New cerebrosides from the marine sponge *Oceanapia* sp.

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New cerebrosides containing *N*-acetylglucosamine were isolated from the marine sponge *Oceanapia* sp. Some of them contain *n*- and *iso*-C_{18}- and *iso*-C_{19}-phytosphingosines N-acylated by *n*-$C_{16:0}$, *n*-$C_{17:0}$, and *n*-$C_{18:0}$ fatty acids, some other are derivatives of *iso*- and *anteiso*-C_{17}-, -C_{18}-, and -C_{19}-phytosphingosines N-acylated by long-chain (C_{24}—C_{28}) α-hydroxy acids. The structures of these compounds were determined by NMR spectroscopy, MALDI-TOF mass spectrometry, and by chemical transformations.

Key words: marine sponge; *Oceanapia* sp.; glycosphingolipids; cerebrosides; NMR; MALDI-TOF mass spectra; GLC-MS.

Many marine invertebrates are plentiful sources of sphingolipids, in particular, cerebrosides, which differ in structures and biological properties from analogous glycosphingolipids of the terrestrial origin. Unusual cerebrosides were isolated from sponges, coelenterates, annelids, and starfishes. As a rule, these contain unique sphingosine bases, in particular, having branched structures, rare fatty acids and sugars that are linked to the base by either β-or α–glycosidic bonds. These compounds exhibit antifungal, cytotoxic, immunostimulating, hypotensive, and wound healing activities; they inhibit the activities of some enzymes.[1,2] Some cerebrosides isolated from sponges possess the most interesting properties. For example, α-galactosylceramides from *Agelas mauritianus* showed a high antitumor activity without being cytotoxic. A synthetic analog of these ceramides, the so-called KRN 7000, is under clinical tests in several countries as a new antitumor drug.[1]

Sponges of the *Oceanapia* genus contain a series of unusual sphingolipids and sphingolipid-like compounds and a series of other biologically active secondary metabolites. The following compounds have previously been isolated from their extracts: a bipolar lipid oceanapiside,[3] a unique alkaloidolipid oceanalin containing tetrahydroisoquinoline and sphingosine-like ends of the molecules,[4] new ceramides,[5] alkaloids of the pyridoacridine, pyrrolopyridine[6—9] and sesquiterpene[10] series dithiocyanates,[11] bromo-substituted polyunsaturated C_{16} fatty acids,[12] acetylenic C_{14} acids,[13] and bromotyrosine alkaloids.[14]

In a continuation of the investigation of biologically active secondary metabolites from the sponge *Oceanapia* sp.,[4] we isolated cerebrosides **1** and **2** and determined their chemical structure. They proved to be new modifications of *N*-acetylglucosamine-containing glycosphingolipids. Previously, *N*-acetylaminoglucopyranosylceramides were found in the sponges *Amphimedon viridis*[15] and *Halichondria cylindrata*.[16]

Results and Discussion

Cerebrosides **1** and **2** were isolated from an ethanolic extract of *Oceanapia* sp. after partition between butanol and water, repeated column chromatography of the butanol-soluble components on silica gel, Sephadex LH-20, and Amberlite XAD-2, and recrystallization of the fraction thus obtained from methanol followed by its HPLC separation.

The ^{1}H NMR spectrum (C_5D_5N) (Table 1) of cerebrosides **1** contains signals for protons of three types of methyl groups, namely, terminal groups in a normal chain, in the isopropyl fragment, and in the *N*-acetyl fragment (δ 0.87 (t, 3 H); 0.86 (d, 6 H), 2.18 (s)). The signals for two amide protons are observed at δ 8.47 (d, J = 8.4 Hz) and δ 8.97 (d, J = 8.4 Hz). The spectrum also contains signals for protons of the methine/methylene groups attached to oxygen or nitrogen atoms, including the anomeric proton of the monosaccharide residue (δ 5.18 (d, J = 8.4 Hz)). An intensive signal at δ 1.20—1.39 for the $(CH_2)_n$ fragment and a signal at δ 2.50 (t, J = 7.5 Hz) for the methylene group located near the carbonyl group suggest the presence of hydrocarbon groups, in particular, fatty acid residues.

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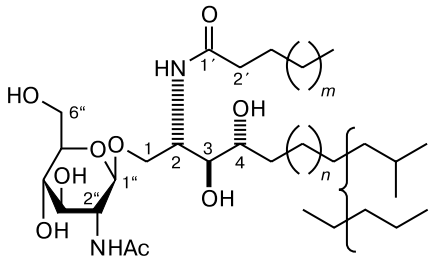


1

m = 12, 13, 14; *n* = 8, 9

Analysis of the ^1H and ^{13}C NMR spectra and a comparison with the spectra of the known cerebrosides suggest that compound **1** is a phytosphingosine derivative N-acylated by a non-hydroxylated saturated fatty acid and O(1)-substituted by *N*-acetyl-β-glucosamine. Indeed, the spectra, in particular, their parts containing signals for the monosaccharide, almost coincided with the spectra of the previously known by *N*-acetyl-β-D-glucosaminylceramides with phytosphingosine as the base.[16]

To determine the chain length of fatty acids and sphingosine bases, we carried out methanolysis of **1**. GLC/MS analysis of the fatty acid methyl esters (Table 2) showed that palmitic acid is the major acid in cerebroside **1** (more than 70% of the total fraction of fatty acids). The other fatty acids (17:0 and 18:0) account for about 12% and 16%, respectively. The NMR spectra of this mixture of methyl esters indicate that these fatty acids are normal (the methyl

Table 1. ^1H and ^{13}C NMR spectra (δ, *J*/Hz, C_5D_5N) of cerebrosides **1** and **2**

Atom, group	**1**			**2**		
	δ_H	δ_C	HMBC[a]	δ_H	δ_C	HMBC[a]
1	4.64 (dd, *J* = 6.6, *J* = 11.0)	68.9	C(2), C(3)	4.64 (dd, *J* = 6.6, *J* = 11.3)	69.0	C(2), C(1″)
	4.59 (dd, *J* = 4.4, *J* = 11.0)		C(2), C(3)	4.60 (dd, *J* = 4.3, *J* = 11.3)		C(2), C(3), C(1″)
2	5.18 (m)	51.9		5.27 (m)	50.7	
3	4.32 (dd, *J* = 6.2, *J* = 10.2)	76.1	C(1), C(2), C(4), C(5)	4.32 (dd, *J* = 6.2, *J* = 10.2)	75.9	C(1), C(2), C(5)
4	4.22 (m)	72.7	C(3)	4.22 (m)	72.6	
5		33.9		3.85 (m)	34.6	
$-CH_2-$	1.20—1.39 (br.s)	29.8—30.5		1.20—1.39 (br.s)	29.8—30.5	
=CH	—	—		5.49 (m)	130.2	C(20), C(23)
Me [b]	0.88 (t, *J* = 7.0)	14.3		0.86 (t, *J* = 7.0)	14.3	
Me [c]	0.87 (d, *J* = 6.6)	22.8			22.9	
		23.5			23.4	
Me [d]	—	—			11.6	
					19.4	
2-NH	8.47 (d, *J* = 8.4)		C(1)	8.53 (d, *J* = 9.3)		
1′		173.7			175.7	
2′	2.50 (t, *J* = 7.5)	36.9	C(3′), C(1′), $-CH_2-$	2.62 (m)	72.6	C(1′)
3′	1.83 (m)	26.4	C(2′), $-CH_2-$	2.01 (m)	35.6	
				2.10 (m)		C(4′)
4′				1.78 (m)	29.7	
				1.71 (m)		
1″	5.18 (d, *J* = 8.4)	102.2	C(1)	5.17 (d, *J* = 8.3)	102.0	
2″	4.54 (dt, *J* = 8.4, *J* = 10.1)	57.7	C(1″), C(3″)	4.52 (dt, *J* = 8.0, *J* = 10.0)	57.8	C(1″), C(3″)
3″	4.28 (dd, *J* = 8.4, *J* = 8.8)	76.9	C(2″), C(4″)	4.27 (dd, *J* = 8.9, *J* = 10.0)	77.2	C(2″), C(4″)
4″	4.18 (t, *J* = 8.8)	72.4	C(3″), C(5″), C(6″)	4,21 (t, *J* = 8.9)	72.3	C(3″), C(5″), C(6″)
5″	3.88 (m)	78.6		3.85 (m)	78.5	
6″	4.49 (dd, *J* = 2.6, *J* = 11.9)	62.6	C(4″), C(5″)	4.47 (dd, *J* = 2.7, *J* = 11.9)	62.4	C(5″)
	4.30 (dd, *J* = 5.5, *J* = 11.9)			4.32 (dd, *J* = 5.3, *J* = 11.9)		
Ac	2.18 (s)	171.8	CO	2.18 (s)	172.1	
			C(3)			
2″-NH	8.97 (d, *J* = 7.9)		—COMe	8.85 (d, *J* = 7.6)		—COMe

[a] 500 MHz in C_5D_5N.
[b] Terminal methyl groups in normal side chains.
[c] Terminal methyl groups in *iso* side chains.
[d] Terminal methyl groups in *anteiso* side chains.

Table 2. Fatty acid composition of cerebrosides **1** from the sponge *Oceanapia* sp.*

Fatty acid methyl ester	Content (%)	M^+, *m/z* (EI)
16:0	72.38	270
17:0	11.51	284
18:0	16.11	298

* M^+ and the percentages are given for the fatty acid methyl esters.

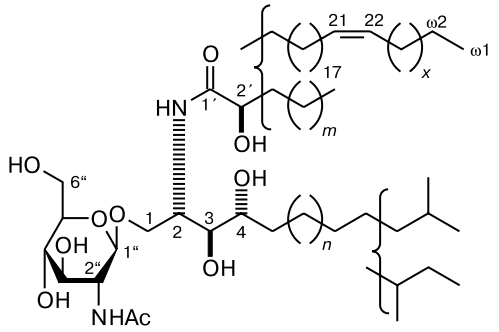
protons and the methyl carbon atom gave only the signals with δ_H 0.88 (t, 3 H, J = 7.1 Hz) and δ_C 14.1, respectively).

The sphingosine bases obtained by methanolysis were analyzed as peracetates by MALDI-TOF mass spectrometry. The pseudomolecular ion peaks $[M + Na]^+$ with *m/z* 508 and 522 corresponded to C_{18}- and C_{19}-phytosphingosines. The ^1H NMR spectrum of the sum of acetylated bases exhibited signals for three methine groups bound to heteroatoms, an NH doublet at δ 5.95, and two doublets of doublets for the CH_2 protons of the CH_2OAc group. A triplet at δ 0.88 and a doublet at δ 0.86 attest to the presence of both normal and *iso*-structured sphingosine bases.

The mutual position of the functional groups in the sphingosine bases was confirmed by HMBC experiments. The spectrum of **1** exhibits cross-peaks (δ): H(1)/C(2), 4.64/51.9 and 4.59/51.9; H(1)/C(3), 4.64/76.1 and 4.59/76.1; H(3)/C(1), 4.32/68.8; H(3)/C(2), 4.32/51.9; and H(3)/C(4), 4.32/72.6. The chemical shifts of the protons at the C(1)—C(4) atoms and of the corresponding carbon atoms are known to be sensitive to the stereochemistry of the C(2), C(3), and C(4) asymmetric centers.[17] The fact that the NMR spectra of cerebrosides **1** coincide with the spectra of cerebrosides from chalicylindroside A(1)—A(4) from *H. cylindrata* suggests that chiral centers in these compounds have the same relative configurations.

Thus, cerebrosides **1** are derivatives of normal and *iso*-C_{18}- and *iso*-C_{19}-phytosphingosines N-acylated by nonhydroxylated unbranched 16:0-, 17:0-, and 18:0-fatty acids and substituted at O(1) by *N*-acetyl-β-glucosamine. Cerebrosides of the type **1** are new variants of natural glycosylceramides differing from chalicylindrosides A_1—A_4, isolated previously[6] only in the length of the fatty acid residues. Indeed, the phytosphingosine amino group in cerebrosides from *H. cylindrata,* unlike compound **1**, is acylated by *n*-21:0-, *n*-22:0-, *n*-23:0-, and *n*-24:0-acids.

The signals in the ^1H and ^{13}C NMR spectra of cerebrosides **2** (see Table 1) were similar to the corresponding signals in the spectra of chalicylindrosides B_1—B_6 (see Ref. 16): *iso*-C_{16}-, *iso*-C_{17}-, *iso*-C_{18}-, and *iso*-C_{19}-phytosphingosines N-acylated by α-hydroxy acids and O(1)-substituted by *N*-acetyl-β-glucosamine. The NMR spectra of **2** differed only in the presence of signals for the double bond (δ_H 5.49 (m) and δ_C 130.3) and signals for the *anteiso*-branching at the end of the hydrocarbon chain (δ_C 11.6 and 19.4).



2

x = 2, 4; *m* = 20, 21, 22; *n* = 6, 7, 8

Methanolysis of cerebrosides **2** resulted in methyl esters of α-hydroxy acids, sphingosine bases, and a mixture of methyl α- and β-glucosaminides. Fatty acid analysis was carried out by GLC/MS. The specific rotation found for this fraction of methyl esters ($[\alpha]_D^{20}$ −5.4) led to the assumption that the acids have *R*-configuration at C(2).[16] According to ^{13}C NMR data, all acids have a normal structure. The results of fatty acid analysis are given in Table 3.

The positions of the double bonds in the carbon chain of the methyl esters of 2 OH-26:1- and 2 OH-28:1-acids were determined by GLC/MS analysis of the corresponding bis(methylthio) adducts of fatty acid methyl esters. The mass spectrum of the adduct of the 2 OH-26:1-acid methyl ester (Fig. 1) exhibits molecular ion peaks with *m/z* 518 and peaks for characteristic ions with *m/z* 401 ($C_{23}H_{45}SO_3^+$, 7%) and *m/z* 117 ($C_6H_{13}S^+$, 6%), indicating that the position of the double bond is C(21)=C(22). The mass spectrum of the adduct of 28:1-acid methyl ester (see Fig. 1) contains peaks for the molecular ion with *m/z* 546 $[M]^+$ and for characteristic ions with *m/z* 401 ($C_{23}H_{45}SO_3^+$, 6%) and *m/z* 145 ($C_8H_{17}S^+$, 7%).

Table 3. Fatty acid composition of cerebrosides **2** from the sponge *Oceanapia* sp.*

Fatty acid methyl ester	Content (%)	M^+, *m/z* (EI)
2OH-24:0	42.71	398
2OH-25:0	25.23	412
2OH-26:1Δ21	5.15	424
2OH-26:0	18.84	426
2OH-28:1Δ21	8.06	452

* M^+ and the percentages are given for the fatty acid methyl esters.

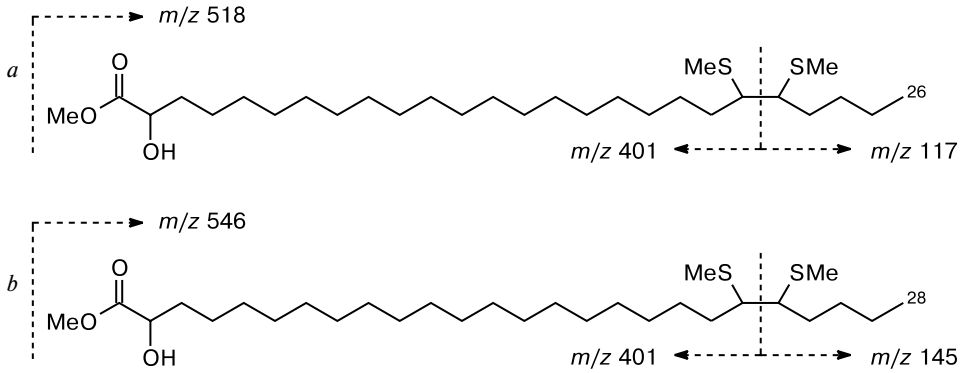


Fig. 1. Fragmentation of bis(methylthio) adducts of the methyl esters of acids 2-OH-26:1Δ21 (*a*) and 2-OH-28:1Δ21 (*b*) in the electron impact mass spectra.

These also indicate that the double bond occurs in the 21(22) position. The positions of the ^{13}C NMR chemical shifts for the carbon atoms (δ_C 27.1) neighboring to the double bond in the fatty acid methyl esters attest to the *cis*-configuration of the double bond.[18] The chemical shifts of carbon atoms neighboring to a *trans*-double bond are known to be about δ 29.5—38.0 and those of the carbon atoms neighboring to a *cis*-double bond, about δ 26.0—28.5.

The MALDI-TOF mass spectra of the peracetates of sphingosine bases exhibit pseudomolecular ion peaks with *m/z* 494, 508, and 522 $[M + Na]^+$, which correspond to C_{17}-, C_{18}-, and C_{19}-phytosphingosines. The signals at δ_C 19.2 and 11.5 in the ^{13}C NMR spectrum were indicative of the presence of *anteiso*-structures, while the signals at δ 39.0, 23.3, and 22.7 implied the presence of *iso*-structured sphingosine bases. A comparison of the ^1H NMR spectrum and the rotation angle ($[\alpha]_D^{20}$ +8.0) for peracetates of the bases with the values for the sphingosine bases obtained from cerebrosides of the sponge *H. cylindrata* suggested a 2*S*,3*S*,4*R* configuration for sphingosine bases.[17]

Cerebrosides of the type **2** also represent a new modification of natural glycosylceramides. They differ from chalicylindrosides B_1—B_6 in the fatty acid composition (the only common fatty acid is 2OH-24:0) and in the presence of *anteiso*-sphingosine bases.

Thus, our communication gives yet another example demonstrating a substantial chemical diversity of marine glycosylceramides as promising physiologically active agents.

Experimental

Optical rotation was measured on a Perkin-Elmer 343 polarimeter. ^1H and ^{13}C NMR spectra were recorded on a Bruker DRX 500 spectrometer (500 and 125 MHz, respectively) using Me_4Si as the internal standard.

MALDI-TOF mass spectra were run on a Bruker Biflex III mass spectrometer (Germany, N_2 laser, 337 nm) using 2,5-dihydroxybenzoic (DHB) and α-cyanohydroxycinnamic (CCA) acid as matrices. GLC analysis was carried out on a Shimadzu GC-17A chromatograph using a SPB-5 capillary column (30 m × 0.25 mm, Supelco, USA). GLC/MS analysis was performed on a Shimadzu QP-5050 Instrument mass spectrometer (ionization energy 70 eV, helium as the carrier gas, a MDW-5S column (30 m × 0.25 mm, Supelco, USA)). Preparative column chromatography was carried out on Amberlite XAD-2 (0.3—1 mm, Serva, Germany), Sephadex LH-20 (Sigma), silica gel KSK (50—160 μm, Sorbpolimer, Russia); TLC was performed on Sorbfil plates with a silica gel CTX-1A layer (5—17 μm, Sorbpolimer, Russia) fixed on a foil; and HPLC was done on an Agilent 1100 Series chromatograph (refractometer as the detector) with a Nucleosil 100 Silica column (5 μm, 4.6 × 250 mm, Supelco, USA) in a chloroform—ethanol system (4 : 1) at a flow rate of 0.8 mL min^{-1}.

A specimen of the marine sponge *Oceanapia* sp. (Haplosclerida order, Phloeodictyidae family) was collected in November 1990 at a 48 m depth near the Scott reef (North-Western Australia (16° 33.6 S; 121° 07.1 E) by means of a dredge during the 12th scientific trip of the research vessel "Academician Oparin." The sponge was identified by V. B. Krasokhin (Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Sciences).

Extraction and isolation of cerebrosides. The freeze-dried crushed sponge (327 g) was subjected to exhaustive extraction with ethanol (3 × 2 L) at room temperature. The combined ethanolic extract was concentrated to dryness *in vacuo*. The residue was dissolved in water and extracted with butanol. The butanolic extract was concentrated *in vacuo* to a crude resinous residue (5.9 g) and chromatographed on a column with silica gel using the following solvent systems: hexane, hexane—ethyl acetate (1 : 1), ethyl acetate, acetone, and ethanol. The fraction eluted with ethanol was then chromatographed on columns with Sephadex LH-20 in a chloroform—ethanol system (1 : 1) and Amberlite XAD-2 in a water→ethanol gradient. The ethanol-eluted fraction (112 mg) was recrystallized from methanol. This gave a sum of cerebrosides (15 mg). The cerebroside-containing fraction was separated by HPLC to give 1.8 mg (0.0006% of the sponge dry weight) of cerebrosides **1** and 3.2 mg (0.001%) of cerebrosides **2**.

Cerebrosides 1. Amorphous powder, $[\alpha]_D^{20}$ −10.0 (*c* 0.18, pyridine). The ^{1}H and ^{13}C NMR spectral data are summarized in Table 1. MALDI-TOF MS (CCA matrix), *m/z* (I_{rel} (%)): 781 (45), 795 (42), 809 (13) $[M + Na]^+$.

Methanolysis of cerebrosides 1. Cerebrosides (1.8 mg) were heated with 5% HCl in MeOH (1 mL) for 4 h at 90 °C. The reaction mixture was extracted with hexane. The hexane extract was concentrated to dryness *in vacuo* to give a mixture of fatty acid methyl esters. The methanolic solution containing sphingosine bases and methyl glycosides was frozen with liquid nitrogen, freeze-dried, and chromatographed on silica gel in a chloroform—methanol (4 : 1) → methanol gradient system to give 0.8 mg of sphingosine bases.

Fatty acid methyl esters obtained from cerebrosides 1. Amorphous powder, ^{1}H NMR ($CDCl_3$), δ: 3.67 (s, 3 H, OMe); 2.30 (t, 2 H, J = 7.0 Hz); 1.60 (m, 2 H); 1.25 (br.s); 0.88 (t, 3 H, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$), δ: 14.1 (terminal methyl groups), 51.4 (OMe).

GLC/MS analysis of the fatty acid methyl esters obtained from 1. The methyl esters were analyzed by GLC/MS in the temperature gradient 170→270 °C, 2 °C min^{-1}, evaporator temperature 260 °C, and interface temperature 260 °C. The fatty acid methyl esters were identified by comparison with authentic samples (see Table 2).

Sphingosine bases obtained from cerebrosides 1. Amorphous powder ^{13}C NMR (C_5D_5N), δ: 73.8 (C(3)), 72.9 (C(4)), 59.5 (C(1)), 57.5 (C(2)), 39.3 ($\underline{C}H_2CHMe_2$), 35.4, 29.9, 26.1 ($\underline{C}HMe_2$), 22,8 ($CH(\underline{C}H_3)_2$), 14.3 (Me). MS MALDI-TOF, *m/z*: 318, 332 $[M + H]^+$.

Acetates of sphingosine bases. The bases were acetylated with a mixture of acetic anhydride and pyridine (1 : 1) for 12 h at room temperature. The solution was concentrated *in vacuo*, and the residue was chromatographed on a column with silica gel in chloroform→chloroform—methanol (40 : 1) to give peracetates of sphingosine bases. ^{1}H NMR ($CDCl_3$), δ: 5.95 (d, 1 H, NH, J = 9.8 Hz); 5.10 (dd, 1 H, H(3), J = 3.3 Hz, J = 8.3 Hz); 4.93 (dt, 1 H, H(4), J = 3.0 Hz, J = 9.4 Hz); 4.44 (m, 1 H, H(2)); 4.29 (dd, 1 H, H(10), J = 4.8 Hz, J = 11.6 Hz); 4.00 (dd, 1 H, H(1), J = 3.0 Hz, J = 11.6 Hz); 2.08 (s, 3 H, NAc); 2.05 (s, 6 H, 2 OAc); 2.03 (s, 3 H, OAc); 1.35—1.2 (br.s); 0.88 (t, $CH_2C\underline{H}_3$ J = 7.0 Hz); 0.86 (d, $CH(C\underline{H}_3)_2$, J = 6.6 Hz). MS MALDI-TOF (DHB matrix), *m/z*: 508, 522 $[M + Na]^+$.

Cerebrosides 2. Amorphous powder; $[\alpha_D^{20}]$ −6.1 (*c* 0.31, pyridine). The ^{1}H and ^{13}C NMR spectral data are given in Table 1. MALDI-TOF MS, *m/z* (I_{rel} (%)): 895 (7), 909 (20), 923 (25), 935 (5), 937 (16), 949 (6), 951 (9), 963 (6), 977 (6.0) $[M + Na]^+$.

Methanolysis of cerebrosides 2. Cerebrosides (2.5 mg) were heated with 5% HCl in MeOH (1 mL) for 4 h at 90 °C. The reaction mixture was extracted with hexane. The hexane extract was concentrated *in vacuo* to dryness to give fatty acid methyl esters (1.1 mg). The methanolic solution was neutralized with 10% aqueous NaOH. The solution was concentrated *in vacuo* to dryness, the residue was dissolved in water (1 mL), and the solution was extracted with ethyl acetate to give sphingosine bases (1.0 mg). Concentration of the aqueous layer gave a mixture of methyl 2-amino-2-deoxy-α- and 2-amino-2-deoxy-β–glucopyranosides.

Fatty acid methyl esters obtained from cerebrosides 2. Amorphous powder, $[\alpha_D^{20}]$ −5.4 (*c* 0.11, $CHCl_3$). ^{13}C NMR ($CDCl_3$), δ: 175.9 (C(1)), 129.9 (=$\underline{C}$H, C(21), C(22)), 70.4 (C(2)), 52.4 (O$\underline{C}H_3$), 34.4 (C(3)), 31.9 (C(24)), 27.1 (C(20), C(23)), 24.7 (C(4)), 22.7 (C(ω2)), 14.1 (C(ω1)).

GLC/MS analysis of the fatty acids methyl esters obtained from cerebrosides 2. The methyl esters were analyzed by GLC/MS at 230 °C, evaporator temperature 260 °C, interface temperature 260 °C. The methyl esters were identified based on their mass spectra and retention times (see Table 3).

Bis(methylthio) derivatives of monoene fatty acids. A mixture of fatty acid methyl esters (0.2 mg) was dissolved in dimethyl disulfide (0.2 mL), and a solution of iodine in diethyl ether ((0.05 mL, 60 mg mL^{-1}) was added. The reaction mixture was kept for 24 h at room temperature. Then hexane (5 mL) was added and the mixture was washed with a dilute aqueous solution of $Na_2S_2O_3$. The filtrate was concentrated to dryness and the residue was dissolved in hexane and analyzed by GLC/MS (300 °C, evaporator temperature 310 °C, interface temperature 290 °C, and detector temperature 300 °C).

Acetylation of sphingosine bases obtained from cerebrosides 2 was carried out under the same conditions as acetylation of sphingosine bases obtained from **1**, $[\alpha_D^{20}]$ +8.0 (*c* 0.10, $CHCl_3$). ^{1}H NMR ($CDCl_3$), δ: 5.91 (d, 1 H, NH, J = 9.4 Hz); 5.10 (dd, 1 H, H(3), J = 3.0 Hz, J = 8.3 Hz); 4.93 (dt, 1 H, H(4), J = 3.0 Hz, J = 9.4 Hz); 4.44 (m, 1 H, H(2)); 4.29 (dd, 1 H, H(1), J = 4.8 Hz, J = 11.6 Hz); 4.00 (dd, 1 H, H(1), J = 3.0 Hz, J = 11.6 Hz); 2.08 (s, 3 H, NAc); 2.05 (s, 6 H, 2 OAc); 2.03 (s, 3 H, OAc); 1.35—1.2 (br.s); 0.85 (t, $CHMeCH_2C\underline{H}_3$, J = 6.7 Hz); 0.84 (d, $CH(C\underline{H}_3)CH_2Me$, J = 6.8 Hz). ^{13}C NMR ($CDCl_3$), δ: 72.9 (C(3)), 71.9 (C(4)), 62.8 (C(1)), 47.6 (C(2)), 39.0 ($\underline{C}H_2CHMe_2$), 36.6 ($CHMe\underline{C}H_2Me$), 34.4 ($\underline{C}HMeCH_2Me$), 30.0—29.2, 23.3, 22.7 ($CH(\underline{C}H_3)_2$), 19.2 ($CH(\underline{C}H_3)CH_2Me$), 11.5 ($CHMeCH_2\underline{C}H_3$). MS MALDI-TOF (DHB matrix), *m/z*: 494, 508, 522 $[M + Na]^+$.

Methyl 2-amino-2-deoxy-α-glucopyranoside and its β-anomer. ^{1}H NMR (D_2O), δ: 4.92 (d, H(1), J = 3.7 Hz); 4.47* (d, J = 8.3). ^{13}C NMR (D_2O), δ: 100.6 [101.3]* (C(1)), 74.4 (C(5)), 72.2 (C(4)), 70.3 (C(3)), 61.1 (C(6)), 55.8 (CN), 55.2 (OMe).

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* Data for the β-anomer.

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