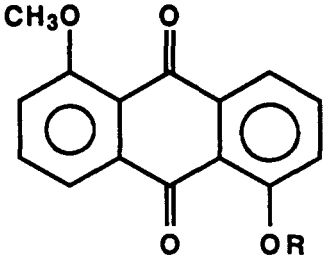
The orange-red pigment separated from **5** by fractional crystallization proved to be 1,3,6,8-tetrahydroxyanthraquinone [**8**], identical with that which we had isolated from the virulent *Leptographium* species we previously investigated (6). Another anthraquinone pigment, compound **9**, was obtained from the fraction eluted with Skellysolve B-EtOAc (4:1). This compound crystallized in the form of pale orange crystals that show a characteristic anthraquinone uv spectrum (14), and in the ir, H-bonded carbonyl (1628 cm^{-1}) and free carbonyl (1668 cm^{-1}) as well as hydroxyl absorption. Both the ^{1}H-nmr spectrum (δ 4.04, 3H, s) and the ^{13}C-nmr spectrum (δ 56.7, q) show the presence of methoxyl, and the ^{1}H-nmr spectrum reveals the presence of two 1,2,3-trisubstituted aromatic rings. The two possible structures, **9** and **12**, were differentiated by analysis of the fully coupled ^{13}C-nmr spectrum that showed the carbonyls as a triplet (δ 182.7, $J = 4.0$ Hz, C-10), and a singlet (δ 188.9, C-9). In confirmation, methylation of **9** provided the ether **10** that shows nine signals in the ^{13}C-nmr spectrum, including two carbonyl signals (δ 184.1 and 182.9). The ether **12** would show but eight signals and only one carbonyl signal. Compound **9** has not previously been reported from natural sources, but it has been prepared by synthesis (15,16).

5-Nonadecylresorcinol, which has previously been reported as a constituent of wheat bran (17), was also isolated from the Et_2O extract. Although we did not detect this compound in our blank extract, we feel that it may be derived from the rye, perhaps released by enzymatic action of the fungus.

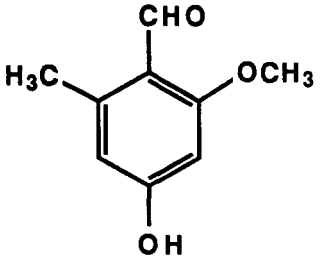
The CH_2Cl_2 extract contained seven components in addition to fatty acids and triglycerides, which were identical with those isolated from the blank rye extract as indicated by tlc, mass spectra, and ^{1}H-nmr spectra. Separation of the remaining components was achieved by flash chromatography over Si gel and led to the isolation of 1,8-dimethoxynaphthalene [**1**], β-sitosterol and its palmitate, palmitic acid, the anthraquinone **9**, and two other compounds. The first of these proved to be 4-hydroxy-2-methoxy-6-methylbenzaldehyde [**13**], previously isolated from the fungus *Guignardia laricina* and called isoevernin aldehyde (18). The other component was shown to be the trihydroxytetralone [**14**] or its enantiomer. The *trans* arrangement of the vicinal diol grouping is indicated by the coupling constant ($J = 7.5$ Hz) between the carbinyl hydrogens and by the fact that no nOe enhancement is observed between these two hydrogens, suggesting that they have a diaxial-like relationship. Compound **14** has previ-



9 R=Me, R'=H
10 R=R'=Me
11 R=R'=H



12 R=H



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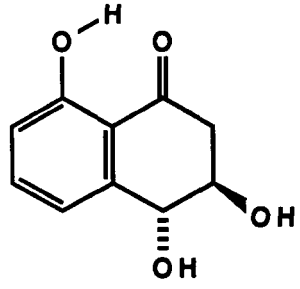
ously been reported as a metabolite of the fungus *Pyricularia oryrze* and has been shown to inhibit the growth of rice seedlings when applied in high concentration (19).

The EtOAc extract provided compounds **1**, **2**, **8**, **9**, and two compounds not present in the other extracts. These related compounds, isolated by charcoal cc followed by Si gel flash chromatography, appear not to have been described previously and have been named vertipyronol [**15**] and vertipyronediol [**17**].

Vertipyronol [**15**], a colorless, optically active oil, possesses the molecular formula $C_9H_{12}O_4$ as indicated by hrms. The base peak in the mass spectrum appears at *m/z* 153 and corresponds to the loss of the primary hydroxyl to give the ion **19** (one canonical form). The uv spectrum shows a 4-methoxy-α-pyrone chromophore (280 nm) (20). The ir spectrum shows both hydroxyl (3417 cm^{-1}) and α-pyrone carbonyl (1699 cm^{-1}) (21) absorption. The ^{1}H-nmr spectrum shows methoxyl (δ 3.90) and hydroxyl (δ 2.15, exchangeable) absorption and alkenic hydrogens as singlets at δ 7.24 and δ 5.51 assigned to the C-6 and C-3 hydrogens, respectively (22). In addition, there is a methine hydrogen (δ 2.95) which is coupled to methyl (δ 1.26) and methylene (δ 3.75) hydrogens. Acetylation of vertipyronol [**15**] gave the acetyl derivative **16** in which the methylene hydrogens (δ 4.15 and 4.08) are shifted downfield by about 0.4 ppm relative



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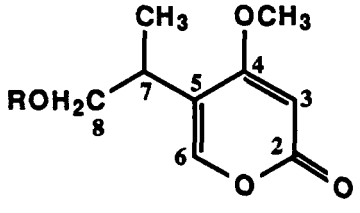
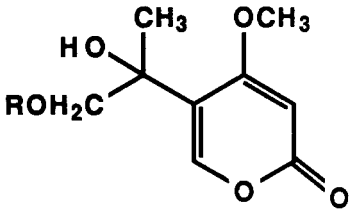
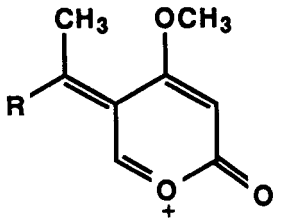


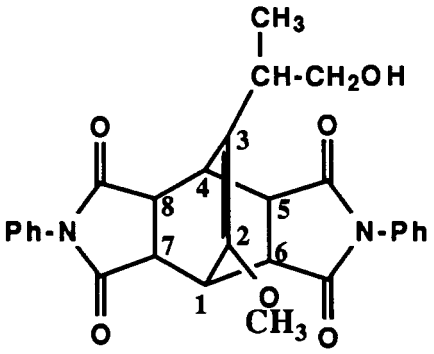
15 R=H
16 R=Ac



17 R=H
18 R=Ac



19 R=H
20 R=OH



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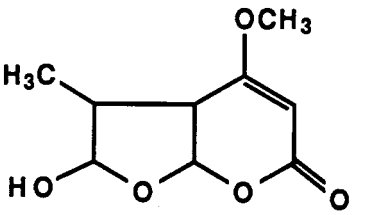

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to **15**, indicating a primary alcohol has been acetylated (23). Difference nOe experiments were used to confirm the location of the substituents on the pyrone ring. On irradiation of H-6, enhancements of the side chain methyl and methine signals are observed. Irradiation of the methoxyl hydrogens brings about enhancement of the signal for H-3, and vice versa. The ^{13}C-nmr spectrum of vertipyronol is consistent with the structure assigned (see Experimental).

α-Pyrones often undergo Diels-Alder reaction with dienophiles to provide adducts which lose CO_2 to form dienes which undergo further reaction with dienophile (24). Indeed, treatment of vertipyronol [**15**] with *N*-phenylmaleimide in hot xylene provides the crystalline, optically active "bis-adduct" **21** in good yield. The molecular formula $C_{28}H_{26}N_2O_6$ was established by ms. The ir and ^{1}H-nmr spectra are consistent with structure **21**. The absolute configuration at the chiral center in vertipyronol has not been established.

Vertipyronediol [**17**], the second new α-pyrone metabolite, is a colorless, crystalline compound with molecular formula $C_9H_{12}O_5$ and with ir and uv characteristics very similar to those of vertipyronol [**15**]. The ^{1}H-nmr spectrum is also very similar to that of **15** except that there are now two exchangeable hydrogens, the *C*-methyl signal is a singlet, and the methylene signals appear as a pair of doublets. The location of a hydroxyl group at C-7 is supported by the ^{13}C-nmr spectrum of **17**, as the chemical shift of C-7 (δ 73.7, s) is downfield relative to C-7 in **15** (δ 32.8, d). Treatment of vertipyronediol [**17**] with Ac_2O in pyridine at room temperature for 12 h provides the monoacetyl derivative **12**. The ^{1}H-nmr spectrum shows a single acetyl methyl, and the C-8 methylene hydrogens are shifted downfield by about 0.4 ppm indicating that the primary alcohol is acetylated. When the *C*-methyl hydrogens are irradiated, nOe enhancement is observed at H-6 and the methylene hydrogens. Irradiation of the methoxyl signal leads to enhancement of the H-3 signal, and vice versa. The base peak in the mass spectra of both **17** and **18** appears at *m/z* 169 corresponding to the ion **20**, consistent with the location of a hydroxyl group at C-7.

Vertipyronol [**15**] and vertipyronediol [**17**] are biogenetically related to astepyrone [**22**] (25), a metabolite of *Aspergillus terreus*, and thus appear to be tetraketides formed by cleavage of an orsellinic acid type precursor (25).

When *L. wageneri* var. *pseudotsugae* was grown in liquid culture on a V-8 juice-glucose medium, the spectrum of metabolites was similar to that obtained from the solid medium with the exception that vertipyronol [**15**] and vertipyronediol [**17**] were not isolated. When the *Leptographium* sp. isolate previously reported by Ayer *et al.* (6) was grown on the solid (rye) medium, compounds **1**, **2**, **8**, and **9** were obtained along with 1,8-dihydroxyanthraquinone [**11**] and a monomethyl ether **7** of vertixanthone [**5**]. The yellow monomethyl ether **7** has previously been reported as a metabolite of *Mycosphaerella rosigena* and named mycoxanthone (26). It thus appears that the previous isolate is closely related to *L. wageneri*.

Preliminary results indicate that the xanthone metabolites **2**, **5**, and **7** cause an inhibition of water transport in pine seedlings (27).

EXPERIMENTAL

All solvents except Et_2O (ACS quality) were distilled prior to use. Skellysolve B refers to Skelly Oil Company light petroleum bp 62–70°. Pyridine was distilled from CaH_2 and stored over molecular sieves; Ac_2O was dried over P_2O_5 and distilled from NaOAc.

Analytical tlc was carried out on aluminum sheets (75 × 25 or 75 × 50) pre-coated (0.2 mm) with Si gel $60F_{254}$ (E. Merck, Darmstadt). Materials were detected by visualization under an uv lamp (254 or 350 nm) or by spraying with a solution of phosphomolybdic acid (5%) containing a trace of ceric sulfate in aqueous H_2SO_4 (5%) followed by charring on a hot plate. Flash column chromatography was performed with Merck Si gel 60 (40-63 mm).

Hrms were recorded on an A.E.I. MS-50 mass spectrometer coupled to a DS 50 computer. Cims were recorded on an A.E.I. MS-9 mass spectrometer using NH_3 as reagent gas. Data are reported as *m/z* (rel. int.). Unless diagnostically significant, peaks with intensities less than 10% of the base peak are omitted. Uv spectra were obtained on a Unicam SP 1700 ultraviolet spectrophotometer. Ir spectra were recorded on a Nicolet 7199 FT interferometer. Optical rotations were measured on a Perkin-Elmer Model 141 polarimeter. ^{1}H-nmr spectra were measured on a Bruker WH-360 spectrometer or a Bruker WH-400 spectrometer with residual $CHCl_3$ in $CDCl_3$ or MeOH in CD_3OD employed as the internal standard (assigned as 7.27 ppm or 3.30 ppm downfield from TMS), and ^{13}C-nmr spectra were measured on a Bruker WH-300 spectrometer or a Bruker WH-400 spectrometer with $CDCl_3$ or CD_3OD employed as the internal standard (assigned as 77.00 ppm or 49.00 ppm downfield from TMS). Chemical shift assignments are based on comparison with calculated values. Melting points were recorded on a Fisher-Johns melting point apparatus and are uncorrected. The isolate, C728, of *Leptographium* sp. (isolated from Douglas fir, Radium Hot Springs, B.C.) used in this study was obtained from Y. Hiratsuka, Northern Forest Research Center (NFRC), Edmonton and deposited at the University of Alberta, Microfungus Collection, UAMH 5029. The isolate, RH50, of *L. wageneri* var. *pseudotsugae* (isolated from Douglas fir, near Kamloops, B.C.) used in the study was supplied by R.S. Hunt, Pacific Forest Center (PFC), Victoria, and is deposited as UAMH 4905.

GROWTH OF *LEPTOGRAPHIUM* ISOLATES RH50 AND C728 ON SOLID MEDIA, EXTRACTION AND ISOLATION OF THE METABOLITES.—Winter rye (500 g) was soaked in warm tap H_2O (400 g) for 8–12 h. Excess H_2O was drained off, the solid substrate was placed in five autoclavable plastic bags, and the media were autoclaved twice for 30 min at 121°. An aqueous suspension of mycelium of *Leptographium* RH50 or C728 was used to inoculate an agar plate (10% filtered V-8 juice, 1% glucose, 2% agar). After 7–10 days at room temperature, the plate culture was blended with sterile H_2O (250 ml). The mycelial suspension (25 ml) was introduced into each bag of solid media, and the culture was kept at room temperature for 6 weeks.

The culture of isolates RH50 or C728 was harvested by extracting successively in a Soxhlet extractor with Skellysolve B, Et_2O, CH_2Cl_2, and EtOAc for 24 h; then the extract was concentrated. Each of the four crude extracts [Skellysolve B 1.25 g (RH50), 2.86 g (C728); Et_2O 4.02 g (RH50), 3.54 g (C728); CH_2Cl_2 3.05 g (RH50), 2.00 g (C728); EtOAc 1.79 g (RH50), 6.40 g (C728)] was separated by flash chromatography utilizing gradient elution with either a Skellysolve B/EtOAc or a CH_2Cl_2/MeOH solvent system. The uninoculated rye medium (blank) was subjected to a similar extraction procedure. Only fatty acids and triglycerides were isolated, and these are identical with those isolated from the crude extracts.

Separation of the extracts from isolate RH50 by chromatography over Si gel with EtOAc Skellysolve B as eluent gave the following compounds: β-sitosteryl palmitate (10 mg), **1** (32 mg), **2** (3 mg), **9** (4 mg), 5-*n*-nonadecylresorcinol (15 mg), **5** (4 mg), and **8** (2 mg) from the crude Et_2O extract. With MeOH/

CH_2Cl_2 as eluent, separation yielded β-sitosteryl palmitate (32 mg), **1** (20 mg), palmitic acid (31 mg), **9** (7 mg), β-sitosterol (15 mg), **13** (4 mg), and **14** (16 mg) from the crude CH_2Cl_2 extract. With EtOAc/Skellysolve B as eluent, separation yielded β-sitosteryl palmitate (21 mg), **1** (8 mg), β-sitosterol (10 mg), **2** (2 mg), **9** (8 mg), 2(3*H*)-benzoxazolone (5 mg), and **8** (2 mg) from the crude EtOAc extract. Further chromatography of the EtOAc extract over charcoal (eluent 10% Me_2CO/H_2O) and Si gel (eluent MeOH/CH_2Cl_2) led to the isolation of compounds **15** (17 mg) and **17** (7 mg).

Separation of the extracts from isolate C728 as described above led to the isolation of the following compounds: **1** (17 mg), **11** (6 mg), **2** (63 mg), **9** (5 mg), and **7** (1 mg) from the Skellysolve B extract; **11** (1 mg), 5-*n*-nonadecylresorcinol (56 mg), **2** (2 mg), **7** (2 mg), **9** (2 mg), and **8** (57 mg) from the Et_2O extract; β-sitosteryl palmitate (40 mg), palmitic acid (51 mg), and **7** (2 mg) from the CH_2Cl_2 extract; and β-sitosteryl palmitate (38 mg), palmitic acid (607 mg), **11** (1 mg), and **8** (7 mg) from the EtOAc extract.

GROWTH OF *LEPTOGRAPHIUM* ISOLATE RH50 IN LIQUID MEDIUM, EXTRACTION AND ISOLATION OF THE METABOLITES.—Cultures of isolate RH50 were maintained at 4° in slant tubes containing Difco potato dextrose agar. The inoculum was prepared as described above and ca. 20 ml aliquots were used to inoculate 5 × 1 liter sterile medium in 2-liter flasks. The still cultures were kept at room temperature for 6 weeks; then the culture broth was decanted from the mycelium, concentrated to ca. 500 ml, and continuously extracted with Skellysolve B then EtOAc for 24 h. The organic extracts were then dried and concentrated. Separation of the Skellysolve B extract (91 mg) by Si gel flash chromatography gave 1,8-dimethoxynaphthalene [**1**] (38 mg). The EtOAc extract (256 mg) was subjected to gradient flash chromatography over Si gel with EtOAc in Skellysolve B. Three metabolites, 1,8-dimethoxynaphthalene [**1**] (20 mg), 1-hydroxy-8-methoxyanthraquinone [**9**] (6 mg), and 3,4-dihydro-3,4,8-trihydroxy-1(2*H*)-naphthalenone [**14**] (23 mg), were isolated.

The mycelium was subjected to successive continuous extraction in a Soxhlet extractor with Skellysolve B and EtOAc. The organic extracts were dried and concentrated. The crude mycelium extracts were separated by flash chromatography over Si gel using gradient elution with EtOAc in Skellysolve B. 1,8-Dimethoxynaphthalene [**1**] (425 mg) was obtained from the crude Skellysolve B extract (650 mg) whereas from the crude EtOAc extract (652 mg), 1,8-dihydroxyanthraquinone [**11**], 1,8-dimethoxynaphthalene [**1**], vertixanthone [**2**], hydroxyvertixanthone [**5**], and 1-hydroxy-8-methoxyanthraquinone [**9**] were isolated.

1,8-DIMETHOXYNAPHTHALENE [**1**].—1,8-Dimethoxynaphthalene [**1**] was isolated from the chromatographic fractions eluted with 2–4% EtOAc in Skellysolve B. It crystallized from Skellysolve B. Recrystallization from 95% EtOH gave 1,8-dimethoxynaphthalene [**1**] as white crystals (60 mg from C50 and 17 mg from C728), mp 154–155° [lit. (7) 157–158°]; tlc R_f 0.63 [Skellysolve B-EtOAc (3:2)]. The compound was identical with an authentic sample prepared by methylation of 1,8-dihydroxynaphthalene.

β-SITOSTERYL PALMITATE.—Impure fractions containing one major compound were obtained by flash chromatography over Si gel with Skellysolve B. The fractions were evaporated under reduced pressure. The residue was dissolved in hot Me_2CO and recrystallized from Me_2CO. White platelets were identified as β-sitosteryl palmitate (62 mg from C50 and 9 mg from C728), mp 92–93° [lit. (8) 92–94°]. Hydrolysis (KOH, MeOH) gave β-sitosterol and palmitic acid.

VERTIXANTHONE [**2**].—Fractions containing vertixanthone were separated by Si gel flash chromatography on elution with 10% EtOAc in Skellysolve B. A yellow solid precipitated from the concentrated fractions. Recrystallization from EtOAc/Skellysolve B gave yellow crystals of vertixanthone [**2**] (7 mg from C50 and 65 mg from C728), mp 152–154°; tlc R_f 0.47 [Skellysolve B-EtOAc (3:1)], R_f 0.37 [Skellysolve B-C_6H_6-MeOH (1:1:0.2)]; uv (95% EtOH) λ max nm (log ε) 232 (4.61), 264 (4.60), 290 (4.50), 384 (3.72); ir ($CHCl_3$, cast), ν max cm^{-1} 3100 (br, OH, hydrogen-bonded), 1730, 1640, 1610, 1600, 1570, 1460, 1280, 1200, 1130, 810; ^1H nmr ($CDCl_3$, 400 MHz) δ 12.24 (1H, s, O*H*, 7.77 (1H, t, J = 8.0 Hz, H-3), 7.61 (1H, t, J = 8.0 Hz, H-6), 7.56 (1H, dd, J = 1.0, 8.0 Hz, H-2), 7.33 (1H, dd, J = 1.0, 8.0 Hz, H-4), 6.94 (1H, dd, J = 1.0, 8.0 Hz, H-5), 6.82 (1H, dd, J = 1.0, 8.0 Hz, H-7), 4.05 (3H, s, OCH_3); ^{13}C nmr ($CDCl_3$, 75 MHz) δ 181.1 (s, C-9), 169.6 (s, C-11), 161.9 (s, C-10a), 156.1 (s, C-4a), 155.8 (s, C-8), 137.2 (d, C-6), 135.1 (d, C-3), 133.7 (s, C-1), 122.7 (d, C-2), 119.5 (d, C-4), 117.6 (s, C-9a), 111.0 (d, C-5), 109.0 (s, C-8a), 106.9 (d, C-7), 53.2 (q, OCH_3); hrms *m/z* (rel. int. %) calcd for $C_{15}H_{10}O_5$ $[M]^+$ 270.0528, found 270.0533 (43), 239 (33), 238 (100), 210 (46), 155 (18), 126 (35), 75 (22), 63 (21), 51 (11).

ACETYLATION OF VERTIXANTHONE.—Ac_2O (0.25 ml) was added to a solution of vertixanthone [**2**] (4 mg) in pyridine (0.5 ml). The reaction mixture was stirred at room temperature overnight. Toluene (5 ml) was added and the solution was evaporated under reduced pressure. The residue was recrystallized from Skellysolve B. A colorless, crystalline monoacetate derivative, compound **6** (2.5 mg) was obtained, mp 170° (dec); tlc R_f 0.51 [Skellysolve B-EtOAc (1:1)]; ir ($CHCl_3$, cast) ν max cm^{-1} 1764, 1730, 1659,

1620, 1602, 1291, 1010; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 7.66 (1H, t, J = 8.5 Hz, H-3), 7.64 (1H, t, J = 8.5 Hz, H-6), 7.48 (1H, dd, J = 1.0, 8.5 Hz, H-2), 7.35 (1H, dd, J = 1.0, 8.5 Hz, H-4), 7.25 (1H, dd, J = 1.0, 8.5 Hz, H-5), 6.95 (1H, dd, J = 1.0, 8.5 Hz, H-7), 3.93 (3H, s, OCH_3), 2.38 (3H, s, $OCOCH_3$); ^{13}C nmr ($CDCl_3$, 75 MHz) δ 174.5 (s, C-9), 169.9 (s, $OCOCH_3$), 169.7 (s, C-11), 156.9 (s, C-10a), 155.3 (s, C-4a), 150.1 (s, C-8), 134.7 (d, C-6), 134.3 (d, C-3), 134.1 (s, C-1), 123.0 (d, C-2), 119.7 (s, C-9a), 119.4 (d, C-4), 118.6 (d, C-5), 116.0 (d, C-7), 115.2 (s, C-8a), 52.6 (q, OCH_3), 21.2 (q, $OCOCH_3$); hrms *m/z* (rel. int. %) calcd for $C_{17}H_{12}O_6$ $[M]^+$ 312.0634, found 312.0639 (13), 270 (59), 239 (25), 238 (100), 210 (14).

HYDROXYVERTIXANTHONE [**5**].—The chromatographic fraction (40% EtOAc in Skellysolve B) from the Et_2O crude extract consisted of two components. Fractional crystallization from Me_2CO gave yellow crystals of hydroxyvertixanthone [**5**] (6 mg from C50), mp 244–245°; tlc R_f 0.55 [CH_2Cl_2-MeOH (9.5:0.5)], R_f 0.35 [Skellysolve B-EtOAc (3:1)]; uv (95% EtOH) λ max nm (log ε) 238 (4.14), 264 (4.25), 290 (3.71), 390 (3.50); ir ($CHCl_3$, cast) ν max cm^{-1} 3300–3100, 1706, 1640, 1605, 1582, 1440, 1380, 1290, 1222, 1050, 817, 740; ^{1}H nmr (DMSO-d_6, 400 MHz) δ 13.82 (1H, s, 8-OH), 10.50 (1H, br, 2-OH), 7.73 (1H, t, J = 8.0 Hz, H-6), 7.65 (1H, d, J = 8.5 Hz, H-4), 7.49 (1H, d, J = 8.5 Hz, H-3), 7.07 (1H, dd, J = 0.8, 8.0 Hz, H-5), 6.80 (1H, dd, J = 0.8, 8.0 Hz, H-7), 3.84 (3H, s, OCH_3); ^{13}C nmr ($CDCl_3$/DMSO-d_6, 75 MHz) δ 180.6 (s, C-9), 170.2 (s, C-11), 160.8 (s, C-10a), 152.6 (s, C-4a), 152.3 (s, C-8), 148.0 (s, C-2), 135.8 (d, C-6), 125.9 (d, C-3), 119.0 (d, C-4), 117.2 (s, C-9a), 116.2 (s, C-1), 109.0 (d, C-5), 107.8 (s, C-8a), 106.8 (d, C-7), 51.9 (q, OCH_3); hrms *m/z* (rel. int. %) calcd for $C_{15}H_{10}O_6$ $[M]^+$ 286.0477, found 286.0480 (32), 255 (20), 254 (100), 226 (12).

ACETYLATION OF HYDROXYVERTIXANTHONE.—Ac_2O (2.5 ml) was added to a solution of hydroxyvertixanthone [**5**] (3 mg) in pyridine (0.5 ml). The solution was stirred at room temperature overnight. Toluene (5 ml) was added to the reaction mixture, and the solvents were evaporated. Recrystallization from Skellysolve B gave acetylhydroxyvertixanthone [**6**] as colorless crystals (2 mg), mp 304–305°; tlc R_f 0.40 [Skellysolve B-EtOAc (1:1)]; ir ($CHCl_3$, cast) ν max cm^{-1} 1771 (d, ester), 1734 (ester), 1658 (CO), 1622, 1214, 1195; ^{1}H nmr ($CDCl_3$, 300 MHz) δ 7.65 (1H, t, J = 8.5 Hz, H-6), 7.48 (2H, s, H-3, H-4), 7.35 (1H, dd, J = 1.5, 8.5 Hz, H-5), 6.95 (1H, dd, J = 1.5, 8.5 Hz, H-7), 3.92 (3H, s, OCH_3), 2.37 (3H, s, C-8 $OCOCH_3$), 2.25 (3H, s, C-2 $OCOCH_3$); ^{13}C nmr ($CDCl_3$, 75 MHz) δ 174.0 (s, C-9), 169.6 (s, C-8 $OCOCH_3$), 168.8 (s, C-2 $OCOCH_3$), 166.9 (s, C-11), 156.9 (s, C-10a), 152.8 (s, C-4a), 150.1 (s, C-8), 144.5 (s, C-2), 135.0 (d, C-6), 129.7 (d, C-3), 115.5 (s, C-9a), 125.8 (s, C-1), 113.5 (s, C-8a), 119.9 (d, C-5), 118.8 (d, C-4), 116.0 (d, C-7), 53.0 (q, OCH_3), 21.2 (q, C-8 $OCOCH_3$), 20.7 (q, C-2 $OCOCH_3$); hrms *m/z* (rel. int. %) calcd for $C_{19}H_{14}O_8$ $[M]^+$ 370.0688, found 370.0699 (1), 328 (18), 286 (33), 255 (24), 254 (100), 226 (7).

DECARBOXYLATION OF HYDROXYVERTIXANTHONE.—Compound **6** (1.8 mg) was dissolved in $CHCl_3$, and 10% K_2CO_3 in MeOH (10 ml) was added. The mixture was stirred at room temperature overnight, then neutralized with 1 N HCl and extracted with EtOAc. The extract was dried (Na_2SO_4) and evaporated under reduced pressure. The residue was dissolved in quinoline (1.5 ml), heated to 240°, and refluxed for 25 min. The solution was cooled to room temperature, and EtOAc (30 ml) was added. Quinoline was removed by extraction (three times) with diluted aqueous HCl. The organic layer was extracted with aqueous 10% NaOH solution to give a yellow solution that was neutralized with concentrated HCl, then extracted with EtOAc. The extract was dried (Na_2SO_4). Evaporation of the solvents gave a yellow solid. Further purification was achieved by Si gel chromatography with 10% EtOAc in Skellysolve B. Crystallization from toluene gave yellow crystals of euxanthone (1.0 mg, 90% yield), mp 236–238° [lit. (13) 236–238°]. The spectral data obtained was identical with that previously reported (13).

MYCOXANTHONE [**7**].—Mycoxanthone [**7**] was isolated as yellow crystals from EtOAc/Skellysolve B by Si gel flash chromatography (eluent, 10% EtOAc in Skellysolve B) (4 mg from C728), mp 221–222° [lit. (26) 222–223°]. Its spectral properties (nmr, ir) were identical with those reported (26).

1,3,6,8-TETRAHYDROXYANTHRAQUINONE [**8**].—An orange-red pigment was separated by repeated Si gel chromatography (elution with 20% EtOAc in CH_2Cl_2) of the hydroxyvertixanthone mother liquors. Crystallization from MeOH/Skellysolve B gave crystalline 1,3,6,8-tetrahydroxyanthraquinone [**8**] (6) (14 mg from RH50 and 57 mg from C728), mp 290° (dec), identical with an authentic sample.

1-HYDROXY-8-METHOXYANTHRAQUINONE [**9**].—Chromatography of the Et_2O extract over Si gel with 20% EtOAc in Skellysolve B or 2% MeOH in CH_2Cl_2 gave an orange-yellow pigment. Crystallization from EtOAc/Skellysolve B gave 1-hydroxy-8-methoxyanthraquinone [**9**] (22 mg from RH50 and 5 mg from C728), mp 182–183° [lit. (26) 185–186°]; tlc R_f 0.44 [Skellysolve B-EtOAc (3:2)], R_f 0.26 [Skellysolve B-C_6H_6-MeOH (1:1:0.2)]; uv (95% EtOH, 1.0 mg/100 ml) λ max nm (log ε) 253 (3.92), 277 (3.72), 412 (3.59); ir ($CHCl_3$, cast) ν max nm cm^{-1} 1668 (CO), 1628 (CO, hydrogen-bonded),

1580, 1480, 1450, 1440, 1350, 1280, 1240, 1070, 1020, 930, 840, 740; ^{1}H nmr ($CDCl_3$, 400 MHz) δ 12.96 (1H, s, OH), 7.96 (1H, dd, J = 1.3, 7.8 Hz, H-5), 7.77 (1H, dd, J = 1.3, 7.8 Hz, H-4), 7.74 (1H, t, J = 7.8 Hz, H-6), 7.60 (1H, t, J = 7.8 Hz, H-3), 7.35 (1H, dd, J = 1.3, 7.8 Hz, H-7), 7.29 (1H, dd, J = 1.3, 7.8 Hz, H-2), 4.04 (3H, s, OCH_3); ^{13}C nmr ($CDCl_3$, 75 MHz) δ 188.7 (s, C-9), 183.0 (s, C-10), 162.6 (s, C-1), 161.0 (s, C-8), 135.9 (s, C-10a), 135.8 (d, C-3), 135.8 (d, C-6), 132.8 (s, C-4a), 124.7 (d, C-4), 120.9 (s, C-8a), 120.2 (d, C-5), 118.8 (d, C-2), 118.2 (d, C-7), 117.1 (s, C-9a), 56.7 (q, OCH_3); hrms *m/z* (rel. int. %) calcd for $C_{15}H_{10}O_4$ $[M]^+$ 254.0579, found 254.0573 (100), 237 (16), 236 (59), 225 (22), 211 (4), 208 (89), 183 (5), 180 (26), 155 (23), 127 (22), 113 (10), 75 (21), 63 (25).

METHYLATION OF 1-HYDROXY-8-METHOXYANTHRAQUINONE.—1-Hydroxy-8-methoxyanthraquinone [**9**] (5 mg) was dissolved in Me_2CO, and saturated methanolic K_2CO_3 (1 ml) and MeI (0.5 ml) were added. The solution changed color from yellow to red when base was added. The solution was refluxed for 12 h. Further additions of K_2CO_3/MeOH and MeI were made at intervals until there was no color change (yellow to red) on addition of the reagent. Excess K_2CO_3 was filtered, and the solvents were evaporated under reduced pressure. The residue was dissolved in H_2O and extracted with CH_2Cl_2. The extract was concentrated to give a yellow solid that was crystallized from Skellysolve B containing a few drops of Me_2CO. 1,8-Dimethoxyanthraquinone [**10**] was obtained as yellow needles (3 mg), mp 219–220° [lit. (29) 219°].

1,8-DIHYDROXYANTHRAQUINONE [**11**].—The crude Et_2O and EtOAc extracts were separated by Si gel flash chromatography (eluent, 5% EtOAc in Skellysolve B). The chromatographic fractions were further purified by extraction with 1% aqueous NaOH solution, neutralized with 1 N HCl, and then extracted with CH_2Cl_2. Evaporation of the solvents and crystallization from EtOAc/Skellysolve B gave yellow needles of 1,8-dihydroxyanthraquinone [**11**] (8 mg from C728), mp 193–194° [lit. (30) 193°].

5-*n*-NONADECYLRESORCINOL.—5-Nonadecylresorcinol was isolated by chromatography over Si gel with 30% EtOAc in Skellysolve B. The white solid was precipitated from hexane (98 mg from RH50 and 56 mg from C728), mp 90–91° [lit. (31) 96.5–97.5°].

ISOEVERNIN ALDEHYDE [**13**].—Compound **13**, isoevernin aldehyde, was isolated from the chromatographic fractions eluted with 15% EtOAc in Skellysolve B. Crystallization from EtOAc afforded compound **13** as pale brown needles (4.4 mg from RH50), mp 196–197° [lit. (18) 196°].

3,4-DIHYDRO-3,4,8-TRIHYDROXY-1(2*H*)-NAPHTHALENONE [**14**].—3,4-Dihydro-3,4,8-trihydroxy-1(2*H*)-naphthalenone [**14**], isolated from the 30% EtOAc in Skellysolve B eluent, was crystallized from EtOAc to give colorless needles (16 mg from RH50), mp 177–178°; tlc R_f 0.12 [Skellysolve B-EtOAc (1:1)]; $[\alpha]_D$ −40° (c = 0.12, MeOH); uv (95% EtOH) λ max nm (log ε) 259 (3.23); ir (KBr, pellet) ν max cm^{-1} 3600–2400, 1650, 1618, 1580, 1335, 790; ^{1}H nmr (MeOH-d_4, 360 MHz) δ 7.55 (1H, t, J = 8.0 Hz, H-6), 7.14 (1H, dd, J = 2.0, 8.0 Hz, H-5), 6.86 (1H, dd, J = 2.0, 8.0 Hz, H-7), 4.62 (1H, d, J = 7.5 Hz, H-4), 4.06 (1H, m, H-3), 3.09 (1H, dd, J = 4.0, 17.5 Hz, H-2e), 2.69 (1H, dd, J = 7.5, 17.5 Hz, H-2a); ^{13}C nmr (MeOH-d_4, 75 MHz) δ 204.4 (s, C-1), 163.2 (s, C-8), 145.8 (s, C-4a), 138.0 (d, C-6), 120.0 (d, C-5), 117.8 (d, C-7), 116.8 (s, C-8a), 73.2 (d, C-4), 71.6 (d, C-3), 44.1 (t, C-2); hrms *m/z* (rel. int. %) calcd for $C_{10}H_{10}O_4$ $[M]^+$ 194.0579, found 194.0582 (80), 176 (15), 150 (70), 147 (20), 122 (63), 121 (100), 93 (17), 65 (26).

VERTIPYRONOL [**15**].—Vertipyronol [**15**], a colorless oil, was separated by Si gel chromatography by elution with 95% EtOAc in Skellysolve B. The chromatographic fraction was purified on a charcoal column (10–50% Me_2CO in H_2O) followed by Si gel flash chromatography (4% MeOH in CH_2Cl_2) (17 mg from RH50), tlc R_f 0.29 [$CHCl_3$-MeOH (1:9)]; $[\alpha]_D$ −1.58° (c = 0.19, $CHCl_3$); uv (95% EtOH) λ max nm (log ε) 280 (3.74); ir ($CHCl_3$, cast) ν max cm^{-1} 3417 (br), 1699 (br), 1645, 1550, 1458, 1419, 1220, 1045, 817; ^{1}H nmr ($CDCl_3$, 360 MHz) δ 7.24 (1H, s, H-6), 5.51 (1H, s, H-3), 3.90 (3H, s, OCH_3), 3.75 (1H, dd, J = 6.0, 10.5 Hz, H-8), 3.65 (1H, dd, J = 6.0, 10.5 Hz, H-8), 2.95 (1H, tq, J = 6.0, 7.5 Hz, H-7), 2.15 (1H, br, O*H*), 1.26 (3H, d, J = 7.5 Hz, CH_3); ^{13}C nmr ($CDCl_3$, 75 MHz) δ 170.0 (s, C-4), 164.4 (s, C-2), 148.7 (d, C-6), 117.1 (s, C-5), 90.0 (d, C-3), 65.8 (t, C-8), 56.1 (q, OCH_3), 32.5 (d, C-7), 15.9 (q, CH_3); hrms *m/z* (rel. int. %) calcd for $C_9H_{12}O_4$ $[M]^+$ 184.0736, found 184.0737 (18), 154 (11), 153 (100), 125 (6), 97 (6), 93 (5).

ACETYLATION OF VERTIPYRONOL.—Vertipyronol (2 mg) was treated with Ac_2O in pyridine at room temperature for 12 h. Workup in the usual way gave acetylvertipyronol, compound **16**, as a yellowish oil (2 mg), $[\alpha]_D$ −2.63° (c = 0.19, $CHCl_3$); ir ($CHCl_3$, cast) ν max cm^{-1} 1745–1734, 1648, 1555, 1458, 1421, 1210, 1043, 817; ^{1}H nmr ($CDCl_3$, 400 MHz) δ 7.22 (1H, s, H-6), 5.56 (1H, s, H-3), 4.15 (1H, dd, J = 6.0, 10.5 Hz, H-8), 4.08 (1H, dd, J = 6.0, 10.5 Hz, H-8), 3.85 (3H, s, OCH_3), 3.05 (3H, tq, J = 6.0, 7.5 Hz, H-7), 2.04 (3H, s, $OCOCH_3$), 1.22 (3H, d, J = 7.5 Hz, CH_3); hrms *m/z*

(rel. int. %) calcd for $C_{11}H_{14}O_5$ $[M]^+$ 226.0841, found 226.0842 (10), 166 (69), 154 (16), 153 (100), 151 (13), 125 (9), 93 (8).

DIELS-ALDER REACTION OF VERTIPYRONOL.—*N*-Phenylmaleimide (14 mg) was added to a stirred solution of vertipyronol [**15**] (5 mg) in xylene. The mixture was refluxed at 140° for 12 h. The solvents were evaporated, and the residue was separated by repeated Si gel chromatography (eluent, 5% MeOH in CH_2Cl_2). Evaporation of the solvent gave a yellowish solid, which was further purified by precipitation from EtOAc with hexane. The bisadduct, compound **21**, was obtained as a yellowish solid (6 mg), $[\alpha]_D$ −3.06° (*c* = 0.36, $CHCl_3$); ir ($CHCl_3$, cast) ν max cm^{-1} 3560–3490, 1715, 1499, 1380, 1219, 1193, 746, 690; ^1H nmr ($CDCl_3$, 360 MHz) δ 7.48 (8H, m, aromatic hydrogens), 7.42 (2H, m, aromatic hydrogens), 4.25 (1H, t, *J* = 3.0 Hz, H-1), 3.91 (1H, t, *J* = 3.0 Hz, H-4), 3.67 (3H, s, OCH_3), 3.40 (2H, t, *J* = 6.5 Hz, 2 × H-1′), 3.23 (2H, dd, *J* = 3.0, 8.0 Hz, H-7, H-7), 3.14 (2H, dd, *J* = 3.0, 8.0 Hz, H-5, H-8), 2.73 (1H, tq, *J* = 6.5, 7.0 Hz, H-2′), 0.86 (3H, d, *J* = 7.0 Hz, CH_3); hrms *m/z* (rel. int. %) calcd for $C_{28}H_{26}N_2O_6$ $[M]^+$ 486.1792, found 486.1775 (1), 456 (100), 455 (90), 442 (19), 441 (21), 174 (30), 135 (18), 77 (18).

VERTIPYRONEDIOL [**17**].—Vertipyronediol was isolated from the crude EtOAc extract by charcoal chromatography (10–50% Me_2CO in H_2O) followed by Si gel flash chromatography (5% MeOH in CH_2Cl_2). Crystallization from Me_2CO/Skellysolve B gave compound **17** as colorless crystals (7 mg from RH50), mp 128–129°; $[\alpha]_D$ −50° (*c* = 0.20, $CHCl_3$); tlc R_f 0.50 [$CHCl_3$-MeOH (2:8)]; uv (95% EtOH) λ max nm (log ε) 279 (3.51); ir ($CHCl_3$, cast) ν max cm^{-1} 3390 (br), 3291, 1711 (br), 1638, 1546, 1347, 1157, 1064, 828; ^1H nmr ($CDCl_3$, 360 MHz) δ 7.65 (1H, s, H-6), 5.56 (1H, s, H-3), 3.98 (1H, d, *J* = 11.0 Hz, H-8), 3.90 (3H, s, OCH_3), 3.64 (1H, d, *J* = 11.0 Hz, H-8), 3.18 (1H, br, *OH*), 1.75 (1H, br, *OH*), 1.52 (3H, s, CH_3); ^{13}C nmr (CD_3OD, 75 MHz) δ 171.6 (s, C-4), 167.4 (s, C-2), 151.9 (d, C-6), 120.9 (s, C-5), 90.6 (d, C-3), 73.7 (s, C-7), 69.2 (t, C-8), 56.7 (q, OCH_3), 24.5 (q, CH_3); hrms *m/z* (rel. int. %) calcd for $C_9H_{12}O_5$ $[M]^+$ 200.0685, found 200.0684 (4), 169 (100), 151 (13), 127 (99), 99 (10); cims (NH_3) *m/z* (rel. int. %) $[M + 18]^+$ 218 (96), $[M + 1]^+$ 201 (100).

ACETYLATION OF VERTIPYRONEDIOL.—Vertipyronediol [**17**] (1.0 mg) was treated with Ac_2O in pyridine at room temperature for 12 h. Workup in the usual manner gave monoacetyl vertipyronediol, compound **18**, as colorless crystals (1.0 mg): $[\alpha]_D$ − 10.0° (*c* = 0.08, $CHCl_3$); ir ($CHCl_3$, cast) ν max cm^{-1} 3420 (br), 1739, 1716, 1642, 1545, 1225, 1043, 1001; ^1H nmr ($CDCl_3$, 400 MHz) δ 7.61 (1H, s, H-6), 5.58 (1H, s, H-3), 4.39 (1H, d, *J* = 11.5 Hz, H-8), 4.27 (1H, d, *J* = 11.5 Hz, H-8), 3.88 (3H, s, OCH_3), 3.01 (1H, br, *OH*), 2.06 (3H, s, $OCOCH_3$), 1.50 (3H, s, CH_3), 1.50 (3H, s, CH_3); hrms *m/z* (rel. int. %) calcd for $C_{11}H_{14}O_6$ $[M]^+$ 242.0790, found 242.0792 (7), 182 (3), 170 (9), 169 (100), 153 (6), 151 (7), 127 (52).

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