Ceramide Constituents from Five Mushrooms

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Five mushrooms, *Panellus serotinus*, *Lyophyllum conatum*, *Amanita pantherina*, *Sarodon aspratus* and *Lepista nuda*, have been investigated chemically. Two new ceramides, (2S,3R,4E,8E)-N-hexadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (1) and (2S,3R,4E,8E,9Z,12Z)-N-9',12'-octadecadienoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (2), have been isolated from *Panellus serotinus*. Compound 2 was also isolated from *Lyophyllum conatum*. Two new ceramides, (2S,2'R,3R,4E,8E)-N-2'-hydroxytetradecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (4) and (2S,2'R,3R,4E,8E)-N-2'-hydroxytetradecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (5), have been isolated from *Amanita pantherina* with (2S,2'R,3R,4E,8E)-N-2'-hydroxyhexadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (3), a known synthetic compound. Compounds 3 and 4 were also isolated from *Sarodon aspratus* and compound 3 was isolated from *Lepista nuda*. The structures of the new compounds were elucidated on the basis of their spectral data.

Key words ceramide; mushroom; structural elucidation; *Amanita pantherina*; *Panellus serotinus*

Recently we reported the isolation and structural elucidation of sterols,1 sesquiterpenoids,2 triterpenoids3 and ceramides4 from seventeen mushrooms. In a continuation of our investigation of chemical constituents from mushrooms, we describe here the isolation and structural elucidation of four new sphingosine-type ceramides, (2S,3R,4E,8E)-N-hexadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (1), (2S,3R,4E,8E,9Z,12Z)-N-9',12'-octadecadienoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (2), (2S,2'R,3R,4E,8E)-N-2'-hydroxytetradecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (4) and (2S,2'R,3R,4E,8E)-N-2'-hydroxyhexadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (5), a known synthetic compound.5 Five mushrooms, *Panellus serotinus* (Pers.: Fr.) Kühn. (Mukitake in Japanese, Tricholomataceae, compound 1), *Lyophyllum conatum* (Schum.: Fr.) Sing. (Oshiroishimeji in Japanese, Tricholomataceae, compound 2), *Amanita pantherina* (DC.: Fr.) Krombh. (Tengutake in Japanese, Amanitaceae, compounds 3, 4 and 5), *Sarodon aspratus* (Berk.) S. Ito (Kotake in Japanese, Thelephoraceae, compound 3) and *Lepista nuda* (Bull.: Fr.) Coke (Murasakishimeji in Japanese, Tricholomataceae, compound 3).

Compound 1 was isolated as an amorphous powder. The molecular formula was determined to be C_{35}H_{67}NO_{3} by high-resolution (HR)-electron ionization (EI)-MS. The IR spectrum showed absorption bands at 3605 cm^{-1} (hydroxyl), 3434, 1657, 1510 cm^{-1} (amide), 2928, 2855 and 1466 cm^{-1} (aliphatic), suggesting it to be a fatty acid amide. The 1H-NMR spectrum (vide Experimental) showed signals due to one hydroxyl group at δ_{H} 5.56 (1H, H-4), 5.80 (1H, H-5) and an amide proton at δ_{H} 3.96 (1H, H-8). The IR spectrum (vide Experimental) showed characteristic signals appearing to be due to an amide carbonyl at δ_{C} 173.9 and a methine carbon linked to amide nitrogen at δ_{C} 54.4.7 These spectral data and molecular formula suggest that compound 1 is a ceramide. Detailed analysis of the ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) spectrum of 1 implied connectivities for H-1 to H-2; H-7 to H-8; H-10 to H-11; H-2 to an amide proton; and H-2’ to H-3’. The lengths of the long chain base and the fatty acid were determined by EI-MS, which showed significant fragment ion peaks at m/z 298 (a) and 281 (b) (Fig. 2). Thus, the long chain base and fatty acid of 1 must be 2-amino-9-methyl-4,8-octadecadiene-1,3-diol and hexadecanoic acid, respectively. The geometry of the double bond at C-4 was deduced to be E from the ^{1}H–^{1}H coupling constant (J=15.8 Hz) between H-4 and H-5. The chemical shift value of the olefinic methyl group at C-9 (δ_{C} 16.0) suggests that the double bond at C-8 is E geometry.9 The same conclusion was derived from the nuclear Overhauser enhancement spectroscopy (NOESY) cross peak observed between H-8 and H-10. The relative stereochemistry at C-2 and C-3 was determined to be erythro, since the 1H-NMR data of 1 was in good agreement with that of (2S,3R,4E,8E)-N-hexadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (6)5 (Table 1). The absolute stereochemistry at C-2 and C-3 was determined to have the 2S, 3R configuration by comparing the optical rotation values of 1 ([α]_{D} -11.6)^{9} and 6 ([α]_{D} -8.0)^{9} On the basis of this evidence, the structure of 1 was determined to be (2S,3R,4E,8E)-N-hexadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol.

Compound 2 was isolated as an amorphous powder. The molecular formula was determined to be C_{35}H_{65}NO_{3} by HR-EI-MS. The ^{1}H- and ^{13}C-NMR spectra of 2 closely resembled those of 1 except for the integration of the aliphatic methyl groups at δ_{H} 1.26–1.32 (26H) and the presence of two additional double bonds [δ_{C} 5.33 (2H, H-10', H-12'), 5.39 (2H, H-9', H-13')]. The 1H–^{1}H COSY spectrum implied connectivities for H-2’ and H-3’ and H-2’ to H-3’. The HMBC spectrum revealed...
correlations from H-11' to C-9', C-10', C-12' and C-13'. Therefore, two double bonds were separated by a methylene. The lengths of the long chain base and the fatty acid were determined by EI-MS, which showed significant fragment ion peaks at m/z 322 (a) and 305 (b) (Fig. 2), indicating that the long chain base was the same as that of 1, and the fatty acid must be octadecadienoic acid. The position of the double bonds of the fatty acid moiety was determined as follows. The EI-MS gave a fragment ion peak at m/z 444 (M+ – C4H15–H2O), resulting from cleavage of the C-10’—C-11’ bond and concomitant H2O loss. In the 13C-NMR spectrum, the signal for C-16 appeared at δC 25.6, 0.4 ppm higher than that of (4E,8E)-N-2'-hydroxyoctadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (7). This is due to the γ-effect of the double bond between C-12' and C-13'. Thus, two double bonds must be between C-9'—C-10' and C-12’—C-13’, respectively. Both of the double bonds were in the Z geometry, according to the chemical shifts of the aliphatic methylenes. The optical rotation values of 2 ([α]D 27.5°) and 1 ([α]D 27.0°) suggested that 2 has the same absolute configuration as that of 1 for the C-2 and C-3 parts. Therefore, the structure of 2 was determined to be (2S,3R,4E,8E,9Z12Z)-N-9'-12'-octadecadienoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol.

Compound 3 was isolated as an amorphous powder, [α]D 27.5°. The molecular formula was determined to be C35H67NO4 by HR-EI-MS. The 1H- and 13C-NMR spectra, and the optical rotation value of 3 were in accord with those of (2S,3R,4E,8E,9Z12Z)-N-9',12'-octadecadienoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol. Thus, compound 3 was as shown in Chart 1. Compound 3 was isolated from a natural source for the first time, although 3 has already been synthesized by Mori and Funaki.6)

Compound 4 was isolated as an amorphous powder. The molecular formula was determined to be C34H65NO4 by HR-EI-MS. The 1H-NMR spectrum was virtually identical with that of 3 except for the integration of the aliphatic methylene protons at δH 1.25—1.33 (32H). The EI-MS gave fragment ion peaks at m/z 300 (a) and 283 (b), indicating that the long chain base was the same as that of 3, and the fatty acid must be 2-hydroxypentadecanoic acid. The optical rotation values of 4 ([α]D 27.0°) and 3 ([α]D 27.0°) suggested that 4 has the same absolute configuration as that of 3 for the C-2, C-3 and C-2' parts. Accordingly, the structure of 4 was determined to be (2S,3R,4E,8E,9Z12Z)-N-2'-hydroxyhexadecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol.

Compound 5 was isolated as an amorphous powder. The molecular formula was determined to be C33H63NO4 by HR-
El-MS. The 1H-NMR spectrum was virtually identical with that of 3 except for the integration of the aliphatic methylene protons at δH 1.25—1.33 (30H). The El-MS gave fragment ion peaks at m/z 286 (a) and 269 (b), indicating that the long chain base was the same as that of 3, and the fatty acid must be 2-hydroxytetradecan-1-yl. The optical rotation values of 5 ([α]D +6.3°) and 3 ([α]D +7.5°) suggested that 5 has the same absolute configuration as that of 3 for the C-2, C-3 and C-2′ parts. Based on this evidence, the structure of 5 is the ceramide part of the glycosphingolipid

Amorphous powder. [ε]D −11.6° (c0.09, CHCl3). IR (CHCl3) cm−1: 3605, 3434, 2928, 2855, 1657, 1510, 1466. HR-El-MS m/z: 549.5113 (M+H, Calcd for C35H66NO3: 549.5114). El-MS m/z (rel. int. %): 179 (M−2, 39%), 176 (M−4, 26), 153 (M−16, 24), 151 (M−18, 29), 145 (M−22, 29), 105 (M−68, 27). EI-MS m/z: 179 (M−2, 39%), 176 (M−4, 26), 153 (M−16, 24), 151 (M−18, 29), 145 (M−22, 29), 105 (M−68, 27).

Experimental

General Procedures Optic rotations were determined using a JASCO DIP-360 digital polarimeter. IR spectra were recorded with a Perkin-Elmer FT-IR 1725X IR spectrophotometer. 1H and 13C-NMR spectra were recorded using a JEOL JNM-A 600 (600 and 150 MHz, respectively) spectrometer. Chemical shifts are given on a δ (ppm) scale, with tetramethylsilane as an internal standard (s, singlet; d, doublet; dd, double doublet; t, triplet; dt, double triplet; br, broad; m, multiplet). The El-, FAB- and HR-El-MS were 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, CCPS; detector, RI-8020) using a TSK gel ODS-120T (7.8 mm i.d.×30 cm) column (Tosoh). HPLC conditions: mobile phase, MeOH; flow rate, 1.0 ml/min; column temperature, 40°C.

Material Panellus serotinus (from Morikita City in Iwate Prefecture, Japan), Lycophyllum connectum (from Morikita City in Iwate Prefecture, Japan), Cortinarius rubromarginatus (from Morikita City in Iwate Prefecture, Japan), and Leptisia nuda (from Morikita City in Iwate Prefecture, Japan) were purchased in a food market. The fresh fruit bodies of Amanita pantherina were collected at Sendai City in Miyagi Prefecture, Japan, in September 1997.

Extraction and Isolation P. serotinus: The fresh fruit bodies of P. serotinus (1.1 kg) were extracted three times with Et2O at room temperature for 2 weeks. The Et2O extract (1.4 g) was chromatographed on a silica gel column (7:3—1:7), EtOAc and MeOH, to afford 20 fractions (frs. 1—20). Fraction 17 was purified by preparative HPLC to give 3 (1.9 mg) and 4 (2.0 mg).

L. connatum: The fresh fruit bodies of L. connatum (0.9 kg) were extracted four times with Et2O at room temperature for 2 weeks. The Et2O extract (1.9 g) was chromatographed on a silica gel column using hexane–EtOAc (7:3—1:7), EtOAc and MeOH, to afford 33 fractions (frs. 1—33). Fraction 17 was purified by preparative HPLC to give 2 (0.8 mg).

A. pantherina: The fresh fruit bodies of A. pantherina (0.6 kg) were extracted three times with Et2O at room temperature for 2 weeks. The Et2O extract (7.3 g) was chromatographed on a silica gel column using hexane–EtOAc (7:3—1:7), EtOAc and MeOH, to afford 21 fractions (frs. 1—21). Fraction 16 was purified by preparative HPLC to give 3 (0.9 mg) and 4 (1.1 mg).

S. asperatus: The fresh fruit bodies of S. asperatus (1.1 kg) were extracted three times with Et2O at room temperature for 2 weeks. The Et2O extract (3.7 g) was chromatographed on a silica gel column using hexane–EtOAc (7:3—1:7), EtOAc and MeOH, to afford 20 fractions (frs. 1—20). Fraction 17 was purified by preparative HPLC to give 3 (1.9 mg) and 4 (1.8 mg).

L. nuda: The fresh fruit bodies of L. nuda (0.3 kg) were extracted five times with Et2O at room temperature for 2 weeks. The Et2O extract (1.4 g) was chromatographed on a silica gel column using hexane–EtOAc (7:3—1:7), EtOAc and MeOH, to afford 29 fractions (frs. 1—29). Fraction 25 was purified by preparative HPLC to give 3 (1.0 mg).

(2S, 2R, 3R, 4E, 8E)-N-Hexadecanoyl-2-amino-9-methyl-4,8-octadecadien-1,3-diol (1): Amorphous powder. [ε]D −11.6° (c0.09, CHCl3). IR (CHCl3) cm−1: 3605, 3434, 2928, 2855, 1657, 1510, 1466. HR-El-MS m/z: 549.5113 (M+, Calcd for C35H66NO3; 549.5121). El-MS m/z (rel. int. %): 179 (M−2, 39%), 176 (M−4, 26), 153 (M−16, 24), 151 (M−18, 29), 145 (M−22, 29), 105 (M−68, 27). EI-MS m/z: 179 (M−2, 39%), 176 (M−4, 26), 153 (M−16, 24), 151 (M−18, 29), 145 (M−22, 29), 105 (M−68, 27).
(2S,2'R,3R,4E,8E)-N-2'-Hydroxytetradecanoyl-2-amino-9-methyl-4,8-octadecadiene-1,3-diol (5): Amorphous powder. $[\alpha]_D^{20} +6.3^\circ$ (c=0.2, CHCl$_3$).

IR (CHCl$_3$) cm$^{-1}$: 3604, 3401, 2928, 2855, 1657, 1526, 1467. HR-EI-MS $m/z$: 537.4728 (M$^+$, Calcd for C$_{33}$H$_{63}$NO$_4$: 537.4757). EI-MS $m/z$ (rel. int. %): 537 (M$^+$, 2), 286 (a, 11), 269 (b, 72). FAB-MS (negative ion mode; matrix, triethanolamine) $m/z$: 536 [M$^-$H$^+$]$_2$. 1H-NMR (600 MHz, CDCl$_3$) $\delta$: 0.88 (6H, t, $J=7.0$ Hz, H$_3$-18, H$_3$-14), 1.25—1.33 (30H, br s, H$_2$-12—H$_2$-17, H$_3$-5—H$_3$-13'), 1.35 (2H, m, H$_2$-11), 1.44 (2H, m, H$_2$-4'), 1.58 (3H, br s, H$_3$-19), 1.65 (1H, m, Ha-3), 1.84 (1H, m, Hb-3'), 1.95 (2H, t, $J=8.1$ Hz, H$_2$-10), 2.08 (2H, m, H$_2$-7), 2.10 (2H, m, H$_2$-6), 2.61 (2H, d, $J=4.4$ Hz, OH-3, OH-2'), 2.67 (1H, br s, OH-1), 3.74 (1H, br d, $J=11.0$ Hz, Ha-1), 3.92 (1H, m, H-2), 3.97 (1H, br d, $J=11.0$ Hz, Hb-1), 4.16 (1H, dd, $J=8.1, 3.3$ Hz, H-2'), 4.33 (1H, br s, H-3), 5.09 (1H, t, $J=6.6$ Hz, H-8), 5.55 (1H, dd, $J=15.4, 6.6$ Hz, H-4), 5.81 (1H, dt, $J=15.4, 6.6$ Hz, H-5), 7.15 (1H, d, $J=8.1$ Hz, NH).

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References and Notes