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Note

Novel Cerebroside, Termitomycesphin I, from the Mushroom, *Termitomyces titanicus*

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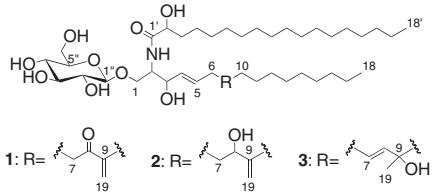
The novel cerebroside, termitomycesphin I (1), and two known cerebrosides (2 and 3) were isolated from the edible mushroom, *Termitomyces titanicus*. The structures of 1–3 were determined and identified by interpreting the spectroscopic data.

Key words: *Termitomyces titanicus*; mushroom; cerebroside; structural determination

Fungi of the genus *Termitomyces* live in obligate mutualistic symbiosis with termites of the subfamily *Macrotermitinae*.[1–3] Termites cultivate the mycelia in their nest, and fruiting bodies can be seen rising on or near the mounds.[4] We have recently reported the isolation of endoplasmic reticulum stress-protective compounds, termitomycamides A to E, from the fruiting bodies of one of the genus fungi *Termitomyces titanicus*.[5] This mushroom with a cap diameter of up to 1 m is the largest edible mushroom in the world according to the Guinness Book of Records. We isolated a new cerebroside during the course of further separation of the extracts of the mushroom. We report here the isolation and structural determination of this novel cerebroside (**1**), together with two known cerebrosides (**2** and **3**).



Compounds **2** and **3** were identified as termitomycesphin B and termitomycesphin D, which had previously been isolated from the Chinese mushroom, *Termitomyces albuminosus*, by comparing their NMR, mass spectral, and specific rotation data with the published values.[6–8]

Termitomycesphin I (**1**) was isolated as a white crystal showing a specific rotation of $[\alpha]_D^{28}$ -2.7° (*c* 0.15, $CHCl_3$). The IR spectrum showed absorption bands at 3348, 2920, 2850, 1468, 1645, and 1539 cm^{-1}. Its molecular formula was determined as $C_{43}H_{79}NO_{10}$ by HRESIMS, *m/z* 792.5572 $[M + Na]^+$ (calcd. for $C_{43}H_{79}NNaO_{10}$, 792.5602). The complete assignment of all the protons and carbons was accomplished by DEPT, HMQC, COSY, and HMBC experiments as shown in Table 1 and Fig. 1. The DEPT experiment indicated the presence of 2 methyls, 28 methylenes, 10 methines, and 3 quaternary carbons. The NMR signals due to two terminal methyl groups [δ_H 0.85 (6H, H18, H18′)] and many aliphatic methylenes (δ_H 1.24, δ_C 23.2–32.5) suggested the presence of two long fatty chains in the molecule. The partial structure of a ceramide moiety was suggested by the NMR data [δ_C 53.8 (C2), 68.9 (C1), 72.2 (C3), 72.6 (C2′), 130.8 (C4), 132.9 (C5), 176.5 (C1′), 202.7 (C8)], the COSY correlations (H1/H2, H2/H3, H3/H4, H4/H5, H5/H6, H6/H7, H17/H18, H2′/H3′, H3′/H4′, H17′/H18′), and the HMBC correlations (H1/C2, H1/C3, H2/C1′, H3/C1, H3/C2, H3/C4, H3/C5, H4/C3, H4/C6, H5/C3, H5/C6, H5/C7, H6/C4, H6/C5, H6/C7, H6/C8, H7/C5, H7/C6, H7/C8, H10/C8, H10/C9, H10/C11, H10/C19, H18/C16, H18/C17, H19/C8, H19/C9, H19/C10, H2′/C1′, H18′/C16′, H18′/C17′) (Fig. 1). These ^{1}H- and ^{13}C-NMR data for **1** were very similar to those for **2** and **3**, especially for **2**.[6,8] A comparison of the chemical shifts of H7 (δ_H 2.78), H19 (δ_H 5.75, 6.03) and C8 (δ_C 202.7) for **1** and its molecular formula with those for **2** indicated **1** to be the oxidized form of the hydroxy at C8 of **2**. The D-glucose moiety was determined by the coupling constants in ^{1}H-NMR (Table 1), and by the COSY correlations (H1″/H2″, H2″/H3″, H3″/H4″), and HMBC correlations (H1″/C2″, H1″/C3″, H2″/C1″, H2″/C3″, H2″/C4″, H3″/C2″, H3″/C4″, H4″/C3″, H4″/C6″, H5″/C3″, H5″/C4″, H6″/C5″) (Fig. 1). The β configuration of the glucosidic linkage was indicated by the coupling constant (8.2 Hz) for the anomeric proton. The connection between the D-glucose and the partial ceramide moiety was confirmed by the HMBC correlations (H1/C1″ and H1″/C1, Fig. 1).

Each carbon-chain length of the long-chain base (LCB) and the fatty acid moiety of **1** were determined by the FABMS fragmentation data. It has been reported that

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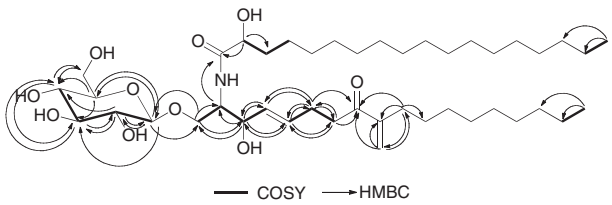
Table 1. ^{1}H- and ^{13}C-NMR Data for **1** (in $CDCl_3$ + CD_3OD)

Position	^{1}H δ (multiplicity, J in Hz)	^{13}C δ
Long-chain base		
1	3.68 (m)	68.9
	4.05 (dd, 10.3, 5.5)	
2	3.96 (m)	53.8
3	4.10 (dd, 7.7, 6.9)	72.2
4	5.48 (dd, 15.5, 7.2)	130.8
5	5.71 (ddd, 15.5, 6.8, 6.8)	132.9
6	2.29 (m)	27.5
7	2.78 (dd, 8.9, 6.2)	37.6
8		202.7
9		149.4
10	2.21 (dd, 6.9, 8.2)	31.4
11	1.50 (m)	29.1
12–17	1.24 (m)	[a]
18	0.85 (t, 6.9)	14.3
19	5.75 (s), 6.03 (s)	124.9
Acyl		
1′		176.5
2′	3.97 (m)	72.6
3′	1.50 (m), 1.70 (m)	35.2
4′	1.37 (m)	25.8
5′–17′	1.24 (m)	[a]
18′	0.85 (t, 6.9)	14.3
Sugar		
1″	4.23 (d, 8.2)	103.8
2″	3.20 (dd, 8.2, 8.9)	74.1
3″	3.36 (dd, 8.9, 9.6)	77.1
4″	3.31 (m)	70.7
5″	3.24 (m)	77.0
6a″	3.67 (dd, 12.0, 5.5)	62.1
6b″	3.84 (dd, 12.0, 2.4)	

[a]23.2, 29.9, 30.0, 30.1, 30.2, 30.3 and 32.5.



1

Fig. 1. COSY and HMBC Correlations for **1**.

cerebrosides give characteristic fragment ions of $[LCB + H]^+$ and $[LCB + H - H_2O]^+$ in positive-mode FABMS.[9] The positive FABMS data for **1** displayed the fragment ions at m/z 308 $[LCB + H]^+$ and 290 $[LCB + H - H_2O]^+$. This result revealed **1** to possess the same carbon-chain length of LCB as those of termitomycesphins A–F.[8,10] Judging from the rest of the molecular formula, the carbon number of the fatty acid part was determined as 18. The planar structure of **1** was determined as a result, but the absolute configuration of **1** remains to be determined.

Compound **1** was obtained during a search for endoplasmic reticulum stress-protective compounds from the mushroom, although it did not show such activity.

Fungal materials. One of authors (M. Kawade) collected the fruiting bodies of *T. titanicus* in Zambia Outside Kasama, the capital of Northern Province, in February 2006. A voucher specimen of the organism is located in Iwade Research Institute of Mycology.

General experiments. ^{1}H-NMR spectra (one- and two-dimensional) were recorded by a Jeol lambda-500 spectrometer at 500 MHz, while ^{13}C-NMR spectra were recorded by the same instrument at 125 MHz. The FABMS and the HRESIMS data were respectively measured by JMS-GC-Mate II and JMS-T100LC mass spectrometers. A Jasco grating infrared spectrophotometer was used to record the IR spectra, and the specific rotation values were measured by a Jasco DIP-1000 polarimeter. Gel permeation was conducted with Toyopearl HW-40F (Tosoh, Japan), and MPLC was done with a Yamazen MPLC system and UltraPack ODS-S50D column (Yamazen, Japan). HPLC separation was performed with a Jasco Gulliver system, using reverse-phase HPLC columns (Develosil C30-UG-5, Nomura Chemical, Japan; and Capcell Pak C18 AQ and Capcell Pak C8 DD, Shiseido, Japan). A silica gel plate (Merck F_{254}) and silica gel 60 N (Merck 100–200 mesh) were respectively used for analytical TLC and flash column chromatography.

Extraction and isolation. The powder of dried fruiting bodies of *T. titanicus* (3.3 kg) was successively extracted with hexane, EtOAc, and then EtOH (5 L, five times). After removing the solvent under reduced pressure, the EtOH-soluble part (100.6 g) was partitioned between 1-BuOH and H_2O. The BuOH-soluble part (22.0 g) was fractionated by gel permeation chromatography (Toyopearl HW-40F, 4.4 × 50 cm, acetone, 1.5 mL/min) to obtain 7 fractions. Fraction 3 (11.1 g) was further fractionated by normal-phase MPLC (Ultrapack SI-40D, $CHCl_3$/MeOH 10:0, 95:5 and 7:3; and MeOH) to obtain 15 fractions. Fraction 3-13 (851 mg) was further fractionated by reversed-phase HPLC (Capcell Pak C18 AQ, 95% MeOH) to obtain 4 fractions. Fraction 3-13-3 (8.2 mg) was further separated by reversed-phase HPLC (Capcell Pak C8 DD, 90% MeOH) to afford compound **1** (1.5 mg). Fraction 3-8-2 (110 mg) was further fractionated by reversed-phase HPLC (Capcell Pak C18 AQ, 90% MeOH) to obtain 4 fractions. Compounds **2** (12.6 mg) and **3** (9.7 mg) were purified from fraction 3-8-2-3 (50.9 mg) by reversed-phase HPLC (Develosil C30-UG-5, 90% MeOH).

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6) Compound **2**. $[\alpha]_D^{23}$ +6.0° (*c* 0.90, MeOH); ^{1}H-NMR (500 MHz, in C_5D_5N) δ: 0.85 (H18, H18′), 1.25 (H12–H17, H5′–H17′), 1.54 (H11), 1.68 (H4′), 1.77 (H4′), 1.88 (H7), 1.98 (H3′), 2.13 (H3′), 2.15 (H10), 2.30 (H10), 2.35 (H6), 2.44 (H6), 3.86 (H5″), 3.99 (H2″), 4.17 (H3″), 4.18 (H4″), 4.20 (H1a), 4.32 (H6″a),

4.40 (H8), 4.47 (H6″b), 4.54 (H2′), 4.67 (H1b), 4.74 (H3), 4.77 (H2), 4.87 (H1″), 4.98 (H19), 5.33 (H19), 6.00 (H4, H5); ^{13}C-NMR (125 MHz, in C_5D_5N) δ: 14.2 (C18, C18′), 25.9 (C4′), 28.5 (C11), 29.5 (C6), 31.7 (C10), 35.5 (C3′), 36.0 (C7), 54.5 (C2), 62.5 (C6″), 70.0 (C1), 71.4 (C4″), 72.3 (C3), 72.4 (C2′), 74.2 (C8), 74.3 (C8), 75.0 (C2″), 78.3 (C3″), 78.4 (C5″), 105.5 (C1″), 108.6 (C19), 131.7 (C4), 132.7 (C5), 153.8 (C9), 175.6 (C1′); ESIMS m/z 794 $[M + Na]^+$.

7) Compound **3**. $[\alpha]_D^{24}$ +8.3° (*c* 0.61, MeOH); ^{1}H-NMR (500 MHz, in C_5D_5N) δ: 0.85 (H18, H18′), 1.24 (H12–H17, H5′–H17′), 1.47 (H19), 1.60 (H11), 1.70 (H4′), 1.71 (H10), 1.78 (H4′), 1.98 (H3′), 2.18 (H3′), 2.90 (H6), 3.87 (H5″), 4.00 (H2″), 4.18 (H3″), 4.19 (H4″), 4.20 (H1a), 4.33 (H6″a), 4.48 (H6″b), 4.54 (H2′), 4.67 (H1b), 4.75 (H3), 4.77 (H2), 4.87 (H1″), 5.92 (H8), 5.96 (H5), 5.97 (H7), 6.02 (H4); ^{13}C-NMR (125 MHz, in C_5D_5N) δ: 14.2 (C18, C18′), 24.5 (C11), 25.8 (C4′), 28.5 (C19), 35.5 (C6, C3′), 43.7 (C10), 54.5 (C2), 62.5 (C6″), 69.9 (C1), 71.4 (C4″), 71.8 (C9), 72.2 (C3), 72.4 (C2′), 75.0 (C2″), 78.3 (C3″), 78.4 (C5″), 105.5 (C1″), 124.9 (C7), 131.0 (C5), 132.4 (C4), 140.1 (C8), 175.6 (C1′); ESIMS m/z 794 $[M + Na]^+$.

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