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Termitomycesphins A–D, Novel Neuritogenic Cerebrosides from the Edible Chinese Mushroom *Termitomyces albuminosus*

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Abstract—Four novel cerebrosides, termitomycesphins A–D, were isolated from the edible Chinese mushroom *Termitomyces albuminosus* (Berk.) Heim. ('Jizong' in Chinese), shown to induce neuronal differentiation in rat PC12 cells. The absolute stereostructures were elucidated by spectroscopic methods and chemical derivatization. These new cerebrosides have a unique C19 hydroxylated sphingosine base with branching around the middle. Termitomycesphins A and C possessing a C16 α-hydroxy fatty acid showed a higher neuritogenic activity than did termitomycesphins B and D possessing a C18 α-hydroxy fatty acid.

Introduction

The nerve growth factor (NGF) is the first and best characterized neurotrophic factor that regulates the growth, differentiation, and survival of neurons, and is thought to be a candidate for treating Alzheimer's disease, one of neurodegenerative diseases of humans.[1–3] Since the NGF molecule is too large in size and too hydrophilic to pass through the blood-brain barrier, there are no other safe ways to be used as a drug except for its intraventricular infusion.[4] NGF-like low-molecular-weight compounds are thus considered to be good candidates for the treatment of Alzheimer's disease. A clonal rat pheochromocytoma cell line, PC12, which expresses neuronal properties, has been used as a model system for such studies.[5]

In the course of our search for such substances using the PC12 cell line system, novel cerebrosides termed termitomycesphins A–D (**1**–**4**) were isolated from the EtOH extract of an edible Chinese mushroom, *Termitomyces albuminosus* (Berk.) Heim. ('Jizong' in Chinese), as neuritogenic substances. We report herein the isolation, structures, and biological activity of these novel cerebrosides.

Results and Discussion

The EtOH extract of *T. albuminosus* was washed with hexane, then partitioned between BuOH and H_2O. The active BuOH fraction was chromatographed on ODS, then on silica gel to give a mixture of cerebrosides, which was purified by reversed-phase HPLC to yield termitomycesphins A (**1**, 0.00026% of dry weight), B (**2**, 0.0003%), C (**3**, 0.00027%), and D (**4**, 0.00057%).

Structural analysis was started with termitomycesphin B (**2**). The molecular formula of termitomycesphin B (**2**), $C_{43}H_{81}NO_{10}$, was determined by HRESIMS measurement [*m/z* 772.5937 $(M+H)^+$, $\Delta -0.2$ mmu]. The IR spectrum of **2** suggests the presence of a secondary amide group (1648 and 1535 cm^{-1}) and hydroxyl groups (3390 cm^{-1}). The ^1H and ^{13}C NMR spectra of **2** showed the presence of eight oxymethines and two oxymethylenes (δ_H 3.9–5.0, δ_C 62.5–78.5 and 105.5), a *trans* disubstituted olefin (δ_H 6.01 and 6.05, J=16.6 Hz), an exomethylene (δ_H 4.99 and 5.35, δ_C 153.8/153.9 (1:1 ratio) and 108.6), and eight exchangeable protons (broad singlets or doublets in the range of δ 6.21–7.61), inculding an NH [δ_H 8.35 (d, J=8.6 Hz)]. The signals at δ 0.84 (t), 0.85 (t) and many aliphatic methylenes (δ_H 1.25, δ_C 22.8–36.0) suggest that there are two long fatty chains in this molecule. The signal at δ 175.6 supports the presence of the amide group.

The six of the oxygenated carbons at δ 62.5 (t), 71.4 (d), 75.0 (d), 78.3 (d), 78.5 (d), and 105.5 (d) suggest the presence of a β-glucoside moiety in **2** by comparison of the observed and reported chemical shifts.[6] The β configuration of the glucoside bond was supported by the coupling constant of 7.7 Hz for the anomeric proton at δ 4.90. The ^1H and ^{13}C NMR data are summarized in Table 1, in which the H–C direct connectivities were determined by an HMQC experiment.

The following partial structures were determined by a DQF-COSY experiment: β-glucoside moiety, C1–C8, C10–C11, C2′–C4′, and two terminal methyls connected to a methylene. An HMBC spectrum showed the correlations from H7, H10, and H19 to the quaternary olefinic carbon (C9),

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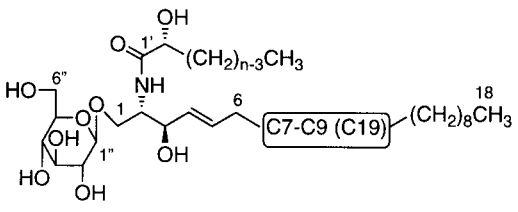
Table 1. ^{1}H and ^{13}C NMR data for termitomycesphins A–D (**1**–**4**) in pyridine-d_5

Carbon no.	**1** and **2**		**3** and **4**	
	^{1}H[a]	^{13}C[b]	^{1}H[a]	^{13}C[b]
Long-chain base				
1a	4.22 dd (10.7, 3.8)	70.7 t	4.22 dd (10.6, 3.8)	69.9 t
1b	4.69 dd (10.7, 4.3)		4.68 dd (10.6, 5.7)	
2	4.80 m	54.5 d	4.79 m	54.5 d
3	4.78 m	72.3 d	4.77 m	72.2 d
3 -OH	6.85 d (4.4)		6.89 d (4.7)	
4	6.05 dd (16.6, 3.5)	131.7 d	6.05 dd(15.4, 5.1)	132.4 d
5	6.01 dd (16.6, 5.1)	132.7 d	5.97 dt (15.4, 6.3)	131.0 d
6	2.38, 2.48 m	29.5 t	2.92 t (6.3)	35.5 t
7	1.91 m	36.0 t	5.98 dt (15.5, 6.3)	124.9 d
8	4.42 m	74.2/74.3 d[c]	5.88 d (15.5)	140.2 d
8-OH	6.21/6.20 d (4.0)[c]		–	
9	–	153.8/153.9 s[c]	–	71.8 s
9-OH			5.68 s	
10	2.16, 2.32 m	31.7 t	1.73 m	43.7 t
11	1.57 m	28.4 t	1.61 m	24.5 t
12–17	1.25 m	[d]	1.24 m	[f]
18	0.85 t (7.5)[e]	14.2 q	0.85 t (7.5)[e]	14.2 q
19	4.99, 5.35 s	108.6 t	1.48 s	28.5 q
2-NH	8.35 d (8.6)		8.35 d (8.4)	
Acyl				
1′		175.6 s		175.6 s
2′	4.56 m	72.4 d	4.57 m	72.4 d
2′-OH	7.61 d (4.3)		7.60 d (5.1)	
3′	2.00, 2.21 m	35.5 t	2.00, 2.24 m	35.5 t
4′	1.69, 1.79 m	25.9 t	1.69, 1.78 m	25.8 t
5′-15′ (or 5′-17′)	1.25 m	[d]	1.24 m	[f]
16′ (or 18′)	0.84 t (7.7)[e]	14.2 q	0.84 t (7.7)[e]	14.2 q
Sugar				
1″	4.90 d (7.7)	105.5 d	4.90 d (7.7)	105.5 d
2″	4.02 dd (7.7, 7.1)	75.0 d	4.02 dd (7.7, 6.3)	75.0 d
2″-OH	7.20 br s		7.19 br s	
3″	4.20 m	78.3 d	4.19 m	78.4 d
3″-OH	7.17 br s		7.17 br s	
4″	4.21 m	71.4 d	4.21 m	71.5 d
4″-OH	7.13 br s		7.13 br s	
5″	3.89 m	78.5 d	3.89 m	78.5 d
6″a	4.34 dd (11.4, 4.5)	62.5 t	4.34 dd (11.6, 4.5)	62.6 t
6″b	4.50 br d (11.4)		4.50 br d (11.6)	
6″-OH	6.37 br s		6.35 br s	

[a] 600 MHz, coupling constants (J, Hz) are in parentheses.
[b] 100 MHz.
[c] 1:1 ratio due to C-8 epimers.
[d] δ 22.8, 29.3, 29.8, 29.9 and 32.0.
[e] Interchangeable.
[f] δ 22.8, 29.5, 29.9, 30.6 and 32.0.

revealing the gross structure of a long-chain base (LCB) (Fig. 2). The HMBC correlations between position 1 and the anomeric position (H1/C1″and H1″/C1) led to the connectivity between glucose and LCB. The correlation between the exchangeable proton at δ 8.35 and the carbonyl carbon (δ 175.6) showed the location of the amide group. Although no correlation between position 2′ and the amide group was observed, the connectivity of C1′–C2′ is self-evident, providing the gross structure of a cerebroside as shown in Fig. 2. The number of other methylene groups



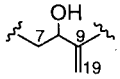
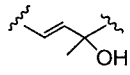
termitomycesphin	C7-C9 (C19)	n
A (**1**)		16
B (**2**)		18
C (**3**)		16
D (**4**)		18

Figure 1. Structures of termitomycesphins A–D (**1**–**4**).

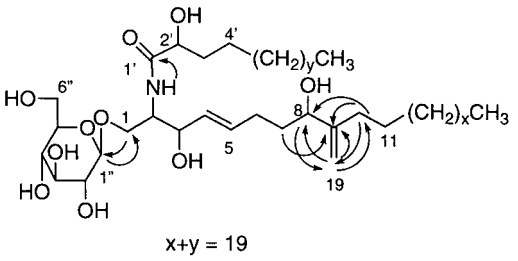


Figure 2. Gross structure of **2** with selected HMBC correlations (arrows are from H to C).

alcohol systems, **2** was first hydrogenated to give a tetrahydro derivative **5**. Methanolysis of **5** gave methyl glucoside (2:1 mixture of α and β anomers), fatty acid methyl ester **6**, and free LCB **7**. The configuration of the methyl glucoside was determined as D by comparison of the optical rotation with the reported one.[7] The methyl ester **6** $[[\alpha]_D^{25}=-4.4$ (c 0.092, $CHCl_3$)] was determined as methyl (*R*)-2-hydroxyoctadecanoate based on the optical rotation[8] and FABMS data. To determine the absolute configurations of **7**, we applied a recent CD exciton chirality method.[9] Thus, the benzoylation of LCB **7** gave tetrabenzoyl derivative **8**. The stereochemistry of **8** was determined as 2*S*,3*R* by

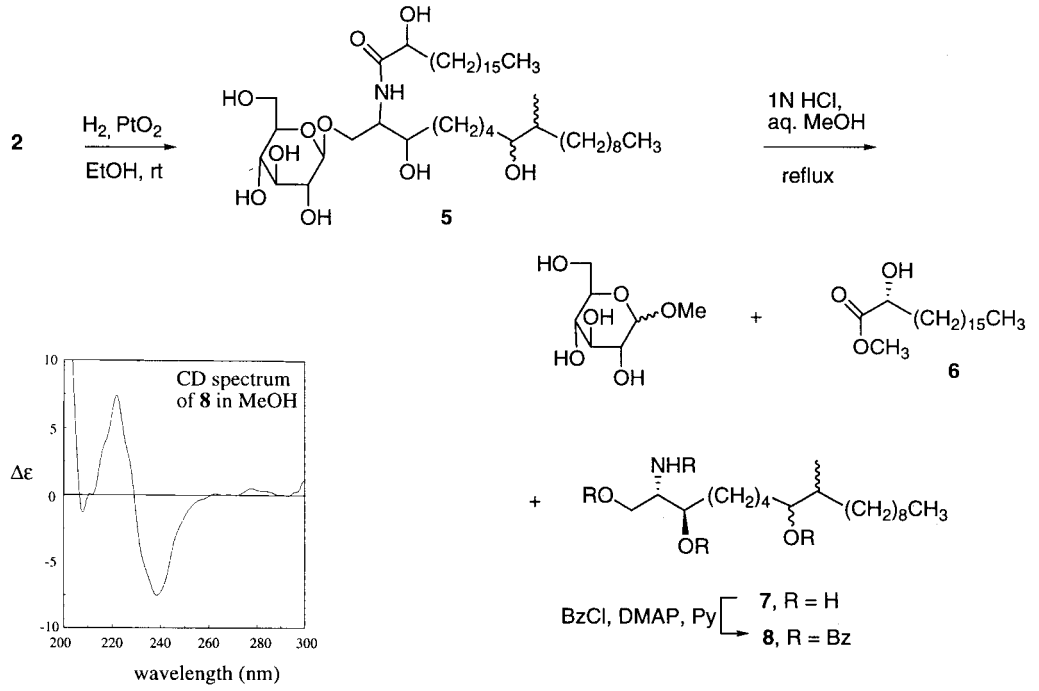


Figure 3. Degradation scheme of **2**.

was determined as 19 from the molecular weight. Since the signals assigned to C8 (d 74.3, 74.2), C9 (153.9, 153.8), and 8-OH (6.21, 6.20) split into two similar chemical shifts in the ratio of 1:1, we presume that 2 is an inseparable 1:1 mixture of C8 epimers.

The degradation of **2** was carried out to determine the stereochemistry and the two carbon chain lengths (Fig. 3). The first trial of acidic methanolysis unsuccessfully led to a complex mixture. To avoid decomposition of the allyl

Table 2. Selected ^{1}H NMR data ($CDCl_3$, 400 MHz) and CD data (MeOH) for tetrabenzoate **8** and tribenzoate **10**

	^{1}H NMR[a]						CD	
	J_{NH-H2}	J_{H2-H3}	δ_{NH}	δ_{H3}	δ_{H2}	δ_{H1}	λ(nm)	(Δε)
8	8.5	3.7	7.07	5.36	4.85	4.58/4.63	238 (−7.8)	222 (+7.4)
10	7.8	4.0	7.08	5.38	4.88	4.61/4.65	239 (−8.4)	222 (+7.2)

[a] Coupling constants J are in Hz, and chemical shifts δ are in ppm.

comparison of the CD spectrum (Fig. 3, Table 2) and ^{1}H NMR data (Table 2) with those reported.[9] Since the exciton interaction between the extra C8 benzoate and others can be neglected due to the long range relation, the CD data are in good agreement with the reported data. The absolute stereostructure of **2**, except for position 8, was finally determined as shown in Fig. 1.

The molecular formula of termitomycesphin A (**1**), $C_{41}H_{77}NO_{10}$, was determined by HRESIMS measurement [m/z 744.5621 $(M+H)^+$, $\Delta-0.5$ mmu]. The ^{1}H and ^{13}C NMR spectra of **1** are superimposable on those of **2**, and its molecular formula is less than that of **2** by C_2H_4, indicating that **1** is a homologue of **2** possessing a shorter carbon chain. It was reported that cerebrosides gave characteristic fragment ions of $(LCB+H-H_2O)^+$ and $(LCB+H)^+$ in the positive mode FABMS measurements.[10] The FABMS spectra of both **1** and **2** showed the same fragment ions at m/z 292 due to $(LCB+H-H_2O)^+$ and at m/z 274 due to $(LCB+H-2H_2O)^+$, though $(LCB+H)^+$ ion peak (m/z 310) was absent due to the instability of an extra OH group at C-8 (Fig. 4), revealing that **1** and **2** possess the

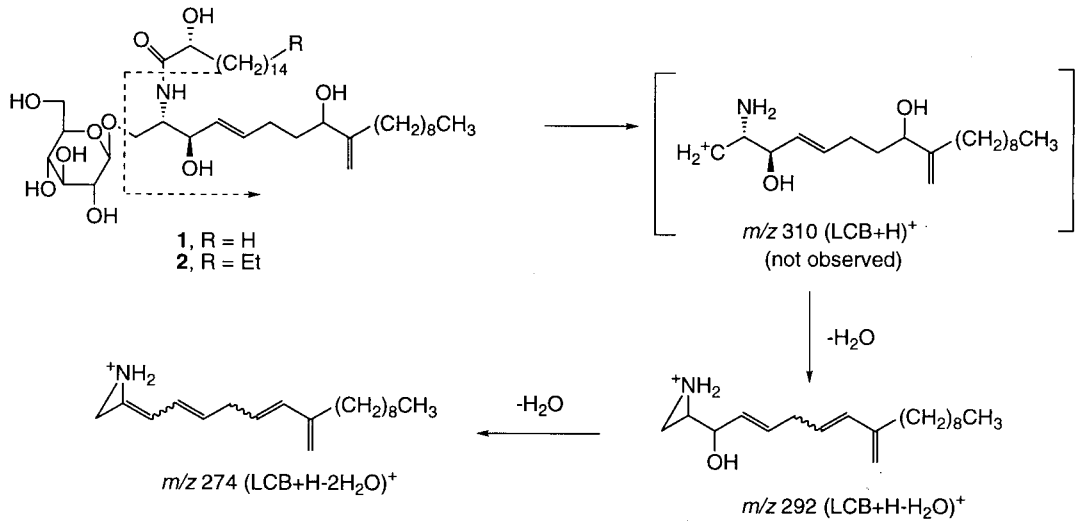


Figure 4. FABMS (+) Fragmentations of **1** and **2** giving the same LCB-derived fragment ions.

same LCB moiety. Thus, the structural difference between **1** and **2** is that **2** possesses two more methylene groups in the fatty acyl chain than **1**. The stereochemistry of **1** seems to be the same as that of **2**, because both compounds showed not only the same NMR data but also quite similar optical rotations.

The molecular formula of termitomycesphin D (**4**), $C_{43}H_{81}NO_{10}$, was determined by a HRFABMS measurement [*m/z* 794.5753 $(M+Na)^+$, $\Delta-0.6$ mmu]. The IR and NMR spectra of **4** are similar to those of **1** and **2**, indicating that **4** is also a cerebroside. The most notable difference in the NMR data between **4** and **2** is that the signals due to the exomethylene (C9–C19) and a secondary alcohol (C8) in **1** are replaced by a *trans* olefin (C7–C8 at δ_H 5.98 and 5.88) and methyl carbinol (C9–C19 at δ_{H19} 1.48, δ_{C9} 71.8 and δ_{C19} 28.5). The other NMR data are quite similar to those of **1** (Table 1). The DQF-COSY spectrum of **4** revealed the following partial structures: the β-glucoside moiety, C1–C8, C10–C11, C2′–C4′, and two terminal methyls connected to methylenes. The HMBC correlations shown in Fig. 5 allowed us to propose the gross structure of **4**.

The absolute stereochemistry and the two carbon-chain lengths of **4** were determined by degradation experiments using the same conditions as those for **2**. The hydrogenation of **4** yielded a saturated cerebroside, acidic methanolysis of which gave methyl D-glucopyranoside (2:1 mixture of α and β anomers), fatty acid methyl ester **6** and free LCB **9**

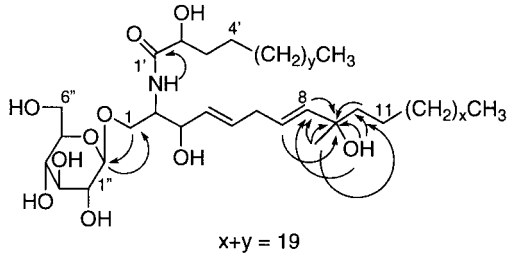


Figure 5. Gross structure of **4** with selected HMBC correlations (arrows are from H to C).

(Fig. 6). LCB **9** was converted to the tribenzoate **10** for purification and stereochemical analysis, being found to be converted into an 8,9-dehydro and/or 9,10-dehydro derivative due to dehydration during methanolysis. The CD spectrum (Fig. 6, Table 2) and ^1H NMR data (Table 2) again agree with those reported for benzoates of (2*S*, 3*R*)-LCB,[9] revealing the 2*S*,3*R* stereochemistry of **10**. The absolute stereostructure of **4**, except for position 9, was thus determined as shown in Fig. 1

The molecular formula of termitomycesphin C (**3**), $C_{41}H_{77}NO_{10}$, was determined by HRFABMS measurement [*m/z* 766.5421 $(M+Na)^+$, $\Delta-2.4$ mmu]. The ^1H and ^{13}C NMR of **3** are superimposable on those of **4**, and the molecular formula of **3** is less than that of **4** by C_2H_4, indicating that **3** is a homologue of **4** possessing a shorter carbon chain. The positive FABMS spectra of both **3** and **4** displayed the same fragment ions at *m/z* 292 $(LCB+H-H_2O)^+$ and at *m/z* 274 $(LCB+H-2H_2O)^+$ as in the case of **1** and **2**, revealing that **3** and **4** possess the same LCB moiety. Thus, the structural difference between **3** and **4** is only the presence of two more methylenes in the fatty acid chain moiety of **4** than that of **3**. The absolute configuration of **3** was assumed to be the same as that of **4** by comparison of the optical rotation and NMR data between **3** and **4** (Fig. 1).

Fig. 7 shows the time course of the neuronal differentiation induced in PC12 cells by termitomycesphins A–D (**1**–**4**). Termitomycesphins A (**1**) and C (**3**) showed the maximum activity of 30% at the concentration of 10 μg/ml, which were similar to the activity of an active control, dibutyryl cAMP, at the concentration of 60 μg/ml, while termitomycesphins B (**2**) and D (**4**) showed the maximum activity of 10% for the same concentration and time. The control cells cultured without the compounds did not show any neuronal differentiation. It is noteworthy that the termitomycesphins possessing a C16-fatty acyl moiety (**1** and **3**) showed a much higher activity than those possessing a longer C18-fatty acyl moiety (**2** and **4**).

An outstanding structural feature of the termitomycesphins

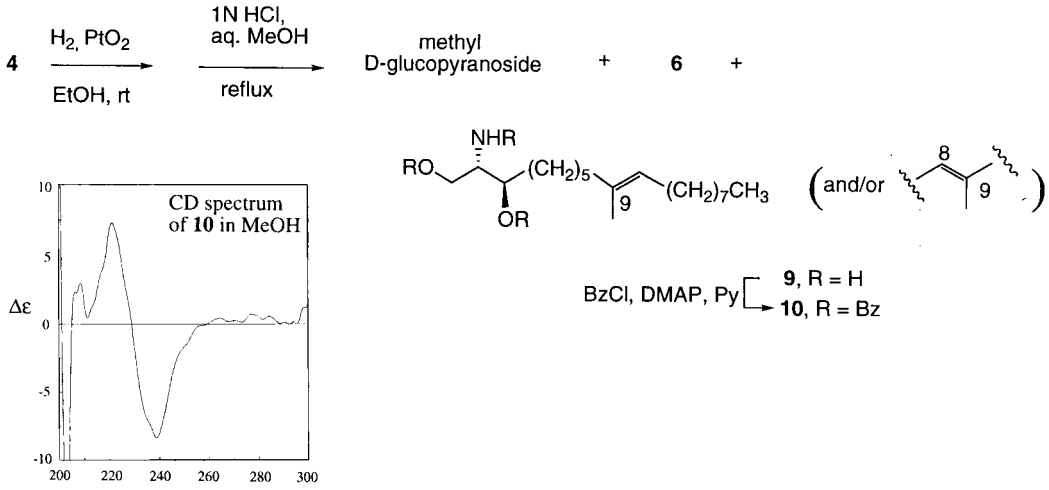


Figure 6. Degradation scheme of **4**.

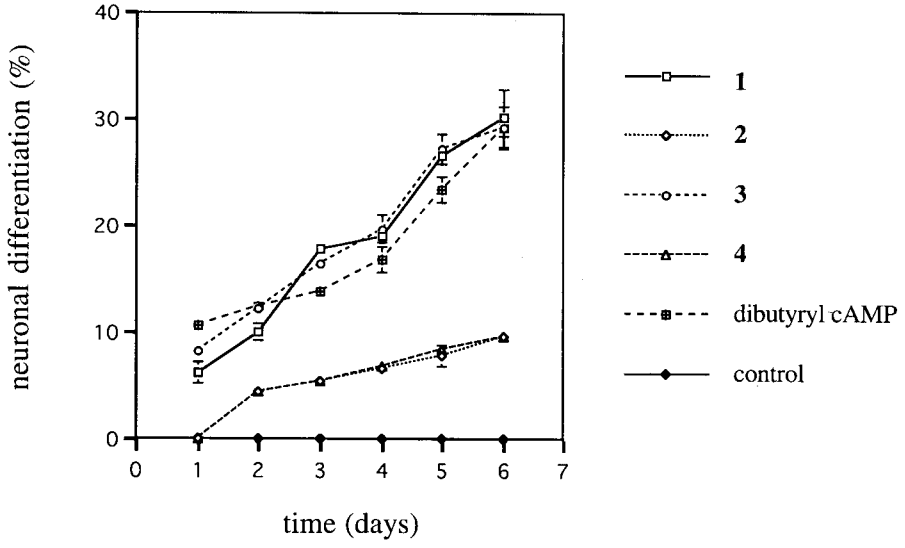


Figure 7. Time course of neuronal differentiation induced in PC12 cells by 10 μg/ml of termitomycesphins A–D (**1**–**4**) in comparison with 60 μg/ml of dibutyryl cAMP as a positive control. The percentages of the cells with longer processes than the cell diameter were plotted.

is the presence of a branch allylic alcohol system in the middle of the LCB, though the chain length of the fatty acyl moiety is rather important for the activity than this characteristic structure of LCB. To the best of our knowledge, termitomycesphins are the first cerebrosides not only possessing such an unusual LCB but also inducing neurite outgrowth in a neuronal cell line.[11]

Experimental

General procedures

HPLC was performed on a JASCO high-pressure gradient system with PU-980 pumps and a JASCO UV-970 detector at 205 nm. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a JASCO FT/IR-7000S. UV spectra were recorded on a JASCO Ubest-50UV/VIS spectrometer. CD spectra were recorded on a JASCO J-720 spectropolarimeter. FABMS (positive) and high-resolution FABMS (positive) were recorded on a JEOL DX-705L and a JEOL Mstation JMS-700 mass spectrometer, respectively, using *m*-nitrobenzyl alcohol as the matrix and PEG 600 as the calibration standard in the positive mode. HRESIMS (positive) were recorded on a PE SCIEX QSTAR mass spectrometer, using a mixture of CsI and sex pheromone inhibitor iPD1 (MW 829) (Bachem) as the calibration standard. One- and two-dimensional (HMBC, HMQC, DQFCOSY) NMR spectra were obtained in a $CDCl_3$ or C_5D_5N solution using a Bruker AMX-600 or a Bruker ARX-400 spectrometer. Chemical shifts are reported in δ (ppm) relative to TMS.

Extraction and purification procedures

The fresh fruiting bodies of *T. albuminosus* were collected in Yanyuan county, southwest of China in July 1999, then dried and sent to Japan.

The dried fruiting bodies (4.30 kg) of the mushroom were powdered and immersed in EtOH with occasional stirring for 10 days. The mixture was filtered and the filtrate was concentrated to produce an EtOH extract (593.3 g) as a viscous brown oil, which was divided into three portions and subjected to solvent partition. In a typical case, a portion (209.7 g) of the extract was dissolved in 90% aq. MeOH (1 liter) and washed twice with hexane (0.8 l). The aq. MeOH layer was concentrated, dissolved in H_2O (0.8 l), and then extracted three times with BuOH (0.4 l). The BuOH fractions from the three portions were combined and concentrated to give a yellowish viscous oil (74.9 g), which was chromatographed on ODS (Cosmosil 75 C18-OPN, 350 g, Nacalai Tesque) eluted with MeOH/H_2O (90:10, 95:5) and then MeOH to give four fractions. The active third fraction (9.9 g), which was eluted with MeOH/H_2O (95:5), was separated by a silica gel (Silica gel 60 230–240 Mesh ASTM, 150 g, Merck) open column eluted with $CHCl_3$/MeOH (95:5, 90:10) and MeOH to give four fractions. The active third fraction (249 mg), which was eluted with $CHCl_3$/MeOH (90:10), was suspended in 90% aqueous MeOH (12 ml) and filtered through a filter (DISMIC-13HP, 0.45 mm, Toyo Roshi). The filtrate (220 mg) was subjected to HPLC [Develosil ODS-10 (ϕ 20/250 mm), flow rate: 8 ml/min; 4 injections] eluted by MeOH/H_2O (90:10) to yield termitomycesphins A (**1**) (11.5 mg, t_R=93 min), crude B (**2**) (25.0 mg, t_R=162 min), C (**3**) (11.9 mg, t_R=100 min), D (**4**) (24.6 mg, t_R=173 min). The pure sample of **2** (13.0 mg, t_R=250 min) was obtained from the crude sample (20 mg) by further HPLC [Develosil ODS-10 (ϕ 20/250), flow rate: 8 ml/min] eluted by CH_3CN/H_2O (82:18).

Termitomycesphin A (1). A colorless powder; $[\alpha]_D^{24}=+6.0$ (*c* 0.233, MeOH); IR (KBr) 3386, 2922, 2853, 1648, 1537, 1467, and 1078 cm^{-1}; HRESIMS *m/z* 744.5621, calcd for $C_{41}H_{78}NO_{10}$ (M+H) 744.5626, for ^1H and ^{13}C NMR see Table 1.

Termitomycesphin B (2). A colorless powder; $[\alpha]_D^{24}=+6.6$ (*c* 0.119, MeOH); IR (KBr) 3390, 2923, 2853, 1648, 1535, 1467, and 1078 cm^{-1}; HRESIMS *m/z* 772.5937, calcd for $C_{43}H_{82}NO_{10}$ (M+H) 772.5939; ^1H and ^{13}C NMR were superimposable on those of **1** (Table 1).

Termitomycesphin C (3). A colorless powder; $[\alpha]_D^{24}=+7.6$ (*c* 0.226, MeOH); IR (KBr) 3387, 2927, 2854, 1637, 1541, 1467, and 1079 cm^{-1}; HRFABMS *m/z* 766.5421, calcd for $C_{41}H_{77}NO_{10}Na$ (M+Na) 766.5445; for ^1H and ^{13}C NMR see Table 1.

Termitomycesphin D (4). A colorless powder; $[\alpha]_D^{24}=+8.0$ (*c* 0.400, MeOH); IR (KBr) 3375, 2924, 2854, 1646, 1540, 1467, and 1079 cm^{-1}; HRFABMS *m/z* 794.5753, calcd for $C_{43}H_{81}NO_{10}Na$ (M+Na) 794.5759; ^1H and ^{13}C NMR were superimposable on those of **3** (Table 1).

Degradation of 2

A solution of **2** (4.6 mg) in EtOH (1 ml) was stirred in the presence of PtO_2 (1 mg) under hydrogen atmosphere for 4 h at room temperature. The reaction mixture was filtered, and the filtrate was concentrated to afford 4.6 mg of tetrahydro derivative **5**: colorless powder, FABMS *m/z* 798 $(M+Na)^+$, ^1H NMR (pyridine-d_5, 400 MHz) δ 8.42 (1H, d, *J*=8.4 Hz, NH), 5.46 to 7.66 (broad singlets or doublets, seven OH), 4.90 (1H, d, *J*=7.7 Hz, H-1″), 4.71 (1H, m, H-2), 4.69 (1H, m, H-3), 4.67 (1H, m, H-1b), 4.59 (1H, m, H-2′), 4.52 (1H, d, *J*=11.4 Hz, H-6″b), 4.36 (1H, dd, *J*=11.4, 5.5 Hz, H-6″a), 4.23 (1H, m, H-1a), 4.20 (1H, m, H-4″), 4.18 (1H, m, H-3″), 4.02 (1H, dd, *J*=7.7, 7.2 Hz, H-2″), 3.91 (1H, m, H-5″), 3.77/3.67 (1H, m, H-8, 1:1 ratio), 2.21 (1H, m, H-3′b), 2.05 (1H, m, H-3′a), 1.63 to 1.90 (m, methylenes), 1.25 (s, methylenes), 1.09/1.07 (3H, d, *J*=7.4 Hz, H-19, 1:1 ratio), 0.86 (3H, t, *J*=6.1 Hz, terminal methyl), 0.85 (3H, t, *J*=6.9 Hz, terminal methyl).

A solution of **5** (4.6 mg) in a mixture of MeOH (1 ml), water (0.1 ml), and 12 N HCl (0.1 ml) was refluxed for 7 h. The reaction mixture was cooled and dried by a N_2 flow. The residue was dissolved in MeOH/H_2O (90:10) (4 ml) and extracted three times with hexane (4 ml). The hexane layers were combined, concentrated, and purified by preparative TLC (silica gel, EtOAc/hexane=1:3, R_f=0.44) to afford the fatty acid methyl ester **6** (1.3 mg): colorless powder, $[\alpha]_D^{25}=-4.4$ (*c* 0.092, $CHCl_3$) [lit.[8] $[\alpha]_D^{24}=-3.6$ ($CHCl_3$)], FABMS *m/z* 315 $(M+H)^+$, ^1H NMR ($CDCl_3$, 400 MHz) δ 4.19 (1H, m, H-2), 3.79 (3H, s, $COOCH_3$), 2.66 (1H, d, *J*=5.8 Hz, OH), 1.78 (1H, m, H-3), 1.63 (1H, m, H-3), 1.26 (30H, br s, methylenes), δ 0.88 (3H, t, *J*=6.8 Hz, terminal methyl).

The aqueous methanolic layer was concentrated, dissolved in MeOH (0.2 ml), and neutralized by passing through an Amberlite CG-4B (OH^- form) resin column (3 ml), which was then eluted with MeOH (20 ml). The eluate was concentrated, dissolved in H_2O (2 ml), and extracted twice with EtOAc (2 ml). The aqueous phase was purified by TLC (silica gel, $CHCl_3$/MeOH=4:1, developed twice, R_f=0.36) to afford a mixture of methyl α- and β-D-glucopyranoside (2:1): $[\alpha]_D^{24}=+75$ (*c* 0.035, MeOH) [lit.[7] $[\alpha]_D^{25}=+77.3$ (*c* 0.1, MeOH)].

The EtOAc phase was concentrated and benzoylated with benzoyl chloride (30 ml) and DMAP (1 mg) by stirring in dry pyridine (1.5 ml) for 5 h at 50°C. The reaction was quenched by adding H_2O (0.5 ml) and stirring for 5 min. The reaction mixture was concentrated in vacuo, dissolved in benzene (1 ml), and passed through an alumina (7 g) column (Aluminiumoxid 90, II–III, Merck). The column was eluted with hexane/EtOAc (9:1 and then 8:2), and the combined eluates were concentrated and purified by preparative TLC (silica gel, EtOAc/hexane=1:9, developed two times, R_f=0.18) to afford (2*S*,3*R*)-2-benzoylamido-1,3,8-tribenzoyloxy-9-methyloctadecane (**8**) (0.6 mg) as a colorless powder: ^1H NMR (400 MHz, $CDCl_3$) δ 8.02 (4H, m), 7.94 (2H, m), 7.77 (2H, m), 7.34–7.55 (12H, m), 7.08/7.06 (1H, d, *J*=8.5 Hz, 1:1 ratio), 5.36 (1H, m), 5.07/5.03 (1H, m, 1:1 ratio), 4.85 (1H, m), 4.63 (1H, m), 4.58 (1H, m), 1.22–1.94 (25H, m), and 0.84–0.96 (6H, m).

Degradation of 4

Termitomycesphin D (**4**) (6 mg) was subjected to hydrogenation followed by acidic methanolysis in the same conditions as those for **2**, yielding methyl α- and

β-D-glucopyranoside (0.7 mg) and **6** (2.0 mg): colorless powder, $[\alpha]_D^{24}=-5$ (*c* 0.13, $CHCl_3$), FABMS, *m/z* 315 $(M+H)^+$. LCB from the EtOAc fraction was subjected to benzoylation in the same conditions as those for **2**. The product was purified by preparative TLC (silica gel, EtOAc/hexane=1:9, developed four times, R_f=0.25) to afford (2*S*,3*R*)-2-benzoylamido-1,3-dibenzoyloxy-9-methyl-8- or -9-octadecene (**10**) (0.3 mg) as a colorless powder: ^1H NMR (400 MHz, $CDCl_3$) δ 8.03 (2H, m), 7.95 (2H, m), 7.78 (2H, m), 7.35–7.58 (9H, m), 7.08 (1H, d, *J*=7.8 Hz), 5.38 (1H, m), 5.06 (1H, m), 4.88 (1H, m), 4.65 (1H, m), 4.61 (1H, m), 1.88–1.96 (6H, m), 1.25–1.62 (21H, m), and 0.86 (3H t, *J*=5.9 Hz).

Bioassay method

The PC 12 cells were purchased from RIKEN Cell Bank (Japan) and cultured in a 1.05% modified minimum essential medium Eagle (MEME) medium (ICN Biomedical) supplemented with 10% fetal bovine serum, 5% horse serum, and premixed antibiotics (100 μg/ml streptomycin and 100 IU/ml penicillin, GIBCOBRL) under a humidified atmosphere of 5% CO_2 at 37°C.

Twenty-thousand of PC12 cells in 1 ml of the medium were placed in each well of a 24-well microplate and precultured under the same conditions. Twenty-four hours later, the medium was replaced by 1 ml of the serum-free MEME medium containing 1% DMSO and a test sample at various concentrations. The morphological changes of cells were monitored by a phase contrast microscope at every 24 h through 6 days. About one hundred cells were counted from a randomly chosen field and this was repeated 3 times.

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