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Halicylindrosides, Antifungal and Cytotoxic Cerebrosides from the Marine Sponge *Halichondria cylindrata*[1]

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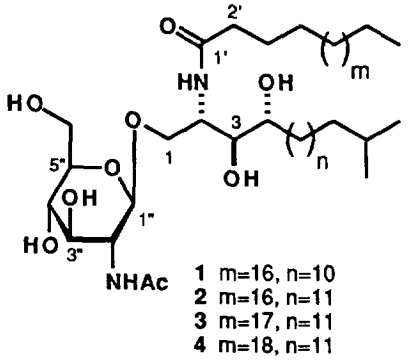
Abstract---Ten new glycosphingolipids, halicylindrosides A_1-A_4 (**1-4**) and B_1-B_6 (**5-10**) have been isolated from the Japanese marine sponge *Halichondria cylindrata*. Their structures were determined by spectroscopic and chemical methods. These cerebrosides were moderately antifungal against *Mortierella remanniana*, and cytotoxic against P388 murine leukemia cells

Marine sponges contain unusual lipid components such as phospholipids with long-chain or branched fatty acids[2] and sterols with unconventional side chains.[3] Sphingolipids or glycosphingolipids are a growing class of sponge metabolites, some of which have promising antitumor activity.[4] In the course of our screening for bioactive metabolites in Japanese marine invertebrates, the EtOH extract of the marine sponge *Halichondria cylindrata* was highly antifungal against *Mortierella remanniana*; the polar fraction contained cyclic tetradecapeptides, halicylindramides[5] and a macrolactam, cylindramide.[6] From the less polar fraction we have isolated *N*-acetylglucosaminyl ceramides, halicylindrosides A_1-A_4 (**1-4**) and B_1-B_6 (**5-10**). We describe the isolation and structure elucidation of these metabolites.

The $CHCl_3$-soluble portion of the EtOH extract of the frozen sponge (2.7 kg) was fractionated on Sephadex LH-20 and silica gel columns, followed by ODS HPLC to yield halicylindrosides A_1-A_4 (**1-4**) and B_1-B_6 (**5-10**) [**1**, 1.7 x 10^{-4}% yield wet weight; **2**, 1.9 x 10^{-4}%; **3**, 2.4 x 10^{-4}%; **4**, 1.5 x 10^{-4}%; **5**, 1.6 x 10^{-4}%; **6**, 1.2 x 10^{-4}%; **7**, 1.3 x 10^{-4}%; **8**, 3.6 x 10^{-4}%, **9**, 1.7 x 10^{-4}%; **10**, 2.4 x 10^{-4}%]. The halicylindrosides were moderately antifungal against *Mortierella remanniana* at 250 μg/disk and cytotoxic against P388 murine leukemia cells at 6.8 μg/mL.



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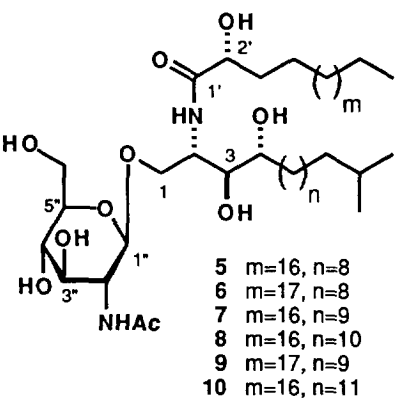

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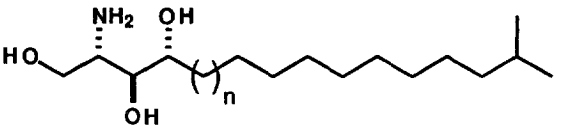
8 m=16, n=10
9 m=17, n=9
10 m=16, n=11

Table I. NMR Data of Halicylindrosides A_3 (**3**) and B_4 (**8**) in C_5D_5N

	3		**8**	
No	^1H	^{13}C	^1H	^{13}C
1	4.61 (dd, 6.7, 9.9)	68.8t	4.63 (m)	69.1t
	4.56 (dd, 4.1, 9.9)		4.59 (dd, 3.5, 10.2)	
2	5.13 (m)	51.8d	5.21 (m)	50.7d
3	4.30 (dd, 6.0, 5.7)	76.0d	4.33 (dd, 5.2, 6.6)	75.8d
4	4.19 (m)	72.6d	4.22 (m)	72.6d
5	2.19 (m), 1.90 (m)	33.8t	2.20 (m), 1.88 (m)	34.2t
6	1.65 (2H, m)	26.6t	1.63 (2H, m)	26.5t
7	1.35 (2H, m)	29.9t	1.34 (2H, m)	30.0t
8~16	1.20~1.32 (m)	29.6t	1.20-1.32 (m)	29.6t
17	1.48 (m)	22.7d	1.49 (m)	22.8d
18, 19	0.85 (6H, d, 6.6)	22.7q	0.86 (6H, d, 6.5)	22.8q
2-NH	8.47 (d, 8.5)	-	8.47 (d, 9.2)	-
1'	-	173.8s	-	175.7s
2'	2.47 (2H, t, 7.5)	36.9t	4.61 (m)	72.6d
3'	1.81 (2H, m)	26.4t	1.98 (m), 2.18 (m)	35.5t
4'	1.34 (2H, m)	29.6t	1.69 (m), 1.75 (m)	29.6t
5'~20'	1.20~1.32 (m)	29.6t	1.20-1.32 (m)	29.6t
21'	1.23 (m)	32.1t	1.23 (m)	32.1t
22'	1.27 (m)	22.9t	1.27 (m)	22.9t
23'	0.84 (3H, t, 7.0)	14.3q	0.85 (3H, t, 6.7)	14.3q
1"	5.31 (d, 8.3)	102.2d	5.12 (d, 8.3)	102.0d
2"	4.52 (dt, 10.2, 8.2)	57.5d	4.52 (m)	57.8d
3"	4.25 (t, 8.8)	76.9d	4.28 (dd, 7.5, 9.3)	77.1d
4"	4.13 (t, 9.3)	72.4d	4.20 (t, 9.2)	72.2d
5"	3.86 (m)	78.5d	3.87 (m)	78.5d
6"	4.47 (dd, 11.8, 2.4)	62.6t	4.47 (brd, 11.9)	62.4t
	4.24 (d, 11.7)		4.29 (m)	
Ac	-	171.8s	-	172.1s
	2.18 (3H, s)	22.9q	2.16 (3H, s)	23.4q
2"-NH	8.94 (d, 7.9)	-	8.77 (d, 7.6)	-

Halicylindroside A_3 (**3**) had a molecular formula of $C_{50}H_{98}N_2O_9$, as established by high resolution FAB mass spectrum and ^{13}C NMR data. IR bands at 3500-3100 and 1640 cm^{-1} suggested the presence of hydroxyl and amide groups. The ^1H NMR spectrum measured in pyridine-d_5 was well dispersed, and exhibited the presence of three methyls [δ 0.84 (3H, t), and 0.85 (6H, d)], an acetyl [δ 2.18 (3H, s)], two amides [δ 8.47 (d, *J*=8.5 Hz) and 8.94 (δ, *J*=7.9 Hz)], twelve oxygen- or nitrogen-bearing methine/methylene protons, including one anomeric proton [δ 5.31 (d, *J*=8.3 Hz)], and huge methylene envelopes at δ 1.24 and 1.30 together with a methylene signal [δ 2.47 (2H, t, *J*=7.5 Hz)] adjacent to a carbonyl group. These spectral data were reminiscent of a glycosphingolipid.

Interpretation of the COSY spectrum starting from the anomeric proton led to assignment of a 2-deoxy-2-amino hexopyranose unit. Coupling constants indicated axial orientation of methine protons in the sugar unit, while the presence of an acetamide at C2 was implied from the HMBC spectrum,[7] thereby identifying the hexose as β-*N*-acetylglucosamine (GlcNAc). Further analysis of the COSY spectrum resulted in connectivities from C1 to C6 of a phytosphingosine moiety with an amide group at C2; a crosspeak was observed between an amide proton (δ 8.47) and H2 methine (δ 5.13). The HMBC



11 n=1
12 n=2
13 n=3
14 n=4

spectrum showed that the amide proton was correlated with an amide carbon at δ 173.8, which was in turn correlated with methylene protons at δ 2.47 (2H, t) and 1.81 (2H, m), thereby indicating the cerebroside nature of **3**.

In order to determine the structure of the sphingosine unit which would lead to a gross structure, **3** was hydrolyzed with 1N HCl/MeOH to afford a C_{19} sphingosine base **14**, which had an isopropyl terminus [δ 0.83 (6H, d)], as revealed by the ^{1}H NMR spectrum. Therefore, the acyl residue of **3** must have a C_{23}-unbranched chain.

Halicylindrosides A_1-A_4 (**1-4**) showed the same R_f values on normal phase TLC, but different retention times in reversed phase HPLC, thus revealing difference in their carbon chains. In fact, the ^{1}H NMR spectra of **1-4** differed only in the shape of the methylene envelopes. Structures of **1**, **2**, and **4** were determined as follows: (1) The molecular formula was determined by high resolution FAB mass spectrum; (2) NMR data were compared with those of **3**, disclosing that they had β-GlcNAc and the C1-C6 portion of the phytosphingosine base in common; (3) The sphingosine base was obtained by methanolysis and the structure was assigned by the ^{1}H NMR and FAB mass spectra.

Due to the paucity of individual samples of **1-4**, stereochemistry of the sphingosine and GlcNAc units was determined by using a mixture of **1-4**. Methanolysis of the mixture followed by silica gel chromatography afforded a mixture of C_{21}-C_{24} fatty acids, sphingosine mixtures, and an anomeric mixture of GlcNAc methyl glycosides. ^{1}H NMR data of the tetraacetyl derivative of the sphingosine base together with its specific rotation allowed the assignment of 2*S*, 3*S*, 4*R* stereochemistry,[8,10] while the specific rotation of the methyl glycosides indicated D-configuration for GlcNAc.[9]

A mixture of halicylindrosides B_1-B_6 (**5-10**) was eluted immediately after halicylindrosides A in silica gel column chromatography, but they could be separated from each other by ODS HPLC with 98% MeOH containing 0.05 % TFA. Halicylindrosides B_1-B_6 had one more oxygen than **1-4**, which was also evident in the ^{1}H NMR spectra; an additional oxygenated methine was observed instead of a methylene adjacent to a carbonyl group. The chemical shifts and splitting patterns of key signals in the ^{1}H NMR spectra were almost superimposable, indicating that the stereochemistry of the sphingosine bases and of the monosaccharide was identical for the six compounds. The chain lengths and termini of the sphingosine bases as well as the structure of the α-hydroxy acid units were determined as described above for **1-4**.

The mixture of **5-10** was hydrolyzed with methanolic HCl to afford a mixture of α-hydroxy acid methyl esters, sphingosines, and anomeric methyl glycosides of GlcNAc, the latter two of which had identical ^{1}H NMR data and the same sign of specific rotation as those obtained from the mixture of **1-4**. An $[\alpha]_D$ value of -5.9° for the mixture of α-hydroxyacid methyl esters indicated 2'*R* stereochemistry for **5-10**.[10]

This work is the third report on the isolation of glycosphingolipids having phytosphingosine bases; previously described are antitumor agelasphins from *Agelas mauritianus*[4b,c] and antifungal acetylated

glycosphingolipids, amphicerebrosides from *Amphimedon viridis*.[4a] Several other ceramides and glycosphingolipids were reported from marine sponges.[11]

Experimental Section

General---Infrared spectra were determined on a JASCO FTIR-5300 IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded either on a Bruker AM-600, a JEOL α-500, a JEOL GSX-500, and a Bruker AC-300P NMR spectrometer. ^{1}H and ^{13}C NMR chemical shifts are referenced to solvent peaks: $\delta_{H/C}$ 7.19/123.5 for C_5D_5N, 7.24/77.0 for $CDCl_3$, and 3.30/49.0 for CD_3OD. Optical rotations were determined by a JASCO DIP-371 digital polarimeter. FAB mass spectra were measured on a JEOL JMX-SX102 mass spectrometer with glycerol or *m*-nitrobenzyl alcohol as a matrix.

Isolation---The sponge was collected by scuba (-5 to -15 m) off Atami, 90 km southwest of Tokyo, in 1992 and 1993. The specimen was identified as *Halichondria cylindrata* Tanita & Hoshino by Dr. Rob van Soest. The frozen sponge (1 kg, wet weight) of the 1992 collection was homogenized and extracted with EtOH (3 x 3 L). The combined extracts were concentrated and partitioned between $CHCl_3$ and H_2O. The 1993 collection (1.67 kg) was extracted with EtOH (3 x 3 L), 70% EtOH (2 x 3 L), EtOH (3 L), and MeOH/$CHCl_3$ (1:1, 2 x 3 L). The EtOH and 70% EtOH extracts were combined, and partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ phase from both collections and the MeOH/$CHCl_3$ extract of the 1993 collection were combined to give a dark semisolid. This material was subjected to Sephadex LH-20 column chromatography to yield eight fractions. The fourth fraction (678 mg) was chromatographed on a silica gel column with a MeOH/$CHCl_3$ solvent system. Two fractions of halicylindrosides A (136 mg) and B (158 mg) were obtained from the 10% MeOH/$CHCl_3$ fraction. The halicylindroside A fraction was separated by reversed-phase HPLC [Cosmosil ODS, 20 x 250 mm, flow rate 8 ml/min.; UV (210 nm) detection] with MeOH to yield halicylindrosides A_1 (**1**, 4.5 mg), A_2 (**2**, 5.2 mg), A_3 (**3**, 6.4 mg), and A_4 (**4**, 4.0 mg). Halicylindroside B fraction was subjected to ODS HPLC with 98% MeOH containing 0.05% TFA to affored halicylindrosides B_1 (**5**, 4.2 mg), B_2 (**6**, 3.1 mg), B_3 (**7**, 3.5 mg), B_4 (**8**, 9.6 mg), B_5 (**9**, 4.5 mg), and B_6 (**10**, 6.6 mg).

Halicylindroside A_1 (1): colorless solid; $[\alpha]_D^{23}$ -20.2° (*c* 0.20, pyridine); FABMS (neg.) *m/z* 841 $(M-H)^-$, 638 (M-GlcNAc-H)$^-$; HRFABMS (neg.) *m/z* 841.6856 (calcd for $C_{48}H_{93}N_2O_9$, Δ -2.5 mmu); IR (neat) 3500-3100, 2930, 2830, 1640, 1550, 1080 cm^{-1}; ^{1}H NMR (C_5D_5N): δ 8.98 (1H, d, *J*=7.9 Hz, NH), 8.59 (1H, d, *J*=8.5 Hz, NH), 5.15 (1H, d, *J*=8.5 Hz, H-1"), 5.15 (1H, m, H-2), 4.56 (1H, dd, *J*=6.7, 11.0 Hz, H-1a), 4.53 (1H, dd, *J*=4.0, 10.7 Hz, H-1b), 4.50 (1H, dt, *J*=9.8, 9.6 Hz, H-2"), 4.45 (1H, dd, *J*=11.9, 2.5 Hz, H-6"a), 4.26 (1H, t, *J*=6.1 Hz, H-3), 4.22 (2H, m, H-3", 6"b), 4.16 (1H, m, H-4), 4.14 (1H, t, *J*=9.9 Hz, H-4"), 3.83 (1H, m, H-5"), 2.46 (2H, t, *J*=7.6 Hz, H-2'), 2.20 (1H, m, H-5a), 2.17 (3H, s, Ac), 1.90 (1H, m, H-5b), 1.80 (2H, m, H-3'), 1.62 (2H, m, H-6), 1.50 (1H, m, H-16), 1.2-1.4 (m), 0.85 (9H, m, terminal Me). ^{13}C NMR (C_5D_5N): δ 102.3d (C-1"), 78.8d (C-5"), 77.0d (C-3"), 76.0d (C-3), 72.7d (C-4), 72.5d (C-4"), 68.9t (C-1), 62.7t (C-6"), 57.7d (C-2"), 51.9d (C-2), 36.9d (C-2'), 33.9d (C-5), 32.2d, 31.9-29.5d, 26.5d (C-6), 26.3d (C-3'), 23.6q (Ac), 22.8 (C-16, 17, 18), 14.3q (C-22).

Halicylindroside A_2 (2): colorless solid; $[\alpha]_D^{23}$ -21.1° (*c* 0.21, pyridine); FABMS (neg.) *m/z* 855 $(M-H)^-$, 652 (M-GlcNAc-H)$^-$; HRFABMS (neg.) *m/z* 855.7053 (calcd for $C_{49}H_{95}N_2O_9$, Δ +1.5

mmu); IR (neat) 3500-3100, 2930, 2830, 1640, 1550, 1080 cm^{-1}; ^1H NMR (C_5D_5N): δ 8.93 (1H, d, *J*=8.6 Hz, NH), 8.45 (1H, d, *J*=8.7 Hz, NH), 5.12 (1H, d, *J*=8.4 Hz, H1"), 5.12 (1H, m, H-2), 4.56 (1H, dd, *J*=6.7, 11.0 Hz, H-1a), 4.53 (1H, dd, *J*=4.0, 10.8 Hz, H-1b), 4.47 (1H, dt, *J*=9.8, 9.6 Hz, H-2"), 4.41 (1H, dd, *J*=11.9, 2.5 Hz, H-6"a), 4.25 (1H, m, H-3), 4.20 (2H, m, H-3", 6"b), 4.15 (1H, m, H-4), 4.12 (1H, t, *J*=9.8 Hz, H-4"), 3.82 (1H, m, H-5"), 2.43 (2H, t, *J*=7.5 Hz, H-2'), 2.17 (1H, m, H-5a), 2.15 (3H, s, Ac), 1.89 (1H, m, H-5b), 1.78 (2H, m, H-3'), 1.60 (2H, m, H-6), 1.50 (1H, m, H-17), 1.2-1.4 (m), 0.83 (9H, m, terminal Me).

Halicylindroside A3 (3): colorless solid; $[\alpha]_D^{23}$ -19.5° (*c* 0.20, pyridine); FABMS (neg.) *m/z* 869 $(M-H)^-$, 666 $(M-GlcNAc-H)^-$; HRFABMS (neg.) *m/z* 869.7187 (calcd for $C_{50}H_{97}N_2O_9$, Δ -0.7 mmu); IR (neat) 3500-3100, 2930, 2830, 1640, 1550, 1080 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table I.

Halicylindroside A4 (4): colorless solid; $[\alpha]_D^{23}$ -22.3° (*c* 0.22, pyridine); FABMS (neg.) *m/z* 883 $(M-H)^-$, 680 $(M-GlcNAc-H)^-$; HRFABMS (neg.) *m/z* 883.7371 (calcd for $C_{51}H_{99}N_2O_9$, Δ +2.0 mmu); IR (neat) 3500-3100, 2930, 2830, 1640, 1550, 1080 cm^{-1}; ^1H NMR (C_5D_5N): δ 8.97 (1H, d, *J*=8.2 Hz, NH), 8.49 (1H, d, *J*=8.7 Hz, NH), 5.15 (1H, d, *J*=8.4 Hz, H-1"), 5.15 (1H, m, H-2), 4.60 (1H, dd, *J*=6.7, 11.0 Hz, H-1a), 4.55 (1H, dd, *J*=3.9, 11.0 Hz, H-1b), 4.50 (1H, dt, *J*=9.8, 9.6 Hz, H-2"), 4.45 (1H, dd, *J*=11.9, 2.5 Hz, H-6"a), 4.27 (1H, m, H-3), 4.20 (2H, m, H-3", 6"b), 4.15 (1H, m, H-4), 4.12 (1H, t, *J*=9.8 Hz, H-4"), 3.82 (1H, m, H-5"), 2.43 (2H, t, *J*=7.4 Hz, H-2'), 2.20 (1H, m, H-5a), 2.18 (3H, s, Ac), 1.91 (1H, m, H-5b), 1.81 (2H, m, H-3'), 1.63 (2H, m, H-6), 1.51 (1H, m, H-17), 1.2-1.4 (m), 0.83 (9H, m, terminal Me). ^{13}C NMR (C_5D_5N): δ 102.2d (C-1"), 78.6d (C-5"), 76.9d (C-3"), 76.1d (C-3), 72.6d (C-4), 72.4d (C-4"), 69.0t (C-1), 62.6t (C-6"), 57.6d (C-2"), 51.9d (C-2), 36.9d (C-2'), 33.9d (C-5), 32.1d, 30.4-30.0d, 26.6d (C-6), 26.4d (C-3'), 23.6q (Ac), 22.9t (C-23'), 22.8 (C-17, 18, 19), 14.3q (C-24').

Halicylindroside B1 (5): colorless solid; $[\alpha]_D^{23}$ -9.2° (*c* 0.15, pyridine); FABMS (neg.) *m/z* 829 $(M-H)^-$, 626 $(M-GlcNAc-H)^-$; HRFABMS (neg.) *m/z* 829.6500 (calcd for $C_{46}H_{89}N_2O_{10}$, Δ -1.7 mmu); IR (neat) 3500-3100, 2930, 2830, 1650, 1550, 1080 cm^{-1}; ^1H NMR (C_5D_5N): δ 8.85 (1H, d, *J*=7.6 Hz, NH), 8.52 (1H, d, *J*=9.4 Hz, NH), 5.24 (1H, m, H-2), 5.14 (1H, d, *J*=8.2 Hz, H-1"), 4.61 (1H, dd, *J*=6.7, 11.1 Hz, H-1a), 4.60 (1H, m, H-2'), 4.57 (1H, dd, *J*=4.5, 11.6 Hz, H-1b), 4.51 (1H, dt, *J*=9.8, 8.2 Hz, H-2"), 4.45 (1H, dd, *J*=11.9, 2.2 Hz, H-6"a), 4.32 (1H, dd, *J*=5.2, 6.7 Hz, H-3), 4.27 (1H, m, H-6"b), 4.24 (1H, t, *J*=9.5 Hz, H-3"), 4.19 (1H, t, *J*=8.8 Hz, H-4"), 4.18 (1H, m, H-4), 3.83 (1H, m, H-5"), 2.21 (1H, m, H-5a), 2.19 (1H, m, H-3'a), 2.17 (3H, s, Ac), 2.00 (1H, m, H-3b'), 1.90 (1H, m, H-5b), 1.75 (1H, m, H-4'a), 1.69 (1H, m, H-4'b), 1.63 (2H, m, H-6), 1.50 (1H, m, H-14), 1.2-1.45 (m), 0.85 (9H, m, terminal Me).

Halicylindroside B2 (6): colorless solid; $[\alpha]_D^{23}$ -9.0° (*c* 0.15, pyridine); FABMS (neg.) *m/z* 843 $(M-H)^-$, 640 $(M-GlcNAc-H)^-$; HRFABMS (neg.) *m/z* 843.6641 (calcd for $C_{47}H_{91}N_2O_{10}$, Δ -3.2 mmu); IR (neat) 3500-3100, 2930, 2830, 1650, 1550, 1080 cm^{-1}; ^1H NMR (C_5D_5N): δ 8.88 (1H, d, *J*=7.9 Hz, NH), 8.52 (1H, d, *J*=8.9 Hz, NH), 5.24 (1H, m, H-2), 5.14 (1H, d, *J*=8.2 Hz, H-1"), 4.62 (1H, m, H-1a), 4.60 (1H, m, H-2'), 4.57 (1H, dd, *J*=3.7, 10.8 Hz, H-1b), 4.54 (1H, dt, *J*=9.2, 8.2 Hz, H-2"), 4.45 (1H, brd, *J*=11.0 Hz, H-6"a), 4.31 (1H, dd, *J*=5.2, 6.7 Hz, H-3), 4.27 (1H, m, H-6"b), 4.25 (1H, t, *J*=9.3 Hz, H-3"), 4.19 (1H, t, *J*=8.8 Hz, H-4"), 4.18 (1H, m, H-4), 3.83 (1H, m, H-5"), 2.21 (1H, m, H-5a), 2.19 (1H, m, H-3'a), 2.17 (3H, s, Ac), 2.00 (1H, m, H-3b'), 1.90 (1H, m,

H-5b), 1.75 (1H, m, H-4'a), 1.69 (1H, m, H-4'b), 1.63 (2H, m, H-6), 1.50 (1H, m, H-14), 1.2-1.45 (m), 0.86 (9H, m, terminal Me).

Halicylindroside B3 (**7**): colorless solid; $[\alpha]_D^{23}$ -9.7° (*c* 0.15, pyridine); FABMS (neg.) *m/z* 843 $(M-H)^-$, 640 $(M\text{-}GlcNAc\text{-}H)^-$; HRFABMS (neg.) *m/z* 843.6641 (calcd for $C_{47}H_{91}N_2O_{10}$, Δ -3.2 mmu); IR (neat) 3500-3100, 2930, 2830, 1650, 1550, 1080 cm^{-1}; ^1H NMR (C_5D_5N): δ 8.97 (1H, d, *J*=7.9 Hz, NH), 8.53 (1H, d, *J*=9.3 Hz, NH), 5.24 (1H, m, H-2), 5.14 (1H, d, *J*=8.5 Hz, H-1"), 4.62 (1H, m, H-1a), 4.60 (1H, m, H-2'), 4.57 (1H, dd, *J*=4.3, 11.3 Hz, H-1b), 4.49 (1H, dt, *J*=9.7, 8.2 Hz, H-2"), 4.44 (1H, dd, *J*=11.6, 2.2 Hz, H-6"a), 4.31 (1H, dd, *J*=5.4, 6.7 Hz, H-3), 4.27 (1H, dd, J=2.7, 12.5 Hz, H-6"b), 4.24 (1H, t, *J*=9.1 Hz, H-3"), 4.19 (1H, t, *J*=9.2 Hz, H-4"), 4.18 (1H, m, H-4), 3.82 (1H, m, H-5"), 2.21 (1H, m, H-5a), 2.18 (1H, m, H-3'a), 2.17 (3H, s, Ac), 2.00 (1H, m, H-3b'), 1.89 (1H, m, H-5b), 1.75 (1H, m, H-4'a), 1.69 (1H, m, H-4'b), 1.63 (2H, m, H-6), 1.50 (1H, m, H-15), 1.2-1.45 (m), 0.85 (9H, brd, *J*=6.7 Hz, terminal Me).

Halicylindroside B4 (**8**): colorless solid; $[\alpha]_D^{23}$ -8.5° (*c* 0.15, pyridine); FABMS (neg.) *m/z* 857 $(M-H)^-$, 654 $(M\text{-}GlcNAc\text{-}H)^-$; HRFABMS (neg.) *m/z* 857.6899 (calcd for $C_{48}H_{93}N_2O_{10}$, Δ +6.9 mmu); IR (neat) 3500-3100, 2930, 2830, 1650, 1550, 1080 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table I.

Halicylindroside B5 (**9**): colorless solid; $[\alpha]_D^{23}$ -8.6° (*c* 0.15, pyridine); FABMS (neg.) *m/z* 857 $(M-H)^-$, 654 $(M\text{-}GlcNAc\text{-}H)^-$; HRFABMS (neg.) *m/z* 857.6788 (calcd for $C_{48}H_{93}N_2O_{10}$, Δ -4.2 mmu); IR (neat) 3500-3100, 2930, 2830, 1650, 1550, 1080 cm^{-1}; ^1H NMR (C_5D_5N): δ 8.90 (1H, d, *J*=7.6 Hz, NH), 8.53 (1H, d, *J*=9.1 Hz, NH), 5.23 (1H, m, H-2), 5.14 (1H, d, *J*=8.6 Hz, H-1"), 4.61 (1H, dd, *J*=7.0, 11.0 Hz, H-1a), 4.60 (1H, m, H-2'), 4.57 (1H, dd, *J*=4.5, 11.6 Hz, H-1b), 4.51 (1H, dt, *J*=9.4, 8.6 Hz, H-2"), 4.45 (1H, dd, *J*=11.9, 2.2 Hz, H-6"a), 4.32 (1H, dd, *J*=5.2, 6.7 Hz, H-3), 4.27 (1H, m, H-6"b), 4.24 (1H, t, *J*=9.5 Hz, H-3"), 4.19 (1H, t, *J*=8.8 Hz, H-4"), 4.18 (1H, m, H-4), 3.83 (1H, m, H-5"), 2.21 (1H, m, H-5a), 2.19 (1H, m, H-3'a), 2.17 (3H, s, Ac), 2.00 (1H, m, H-3b'), 1.90 (1H, m, H-5b), 1.75 (1H, m, H-4'a), 1.69 (1H, m, H-4'b), 1.63 (2H, m, H-6), 1.50 (1H, m, H-15), 1.2-1.45 (m), 0.85 (9H, m, terminal Me).

Halicylindroside B6 (**10**): colorless solid; $[\alpha]_D^{23}$ -8.3° (*c* 0.14, pyridine); FABMS (neg.) *m/z* 871 $(M-H)^-$, 668 $(M\text{-}GlcNAc\text{-}H)^-$; HRFABMS (neg.) *m/z* 871.7037 (calcd for $C_{49}H_{95}N_2O_{10}$, Δ +5.0 mmu); IR (neat) 3500-3100, 2930, 2830, 1650, 1550, 1080 cm^{-1}; ^1H NMR (C_5D_5N): δ 8.92 (1H, d, *J*=7.6 Hz, NH), 8.53 (1H, d, *J*=9.4 Hz, NH), 5.24 (1H, m, H-2), 5.14 (1H, d, *J*=8.4 Hz, H-1"), 4.61 (1H, m, H-1a), 4.60 (1H, m, H-2'), 4.57 (1H, dd, *J*=3.9, 11.0 Hz, H-1b), 4.51 (1H, dt, *J*=9.8, 8.9 Hz, H-2"), 4.45 (1H, brd, *J*=11.5 Hz, H-6"a), 4.30 (1H, dd, *J*=5.2, 6.7 Hz, H-3), 4.27 (1H, m, H-6"b), 4.24 (1H, t, *J*=9.4 Hz, H-3"), 4.18 (1H, t, *J*=8.8 Hz, H-4"), 4.17 (1H, m, H-4), 3.82 (1H, m, H-5"), 2.21 (1H, m, H-5a), 2.19 (1H, m, H-3'a), 2.17 (3H, s, Ac), 2.00 (1H, m, H-3b'), 1.90 (1H, m, H-5b), 1.75 (1H, m, H-4'a), 1.69 (1H, m, H-4'b), 1.63 (2H, m, H-6), 1.50 (1H, m, H-17), 1.2-1.45 (m), 0.85 (9H, m, terminal Me).

Acid Methanolysis of a Mixture of 1-4: A 15 mg portion of a mixture of **1-4** was heated at 80 °C in 2.0 mL of 1N HCl/MeOH (18:82) overnight. The reaction mixture was evaporated by freeze-drying. The residue was subjected to silica gel column chromatography with a MeOH/$CHCl_3$ solvent system. The $CHCl_3$ eluate gave a mixture of the methyl esters of C_{21}-C_{24} fatty acids; FABMS (pos.) *m/z* 341, 355, 369, 383 $(M+H)^+$; ^1H NMR ($CDCl_3$): δ 3.61 (3H, s, COOMe), 2.23 (2H, t, *J*=7.6 Hz,

H-2), 1.50 (2H, m, H-3), 1.2-1.4 (m), 0.89 (3H, t, *J*=6.9 Hz, terminal Me). MeOH/$CHCl_3$ (1:4) fraction afforded a mixture of sphingosine bases (3.2 mg); FABMS (pos.) *m/z* 290, 304, 318, 332 $[M+H]^+$; ^1H NMR (C_5D_5N): δ 4.72 (3H, m), 4.59 (1H, m), 4.18 (1H, brt, *J*=6.1, 4.6 Hz), 2.26 (1H, m), 1.85 (1H, m), 1.77 (1H, m), 1.60 (1H, m), 1.45 (1H, m), 1.37-1.20, 0.83 (3H, d, *J*=6.7 Hz, terminal Me); ^1H NMR (500 MHz, in CD_3OD:$CDCl_3$/1:1): δ 3.88 (1H, dd, *J*=11.6, 3.4 Hz), 3.75 (1H, dd, *J*=11.3, 7.6 Hz), 3.51 (1H, dd, *J*=6.1, 4.6 Hz), 3.45 (2H, m), 1.75 (1H, m), 1.50 (1H, m), 1.37-1.20, 0.81 (6H, t, *J*=6.5 Hz, terminal Me); ^{13}C NMR (C_5D_5N): δ 73.7, 72.8, 59.3, 57.2, 39.2, 34.5, 30.2, 29.9, 26.0, 22.7. The MeOH eluate yielded an anomeric mixture of the methyl glycosides of glucosamine (2.6 mg); $[\alpha]_D^{23}$ +120.3° (*c* 0.13, H_2O); FABMS (pos.) *m/z* 194 $[M+H]^+$, 216 $[M+Na]^+$; ^1H NMR (C_5D_5N): δ 5.47 (0.9H, brs, H-1α), 4.79 (0.1H, d, *J*=7.9 Hz, H-1β), 4.38 (brd, *J*=10.4 Hz), 4.30 (dd, *J*=11.5, 4.7 Hz), 4.13 (m), 3.53 (brd, *J*=10.4 Hz), 3.37 (3H, s, OMe); ^{13}C NMR (C_5D_5N): δ [104.1], 100.4, [78.5, 76.8], 74.5, 74.3, 71.8, 62.3, [58.4], 56.5, 54.9. [Small peaks ascribed to the β-anomer are bracketed.]

Acetylation of a Mixture of the Sphingosine Bases: A mixture of the sphingosine bases (3.2 mg) isolated from the acid hydrolysate of a mixture of **1-4** was treated with Ac_2O/pyridine (1:1, 0.5 mL) overnight at rt. The reaction mixture was diluted with 2 mL of H_2O and extracted with $CHCl_3$ (2 mL x 3). The $CHCl_3$ extract was subjected to silica gel column chromatography to furnish a mixture of peracetylated sphingosine bases (3.8 mg); $[\alpha]_D^{23}$ +29.6° (*c* 0.19, $CHCl_3$); FABMS (pos.) *m/z* 458, 472, 486, 550 $[M+H]^+$; ^1H NMR ($CDCl_3$): δ 5.99 (1H, d, *J*=9.5 Hz), 5.08 (1H, dd, *J*=8.2, 3.1 Hz), 4.92 (1H, dt, *J*=10.0, 3.1 Hz), 4.46 (1H, m), 4.28 (1H, dd, *J*=11.9, 4.1 Hz), 3.98 (1H, dd, *J*=11.6, 3.0 Hz), 2.07 (3H, s), 2.04 (6H, s), 2.02 (3H, s), 1.62 (2H, m), 1.47 (1H, m), 1.20-1.42 (m), 0.85 (6H, d, *J*=6.8 Hz); ^{13}C NMR ($CDCl_3$): δ 171.2, 170.9, 170.1, 169.8, 73.0, 71.9, 62.8, 47.6, 39.1, 31.9, 29.6, 29.3, 28.1, 25.5, 23.3, 22.7, 21.1, 20.8.

Acid Methanolysis of Each Cerabroside: A 2.0 mg portion of **3** was heated at 80 °C in 1.0 mL of 1N HCl/MeOH (18:82) overnight. The reaction mixture was evaporated and chromatographed on a short silica gel column. Sphingosine **14** was obtained from the 25% MeOH/$CHCl_3$ fraction as a colorless solid. **14**: FABMS (pos.) *m/z* 332 $[M+H]^+$; ^1H NMR (C_5D_5N): δ 4.72 (3H, m), 4.59 (1H, m), 4.18 (1H, brt, *J*=6.1, 4.6 Hz), 2.26 (1H, m), 1.85 (1H, m), 1.77 (1H, m), 1.60 (1H, m), 1.45 (1H, m), 1.37-1.20, 0.83 (6H, d, *J*=6.7 Hz, terminal Me). The compounds **1**, **2**, **4-10** were treated similarly to yield **13** [*m/z* 318 $(M+H)^+$], **14** [*m/z* 332 $(M+H)^+$], **14** [*m/z* 332 $(M+H)^+$], **11** [*m/z* 290 $(M+H)^+$], **11** [*m/z* 290 $(M+H)^+$], **12** [*m/z* 304 $(M+H)^+$], **13** [*m/z* 318 $(M+H)^+$], **12** [*m/z* 304 $(M+H)^+$], and **14** [*m/z* 332 $(M+H)^+$], respectively.

Acid Methanolysis of a Mixture of 5-10: A mixture of **5-10** (14 mg) was methanolyzed as described above to yield a mixture of sphingosine bases, an anomeric mixture of the methyl glycosides of glucosamine, and a mixture of methyl esters of α-hydroxy C_{22}-C_{24} fatty acids (3.1 mg); $[\alpha]_D^{23}$ -5.9° (*c* 0.16, $CHCl_3$); FABMS (pos.) *m/z* 371, 385, 399 $(M+H)^+$; ^1H NMR ($CDCl_3$): δ 4.17 (1H, dd, *J*=7.4, 4.0 Hz, H-2), 3.73 (3H, s, COOMe), 1.77 (2H, m), 1.61 (2H, m), 1.2-1.4 (m), 0.88 (3H, t, *J*=7.0 Hz, terminal Me). The mixture of sphingosine bases was acetylated to yield the mixture of tetraacetyl derivatives which showed an ^1H NMR spectrum essentially identical with that of the tetraacetyl sphingosine mixture of **1-4**.

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