Structures of New Marasmane Sesquiterpenoids from *Lactarius piperatus* (Scop.: Fr.) S. F. Gray[1)]

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Received January 11, 1999; accepted March 26, 1999

Four new marasmane sesuquiterpenoids, lactapiperanols A—D (1—4), were isolated from the fruit bodies of *Lactarius piperatus* (Scop.: Fr.) S. F. Gray (Russulaceae) along with two known compounds. The structures of the new compounds were elucidated on the basis of spectroscopic evidence.

Key words *Lactarius piperatus*; Russulaceae; marasmane sesuquiterpenoid

Recently we reported the isolation and structural elucidation of sterols from six edible mushrooms.[1,2)] In a continuation of our investigation of the chemical constituents of mushrooms, we describe here the isolation and the structural elucidation of four new marasmane-type sesuquiterpenoids, lactapiperanols A—D (**1**—**4**), as well as two known compounds, lactarorufin A (**5**)[3)] and furosardonin A (**6**)[4)] from the fruit bodies of *Lactarius piperatus* (Scop.: Fr.) S. F. Gray (tsuchikaburi in Japanese, Russulaceae). This is the first report of the isolation of **5** and **6** from *L. piperatus*. Extraction and isolation were carried out as described in the Experimental section.

Compound **1** was isolated as an amorphous powder, $[\alpha]_D$ +26.3°. The molecular formula was determined to be $C_{16}H_{26}O_4$ by high-resolution (HR)-MS. The IR spectrum showed the presence of hydroxyl groups (3569 cm^{-1}). The ^1H- (Table 1) and ^{13}C-NMR (Table 2) spectra showed signals due to three tertiary methyl groups [δ_H 1.01 (3H, H_3-14), 1.07 (3H, H_3-12), 1.11 (3H, H_3-15); δ_C 21.2 (C-12), 31.8 (C-14), 32.3 (C-15)], a cyclopropane ring [δ_H 0.56 (1H, H-4α), 0.85 (1H, H-4β); δ_C 17.6 (C-4), 22.4 (C-3), 42.0 (C-6)], two methylenes [δ_H 1.44 (1H, H-1α), 1.54 (1H, H-10β), 1.58 (1H, H-1β), 1.74 (1H, H-10α); δ_C 42.2 (C-10), 45.1 (C-1)], two methines [δ_H 1.77 (1H, H-9), 2.54 (1H, H-2); δ_C 38.7 (C-9), 45.2 (C-2)], an oxygenated methine [δ_H 3.18 (1H, H-8); δ_C 73.2 (C-8)], a methoxyl group [δ_H 3.36 (3H); δ_C 54.6], an oxygenated methylene [δ_H 3.95 (1H, H-13β), 4.27 (1H, H-13α); δ_C 77.7 (C-13)], an oxygenated quaternary carbon [δ_C 77.6 (C-7)] and an acetal group [δ_H 4.63 (1H, H-5); δ_C 106.4 (C-5)]. Four degrees of unsaturation derived from the molecular formula suggested that **1** was a tetracyclic compound. Detailed analysis of the ^1H–^1H shift correlation spectroscopy (^1H–^1H COSY) spectrum of **1** implied connectivities for H_2-1–H-2, H-2–H-9, H-8–H-9 and H-9–H_2-10 (Fig. 1). In the ^1H-detected heteronuclear multiple bond connectivity (HMBC) spectrum, the C–H long-range correlations observed are shown in Fig. 1. These spectral data suggested that **1** was a marasmane sesquiterpenoid derivative.[5)] The stereostructure was determined with a nuclear Overhauser effect correlation spectroscopy (NOESY) spectrum in pyridine-d_5 (Fig. 2). The NOESY cross peaks observed between H-4β and H-2; H-4β and H-9; and H_3-14 and H-2 implied a *cis*-junction for the A/B rings, and that the cyclopropane ring, H-2, H-9 and H_3-14 methyl group occurred on the same face (β) on the ring system. The NOESY cross peak observed between H_3-12 and H-5α suggested that the methoxyl group at C-5 had β configuration. The coupling pattern and the constants for H-8 (dd, J=11.7, 9.3 Hz) suggested that the hydroxyl group at C-8 had β configuration, which was supported by the NOESY cross peak between H-

Table 1. ^1H-NMR Chemical Shifts of Compounds **1**—**4** (600 MHz, $CDCl_3$)

Hydrogen	**1**[a)]	**2**	**3**	**4**
1α	1.44 dd (13.2, 12.7)	1.56 dd (13.2, 12.8)		
1β	1.58 dd (12.7, 6.8)	1.61 dd (12.8, 7.0)	1.57 dd (12.5, 7.0)	1.60 dd (12.5, 6.6)
2	2.54 ddd (13.2, 6.8, 6.6)	2.61 ddd (13.2, 7.0, 6.6)	2.50 ddd (13.2, 7.0, 6.6)	2.57 ddd (12.8, 7.0, 6.6)
4α	0.56 d (5.1)	0.61 d (5.1)	0.61 d (4.8)	0.65 d (4.8)
4β	0.85 d (5.1)	0.94 d (5.1)	0.93 d (4.8)	1.03 d (4.8)
5	4.63 s	4.64 s	4.73 s	4.72 s
8	3.18 dd (11.7, 9.3)	4.84 d (12.5)	3.29 dd (11.7, 8.4)	4.84 d (12.1)
9	1.77 m	2.10 m	1.78 m	2.09 m
10α	1.74 dd (14.1, 1.7)	1.21 dd (13.9, 1.5)	1.70 dd (13.9, 1.5)	1.24 dd (13.9, 1.5)
10β	1.54 dd (14.1, 7.8)	1.49 dd (13.9, 7.7)		1.50 dd (13.9, 7.7)
12	1.07 s	1.07 s	1.06 s	1.07 s
13α	4.27 d (9.5)	4.01 d (9.5)	4.11 d (9.5)	4.08 d (9.5)
13β	3.95 d (9.5)	3.90 d (9.5)	4.05 d (9.5)	3.85 d (9.5)
14	1.01 s	1.00 s	1.02 s	1.00 s
15	1.11 s	1.08 s	1.12 s	1.08 s
OCH_3	3.36 s	3.36 s	3.29 s	3.27 s
8-OH	1.81 d (9.3)		1.76 d (8.4)	
$COCH_3$		2.13 s		2.12 s

Coupling constants (J in Hz) are given in parentheses. *a*) Measured at 400 MHz.

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Table 2. ^{13}C-NMR Chemical Shifts of Compounds **1**—**4** (150 MHz, $CDCl_3$)

Carbon	**1**[a]	**2**	**3**	**4**
1	45.1	44.9	44.9	44.7
2	45.2	45.2	45.8	45.9
3	22.4	22.2	23.7	23.7
4	17.6	17.7	20.6	20.6
5	106.4	106.0	109.9	109.5
6	42.0	42.1[b]	42.2	42.5
7	77.6	77.4	78.1	77.9
8	73.2	75.1	73.9	75.7
9	38.7	36.2	40.1	37.4
10	42.2	42.1[b]	41.9	41.8
11	37.1	36.7	37.1	36.7
12	21.2	21.1	23.0	22.9
13	77.7	76.9	79.0	78.3
14	31.8	31.5	31.9	31.8
15	32.3	31.8	32.3	32.0
OCH_3	54.6	54.7	54.6	54.4
$CO\underline{C}H_3$		21.0		21.0
$\underline{C}OCH_3$		170.9		170.6

a) Measured at 100 MHz. *b*) Signals overlapped.

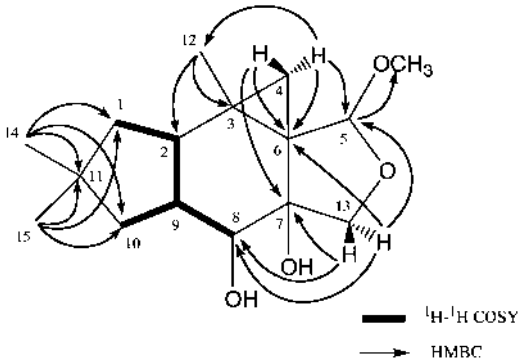

Fig. 1. ^{1}H–^{1}H COSY and HMBC Correlations for **1**

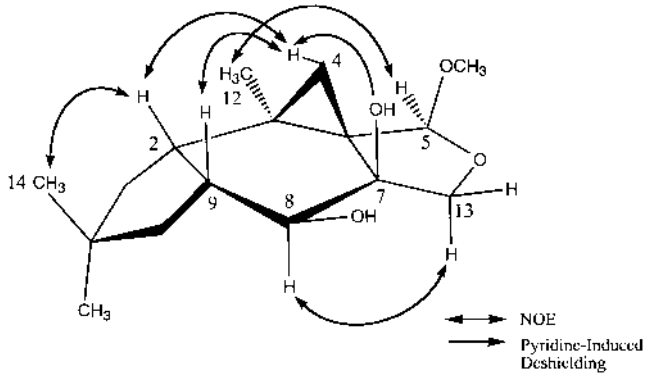

Fig. 2. NOEs and Pyridine-Induced Deshielding for **1**

13α and H-8α. In the ^{1}H-NMR spectrum, the chemical shift of the H-4β in pyridine-d_5 was shifted downfield by the pyridine-induced deshielding effect[6] ($\delta_{C_5D_5N}-\delta_{CDCl_3}$; $\Delta\delta$, H-4β, +0.42 ppm). This deshielding effect indicated that the hydroxyl group at C-7 had β configuration (Fig. 2). On the basis of the above data, the structure of lactapiperanol A (**1**) was determined to be as shown in Chart 1.

Compound **2** was isolated as a colorless oil, $[\alpha]_D$ +33.3°. The molecular formula was determined to be $C_{18}H_{28}O_5$ by HR-MS. The IR spectrum showed the presence of hydroxyl

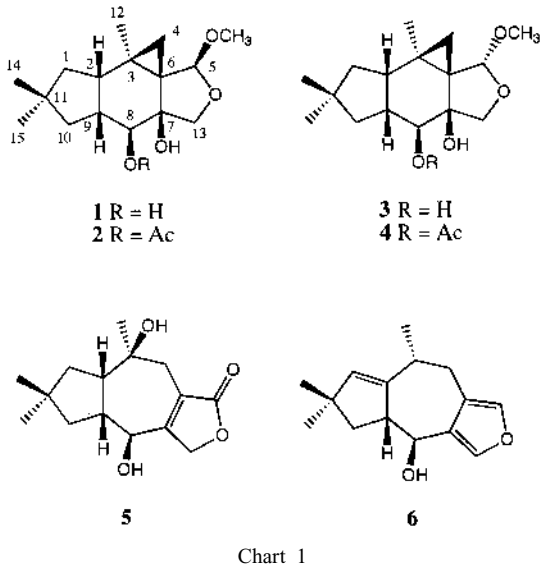

Chart 1

group (3588 cm^{-1}) and acetyl group (1731 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectral data of **2** were closely related to those of **1**, except for the presence of an acetyl group [δ_H 2.13 (3H); δ_C 21.0, 170.9]. The ^{1}H- and ^{13}C-NMR chemical shifts at C-8 position of **2** were shifted downfield by +1.66 ppm (H-8) and +1.9 ppm (C-8) compared with those of **1**, suggesting that the acetyl group is located at the C-8 hydroxyl group. This finding was supported by HMBC correlation from H-8 (δ 4.84) to the carbonyl carbon in the acetyl group (δ 170.9). The coupling pattern and the constants for H-8 (d, J=12.5 Hz) suggested that the acetoxyl group at C-8 had β configuration. From the above data, the structure of lactapiperanol B (**2**) was determined to be as shown in Chart 1.

Compound **3** was isolated as an amorphous powder, $[\alpha]_D$ −52.6°. The molecular formula was determined to be $C_{16}H_{26}O_4$ by HR-MS. The IR spectrum showed the presence of hydroxyl groups (3565 cm^{-1}). Comparison of the ^{1}H- and ^{13}C-NMR spectral data with those of **1** revealed that they were identical except at C-5. In the NOESY spectrum, cross peaks were observed between the H_3-12 methyl group and the methoxyl group; and H-4α and H-5β, and the configuration of the methoxyl group at C-5 was determined to be α. Thus, **3** was the 5-epimer of **1**. From the above data, the structure of lactapiperanol C (**3**) was determined to be as shown in Chart 1.

Compound **4** was isolated as a colorless oil, $[\alpha]_D$ −105.3°. The molecular formula was determined to be $C_{18}H_{28}O_5$ by HR-MS. The IR spectrum showed the presence of hydroxyl group (3587 cm^{-1}) and acetyl group (1732 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **4** were very similar to those of **3**, except for the presence of an acetyl group [δ_H 2.12 (3H); δ_C 21.0, 170.6]. The ^{1}H- and ^{13}C-NMR chemical shifts at C-8 of **4** were shifted downfield by +1.55 ppm (H-8) and +1.8 ppm (C-8) compared with those of **3**, suggesting that the acetyl group is located at the C-8 hydroxyl group, which was supported by HMBC correlation from H-8 (δ 4.84) to a carbonyl carbon in the acetyl group (δ 170.6). The coupling pattern and constants for H-8 (d, J=12.1 Hz) suggested that the acetoxyl group at C-8 was β configuration. From the above data, the structure of lactapiperanol D (**4**) was determined to be as shown in Chart 1.

Although the absolute structures of compounds **1**—**4** have not been determined, it is probably the same as compounds **5** and **6** from a biogenetic point of view.

Experimental

General Procedures Optical rotations were determined with JASCO DIP-360 digital polarimeter. IR spectra were recorded with a Perkin-Elmer FT-IR 1725X IR spectrophotometer and UV spectra with a Beckman DU-64 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded with JEOL JNM-LA 600 (600 and 150 MHz, respectively) and JEOL JNM-LA 400 (400 and 100 MHz, respectively) spectrometers. Chemical shifts are given on a δ (ppm) scale with tetramethyl silane as an internal standard (s, singlet; d, doublet; dd, double doublet; ddd, double double doublet; m, multiplet). The HR-MS was recorded on a JEOL JMS-DX 303 mass spectrometer. Column chromatography was carried out on Kieselgel 60 (Merck; 230—400 mesh). Preparative HPLC was carried out on a Tosoh HPLC system (pump, CCPD; detector, RI-8010) using a TSK gel ODS-120T (7.8 mm i.d.×30 cm) column (Tosoh).

Material The fresh fruit bodies of *Lactarius piperatus* were collected at Sendai City in Miyagi Prefecture, Japan, in July 1997.

Extraction and Isolation The fresh fruit bodies of *L. piperatus* (120 g) were extracted three times with Et_2O at room temperature for 2 weeks. The Et_2O extract (1.7 g) was chromatographed on a silica-gel column using *n*-hexane–AcOEt (7 : 3—1 : 7), AcOEt and MeOH, to afford 24 fractions (frs. 1—24) . Fraction 4 was purified by preparative HPLC [MeOH–H_2O (4 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give **6** (0.5 mg). Fraction 9 was purified by preparative HPLC [MeOH–H_2O (4 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give a mixture of **1** and **3**, **2** (0.6 mg) and **4** (0.4 mg). The mixture of **1** and **3** was purified by preparative HPLC [MeOH–H_2O (3 : 1); flow rate, 1.0 ml/min; column temperature, 40 °C] to give **1** (0.4 mg) and **3** (0.4 mg). Fraction 12 was purified by preparative HPLC [MeOH, flow rate, 1.0 ml/min; column temperature, 40 °C] to give **5** (0.7 mg).

Known compounds (**5**, **6**) were identified by comparison of their physical data with reported values.

Lactapiperanol A (**1**): Amorphous powder. $[\alpha]_D^{21}$ +26.3° (c=0.04, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3569. HR-MS *m/z*: 282.1837 (M^+, Calcd for $C_{16}H_{26}O_4$: 282.1831). ^{1}H-NMR (400 MHz, $CDCl_3$): see Table 1. (400 MHz, C_5D_5N) δ: 0.76 (1H, d, J=4.9 Hz, H-4α), 0.97 (3H, s, H_3-14), 1.07 (3H, s, H_3-12), 1.10 (3H, s, H_3-15), 1.27 (1H, d, J=4.9 Hz, H-4β), 1.62 (1H, dd, J=13.8, 7.8 Hz, H-10β), 2.15 (1H, dd, J=13.8, 2.0 Hz, H-10α), 2.27 (1H, m, H-9), 2.57 (1H, m, H-2), 3.39 (3H, s, OCH_3), 3.55 (1H, dd, J=10.9, 7.9 Hz, H-8), 4.42 (1H, d, J=8.9 Hz, H-13β), 4.72 (1H, d, J=8.9 Hz, H-13α), 4.93 (1H, s, H-5). ^{13}C- NMR (100 MHz, $CDCl_3$): see Table 2.

Lactapiperanol B (**2**): Colorless oil. $[\alpha]_D^{20}$ +33.3° (c=0.06, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3588, 1731. HR-MS *m/z*: 324.1949 (M^+, Calcd for $C_{18}H_{28}O_5$: 324.1937). ^{1}H-NMR (600 MHz, $CDCl_3$): see Table 1. ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 2.

Lactapiperanol C (**3**): Amorphous powder. $[\alpha]_D^{22}$ −52.6° (c=0.04, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3565. HR-MS *m/z*: 282.1840 (M^+, Calcd for $C_{16}H_{26}O_4$: 282.1831). ^{1}H-NMR (600 MHz, $CDCl_3$): see Table 1. ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 2.

Lactapiperanol D (**4**): Colorless oil. $[\alpha]_D^{21}$ −105.3° (c=0.04, $CHCl_3$). IR ν_{max} ($CHCl_3$) cm^{-1}: 3587, 1732. HR-MS *m/z*: 324.1945 (M^+, Calcd for $C_{18}H_{28}O_5$: 324.1937). ^{1}H-NMR (600 MHz, $CDCl_3$): see Table 1. ^{13}C-NMR (150 MHz, $CDCl_3$): see Table 2.

Acknowledgments We are grateful to Mrs. S. Sato and T. Matsuki of this university for measurement of the mass and NMR spectra.

References and Notes

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