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MINOR CONSTITUENTS OF *GYMNOPILUS SPECTABILIS*

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ABSTRACT.—Three new metabolites have been isolated from the mushroom *Gymnopilus spectabilis*, namely, gymnopilene [**1**] ($C_{50}H_{96}O_8$), a novel polyisoprenol, 4,6-decadiyne-1,3,8,10-tetraol [**6**], and methyl 5-hydroxy-7-*p*-hydroxyphenyl-3-keto-4*Z*,6*E*-heptadienoate [**3**]. Their structures have been determined by spectral means.

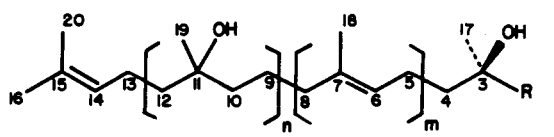
Japanese specimens of the hallucinogenic "big laughter" mushroom (ohwaraitake) *Gymnopilus spectabilis* (Fr.) Singer (Cortinariaceae) have been a rich source of unique metabolites (1–5). Study of the extracts of the fruiting bodies by Japanese workers has brought to light the presence of novel polyisoprenepolyols designated gymnoprenols (1) and gymnopilins (2) for the related esters. In addition, cerevisterol, ergosterol, ergosteryl peroxide, choline, 4,6-decadiyne-1,3,8-triol (6), α,α'-trehalose (7), psilocybin (8), bisnoryangonin [**8**], and hispidin [**10**] (9,10) have been isolated from this source.

In examining Canadian specimens of this fungus, we have isolated, in addition to most of the compounds named above, galactitol, together with three new metabolites **1**, **3**, and **6**, whose structures have been deduced by spectroscopic means.

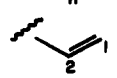
Compound **1**, which we designate gymnopilene, displays significant pseudomolecular ions at *m/z* 823 $[M-H]^-$, 977 $[M+\text{matrix}-H]^-$, and 1131 $[M+2\times\text{matrix}-H]^-$ in a negative fabms using "magic bullet" matrix (11). The negative fabms employing a glycerol matrix shows a molecular ion at *m/z* 824 $[M]^-$ ($C_{50}H_{96}O_8$). The structure of **1** was deduced on the basis of ^{1}H- and ^{13}C-nmr data (Table 1). The distinguishing feature of the ^{1}H spectrum is the presence of an ABX system in the downfield region typical for a vinyl group attached to a fully substituted carbon. A broad multiplet (2H) at δ 5.10 ppm is ascribed to H-6 and H-14. Comparison of the ^{13}C chemical shifts with those of gymnopilin A9 [**2**] (3) attests to the identity of the structural elements from C-3 to C-20 of gymnopilene [**1**]. The attachment of the vinyl moiety to C-3 completes the structure of this novel polyisoprenepolyol.

Confirmation of the location of a double bond at C-6 is provided by a selective INEPT (12) experiment in which irradiation of the allylic protons signal centered at δ 2.0 ppm produced significant enhancement of the signal for C-3 at δ 73.8 ppm as well as signals for C-6, C-7, C-11, C-14, and C-15. Thus we formulate gymnopilene as **1** in which the stereochemistry at the chiral centers is yet to be determined.

The yellow crystalline compound **3** shows a molecular ion at *m/z* 262 ($C_{14}H_{14}O_5$) in



1 n=7, m=1, R=



2 n=5, m=2, R=

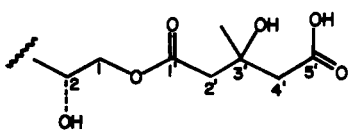
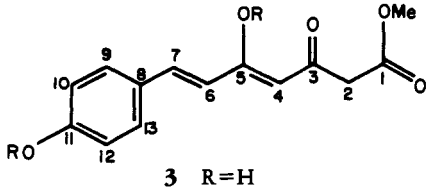


TABLE 1. ^{13}C-nmr Data for Gymnopilene [**1**] and Gymnopilin A9 [**2**] in CD_3OD.[a]

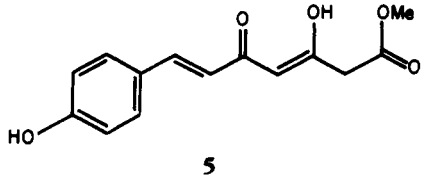
Carbon	Compound	
	1[a]	**2**[a]
C-1	112.0	67.1
C-2	146.3	75.7
C-3	73.8	74.4
C-4	42.7[b]	42.7[b]
C-5	23.2[c]	23.2[c], 27.8
C-6	125.8	125.8(×2)[d]
C-7	136.0	136.0(×2)
C-8	41.3[b]	40.3[b], 40.9[b]
C-9	19.4(×7)	19.4(×5)
C-10	43.4(×7)[b]	43.4(×5)[b]
C-11	73.4(×6), 73.8	73.5(×5)
C-12	43.4(×6)[b], 42.4[b]	43.4(×2)[b], 43.3[b], 42.2
C-13	23.7[c]	23.7[c]
C-14	125.8	125.5[d]
C-15	132.0	132.0
C-16	25.9	25.9
C-17	27.6	27.6
C-18	15.9	16.0, 16.1
C-19	27.0(×7)	27.0(×5)
C-20	17.8	17.8
C-1′		172.9
C-2′		46.8
C-3′		70.9
C-4′		46.8
C-5′		177.4
C-6′		27.0

[a]Chemical shifts in δ (ppm); assignments from DEPT, selective INEPT experiments, and comparison with reference data (3).

[b–d]Values in same column with the same superscript may be interchanged.



3 R=H
4 R=Ac



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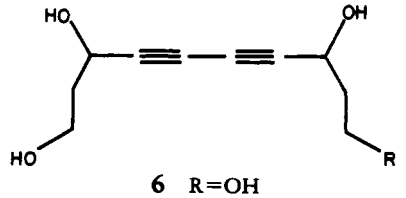
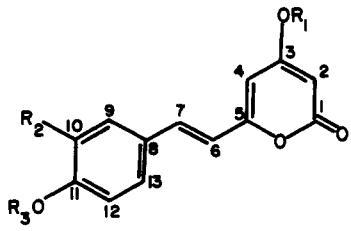


6 R=OH
7 R=H



8 R_1,R_2,R_3=H
9 R_1,R_2=H, R_3=Ac
10 R_1,R_3=H, R_2=OH
11 R_1=H, R_2=OAc, R_3=Ac

its eims. The base peak *m/z* 147 corresponds to the phenolic acylium ion formed by cleavage at the C-4–C-5 bond and is also observed with bisnoryangonin [**8**] (7). The presence of a para substituted phenol moiety was evident from ^{1}H and ^{13}C chemical shift data for the structurally related bisnoryangonin [**8**] and hispidin [**10**] and their respective acetates **9** and **11**. Compound **3** was converted into diacetate **4** which, after equilibration in CD_3OD, gave a molecular ion *m/z* 348 ($C_{18}H_{16}O_7D_2$). Thus, the presence of two hydroxyls in addition to two other exchangeable hydrogens in **3** is confirmed. The alternate formulation **5** is excluded for the following reasons: the ^{1}H-^{1}H COSY spectrum shows no evidence of allylic coupling between H-2 and H-4; the uv spectrum ($CHCl_3$) λ max 323 of **4** is consistent with the more extended conjugation in structure **3** but less so than in bisnoryangonin, whose uv spectrum shows λ max 364 (7); conversion to the diacetate **4** is accompanied by substantial changes in chemical shift for H-6 and H-7, indicating they are close to a site of acylation. Thus, compound **3** is methyl 5-hydroxy-7-*p*-hydroxyphenyl-3-keto-4*Z*,6*E*-heptadienoate and corresponds to the methyl ester of a lactone opened form of bisnoryangonin **8**.

The hreims of compound **6** shows a molecular ion at *m/z* 198.0891 ($C_{10}H_{14}O_4$) and significant ions at *m/z* 180 $[M-H_2O]^+$, 162 $[M-2H_2O]^+$, 153 $[M-CH_2CH_2OH]^+$, and 135 $[153-H_2O]^+$. The ^{13}C-nmr spectrum shows only five signals, indicating a symmetrical C-10 skeleton. Analysis of the ^{1}H and ^{13}C chemical shift data (Table 3) leads to structure **3**. Comparison of chemical shift data with that of the known 4,6-decadiyne-1,3,8-triol [**7**], which we also isolated from *G. spectabilis*, confirmed our structural conclusions for **6**, whose stereochemistry remains to be deduced.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on a Varian XL200 instrument using TMS as internal standard. Mass spectra were recorded with a Kratos MS50 instrument. Preparative tlc was performed on E. Merck precoated 20 × 20 cm glass plates of Si gel 60 F254 and RP-18 F254. Known compounds were identified by comparison of their spectra with those of authentic samples or with literature data.

PLANT MATERIAL.—Fresh fruiting bodies (310 g) of *G. spectabilis* were collected in Sunbury County, New Brunswick, Canada, in September 1989. The material was identified by N.J. Whitney, Department of Biology, University of New Brunswick, where a voucher specimen is maintained.

EXTRACTION AND ISOLATION.—The fresh mushrooms were extracted with cold MeOH (1000 ml × 3). The MeOH solution was concentrated at 30° under reduced pressure, and the residual extract was partitioned between $CHCl_3$ (500 ml × 3) and H_2O (500 ml) to give, after evaporation, the $CHCl_3$ residue (1.8 g). The aqueous layer was further extracted with EtOAc (500 ml × 3) to give an EtOAc residue (1.6 g). The aqueous layer was evaporated to dryness, and α,α′-trehalose (120 mg) was recovered from the residue by recrystallization from MeOH.

The $CHCl_3$ extract (1.8 g) was dissolved in $CHCl_3$, filtered, and chromatographed on a Si gel (200 g) column, eluting with $CHCl_3$→$CHCl_3$-MeOH (9:1). Fractions were collected and combined on the basis of

TABLE 2. Nmr Data for Compounds **3**, **4**, and **8–11**.[a]

Position	Compound										
	3		**4**		**8**		**9**	**10**		**11**	
	^{13}C $(CD_3)_2CO$	^1H (J Hz) $CDCl_3$	^1H pyridine-d_5	^1H $CDCl_3$	^{13}C CD_3OD	^1H CD_3OD	^1H CD_3OD	^{13}C CD_3OD	^1H (J Hz) CD_3OD	^1H $(CD_3)_2CO$	^1H (J Hz) CD_3OD
1	168.30				173.50			173.46			
2	46.79	3.45 s	3.59 s	3.65 s	[b]	[b]	[b]	[b]	[b]	5.38 d (2.0)	[b]
3	193.46				162.05			162.08			
4	116.63	5.70 s	6.48 s	6.30 s	116.93	6.12 s	6.18 s	116.87	6.09 s	6.19 d (2.0)	6.20
5	141.26				167.71			167.72			
6	101.13	7.57 d (15.9)	7.50 d	7.37 d	101.90	7.37 d	7.44 d	101.78	7.29 d	7.35 d	7.38 d
7	119.91	6.31 d (15.9)	6.84 d	6.61 d	136.91	6.65 d	6.80 d	137.33	6.57 d	6.91 d	6.83 d
8	127.55				128.25			128.82			
9	116.75	7.42 d (8.3)	7.66 d	7.55 d	116.80	7.45 d	7.62 d	114.82	7.02 d (2.0)	7.53 d	7.47 d
10	130.97	6.85 d (8.3)	7.30 d	7.15 d	130.39	6.80 d	7.13 d	146.81			
11	160.55				160.42			148.71			
12	130.97	6.85	7.30 d	7.15 d	130.39	6.80 d	7.13 d	116.55	6.76 (8.3)	7.25 d	7.23 d
13	116.75	7.42	7.66 d	7.55 d	116.80	7.45 d	7.62 d	121.95	6.93 (8.3,2.0)	7.55 d	7.49 dd
Ac			2.22 2.26	2.32 s 2.48 s			2.29			2.25 2.26	2.28 2.28
Me	52.32	3.77 s	3.83	3.92 s							

[a]Chemical shifts in δ (ppm).
[b]Signal not observed because H exchanged for D.

TABLE 3. ^{1}H- and ^{13}C-nmr Data for Compounds **6** and **7**.[a]

Position	Compound				
	6			**7**	
	^{13}C[b]	^{1}H[c]	^{1}H[b]	^{13}C[c]	^{1}H[c]
1	60.1	4.17	3.68	63.2	4.15
2	41.3	2.35	1.88	41.8	2.34
3	59.0	5.22	4.55	59.5	5.23
4	81.5			82.8	
5	69.0			68.5	
6	69.0			58.3	
7	81.5			82.9	
8	59.0	5.22	4.55	58.3	4.67
9	41.3	2.35	1.88	31.4	1.84
10	60.1	4.17	3.68	9.9	1.04
10H		6.21 t, J = 6 Hz			
30H		7.60 d, J = 5.6 Hz			

[a]Chemical shifts in δ (ppm).
[b]In CD_3OD.
[c]In pyridine-d_5.

tlc scrutiny to give 5 major fractions. Fraction 1 (180 mg) was recrystallized with MeOH, yielding crystalline ergosterol (20 mg). The mother liquor was subjected to preparative tlc (0.5 mm, 20 mg/plate) yielding ergosterol peroxide (2 mg). Fraction 2 (120 mg) was rechromatographed on a Si gel column (20 g), eluting with $CHCl_3$-MeOH (19:1), yielding pure palmitic acid (ca. 10 mg). Fraction 3 (250 mg) was repeatedly chromatographed on a Si gel column, yielding a minor uv-active compound **3** (5 mg) and bisnoryangonin [**8**] (70 mg). Fraction 4 (260 mg), which contained many gymnoprenols, was subjected to repeated cc on Si gel, using $CHCl_3$-MeOH (9:1) as solvent, to yield compound **1** (40 mg), which was finally purified by reversed-phase preparative tlc using MeOH-H_2O (9:1) as solvent. Fraction 5 (200 mg) gave a mixture of gymnopilins.

The EtOAc extract (1.6 g) was chromatographed on a Si gel (200 g) column eluting with $CHCl_3$-MeOH-H_2O (10:3:0.5) to give 9 fractions. Fraction 2 (160 mg) was recrystallized with MeOH yielding bisnoryangonin [**8**] (30 mg), and the filtrate was separated on reversed-phase preparative tlc [MeOH-H_2O (7:3)], yielding 4,6-decadiyne-1,3,8-triol [**7**] (10 mg). Fraction 4 (148 mg) was rechromatographed on a Si gel column followed by further purification on reversed-phase preparative tlc [MeOH-H_2O (7:3)] yielding a minor compound **6** (ca. 10 mg). Hispidin (5 mg) was obtained from fraction 5. Fractions 6–9 contained many gymnoprenols and gymnopilins; pure gymnopilin A9 was obtained from fraction 7 after repeated chromatography on Si gel columns and recrystallization from MeOH. Fraction 9 also gave pure galactitol as a colorless powder after repeated chromatography on Si gel and recrystallization from MeOH.

GYMNOPILENE [**1**].—Colorless crystals: mp 106–109°; negative fabms (glycerol) *m/z* 824 (8), 786 (28), 657 (21), 639 (100), 625 (20), 493 (26), 457 (13), 127 (36), 89 (45), 71 (46), 59 (73), 41 (58); negative fabms (magic bullet) *m/z* $[M + 2 \times \text{matrix} - H]^-$ 1131 (1), $[M + \text{matrix} - H]^-$ 977 (3), $[M - H]^-$ 823 (1), $[\text{matrix} - H]^-$ 153 (100); ^{1}H nmr (CD_3OD) δ (ppm) 5.91 (1H, dd, $J_{1a,2}$ = 17.4 Hz, $J_{1b,2}$ = 10.7 Hz, H-2), 5.20 (1H, dd, $J_{2,1a}$ = 17.4 Hz, $J_{1b,1a}$ = 1.7 Hz, H-1a), 5.10 (2H, m, H-6, H-14), 5.02 (1H, dd, $J_{2,1b}$ = 10.7 Hz, $J_{1a,1b}$ = 1.7 Hz, H-1b), 2.00 (6H, m, H-5, H-8, H-13), 1.67, 1.62, 1.60 (3H × 3, 3s, H-16, H-18, H-20), 1.55–1.27 (44H, br s centered at δ 1.42, H-4, H-9 × 7, H-10 × 7, H-12 × 7), 1.24 (3H, s, H-17), 1.15 (21H, br s, H-19 × 7); ^{13}C nmr see Table 1.

METHYL 5-HYDROXY-7-*p*-HYDROXYPHENYL-3-KETO-4*Z*,6*E*-HEPTADIENOATE [**3**].—Yellow prisms: mp 174–177°; eims *m/z* $[M]^+$ 262 (11), 213 (26), 200 (41), 185 (32), 171 (50), 157 (48), 147 (100), 129 (95); ^{1}H nmr and ^{13}C nmr see Table 2.

COMPOUND **4**.—Compound **3** (4 mg) in pyridine (2 ml) and Ac_2O (1 ml) was warmed at 50° for 2 h, and the mixture was poured into cold H_2O and extracted with $CHCl_3$ (50 ml). Compound **4** was obtained from the $CHCl_3$ extract after preparative tlc [$CHCl_3$-MeOH (19:1)]: yellow amorphous solid; eims *m/z* $[M + 2]^+$ 348 (2), 322 (3), 308 (3), 296 (5), 204 (10), 162 (39), 147 (54), 122 (88), 121 (100); uv λ max ($CHCl_3$) 323 (ε 25,000); ^{1}H nmr see Table 2.

4,6-DECADIYNE-1,3,8,10-TETRAOL [**6**].—Colorless oil: hreims 198.0891 (calcd for $C_{10}H_{14}O_4$, 198.0892), eims *m/z* $[M]^+$ 198 (8), 180 (15), 162 (50), 153 (38), 135 (69), 121 (39), 105 (100), 95 (43), 91 (50), 79 (67), 77 (83), 73 (61), 69 (30), 55 (37), 51 (33), 43 (62), 31 (34); ir (KBr) ν max cm^{-1} 3400 (OH), 630 (C≡C); ^1H nmr and ^{13}C nmr see Table 3.

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