Three New 2,3-Dihydroxy Fatty Acid Glycosphingolipids from the Mediterranean Tunicate *Microcosmus sulcatus*

Anna Aiello,[a] Ernesto Fattorusso,*[a] Alfonso Mangoni,[a] and Marialuisa Menna[a]

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Three new glycosphingolipids (**2a**–**4a**) were isolated as their peracetates (**2b**–**4b**) from the marine ascidian *Microcosmus sulcatus*, and their structures were elucidated by spectroscopic and chemical analysis. Compounds **2a**–**4a** are the first natural glycosphingolipids whose ceramide contains a 2,3-dihydroxy acid.

Introduction

A knowledge of the occurrence and structure of marine invertebrate glycosphingolipids (GSLs) is of interest in the area of biochemical evolution, especially after the findings that some GSLs serve as differentiation and/or growth regulators.[1] All the recent studies on the matter highlighted that each marine invertebrate is characterised by a distinctive GSL profile, thus suggesting they could actually have a specific biological function for the producing organism. Moreover, much attention has been directed to the potential pharmacological activities of these compounds on account of their important biological properties, such as the effect on the mammal immune system[2] and on the angiogenic process.[3]

We recently described[4] the isolation of sulcaceramide (**1a**), a new triglycosylceramide characterised by an unprecedented fucosylated carbohydrate moiety, from the Mediterranean tunicate *Microcosmus sulcatus*. This interesting finding induced us to a further examination of GSLs from this species. From some minor GSL fractions of the methanol extract of *M. sulcatus* we have now obtained three novel GSLs, **2a**−**4a**, as their respective peracetates **2b**−**4b**, and their isolation and structural elucidation are reported here (Figure 1). These compounds differ from all the previously isolated natural cerebrosides in that a 2,3-dihydroxylated fatty acid is present in the ceramide moiety. To date, the GSL structures that have been elucidated contain either non-hydroxylated or 2-hydroxylated fatty acids, whereas 2,3-dihydroxy fatty acids have only been found as constituents of ceramides of fungi and plants.[5] In these organisms, they are predominantly linked to trihydroxy long-chain bases (phytosphingosines) to give highly hydroxylated ceramides, which have been considered to be of significance for the regulation of barrier properties and stability of cell surface membranes due to the formation of lateral hydrogen bonds.[6]

[a] Dipartimento di Chimica delle Sostanze Naturali,
Via D. Montesano 49, 80131 Napoli, Italy
Fax: (internat.) + 39-081/678-552
E-mail: fattoru@unina.it

Results and Discussion

The *n*BuOH-soluble portion of the MeOH extract of *M. sulcatus* was fractionated by successive reversed- and direct-phase column chromatography, thus affording a mixture of glycolipids easily recognised from the ^{1}H NMR spectrum. This fraction was acetylated with Ac_2O/pyridine and successively separated and purified by repeated HPLC on SiO_2 columns. The main constituent of the glycolipid fraction was the previously described sulcaceramide peracetate (**1b**). In addition, three new glycosphingolipids were isolated, as their respective peracetates **2b** (2 mg), **3b** (3 mg), and **4b** (2 mg).

The negative-ion FAB-MS spectrum of the GSL peracetate **2b** showed eight 14-amu-apart quasimolecular ion peaks ([M − H]$^-$: *m*/*z* = 1672, 1686, 1700, 1714, 1728, 1742, 1756, and 1770), indicating that **2b** is a complex mixture of homologues; these components could not be separated. These mass spectrometric data are in accordance with the molecular formula $C_{83}H_{135}NO_{33}$ + *n*CH_2 (*n* = 0−7) for **2b**. Examination of the ^{1}H NMR spectrum of **2b** revealed a strong resemblance with that of sulcaceramide peracetate **1b**,[4] suggesting a close structural relationship. Thus, comparison of the various spectral features of the two compounds was useful to define the differences between them.

Consideration of the molecular formula of **2b** suggested that it should possess an extra acetoxy group compared to sulcaceramide peracetate **1b**; this hypothesis was corroborated by the observation of one additional acetyl methyl singlet in the ^{1}H NMR spectrum of **2b**, as well as one additional oxymethine signal in its ^{13}C NMR spectrum. However, **1b** and **2b** show virtually identical ^{1}H and ^{13}C NMR resonances for the sugar component of the molecule (see Tables 1 and 2); the ^{1}H-^{1}H connectivities observed for each

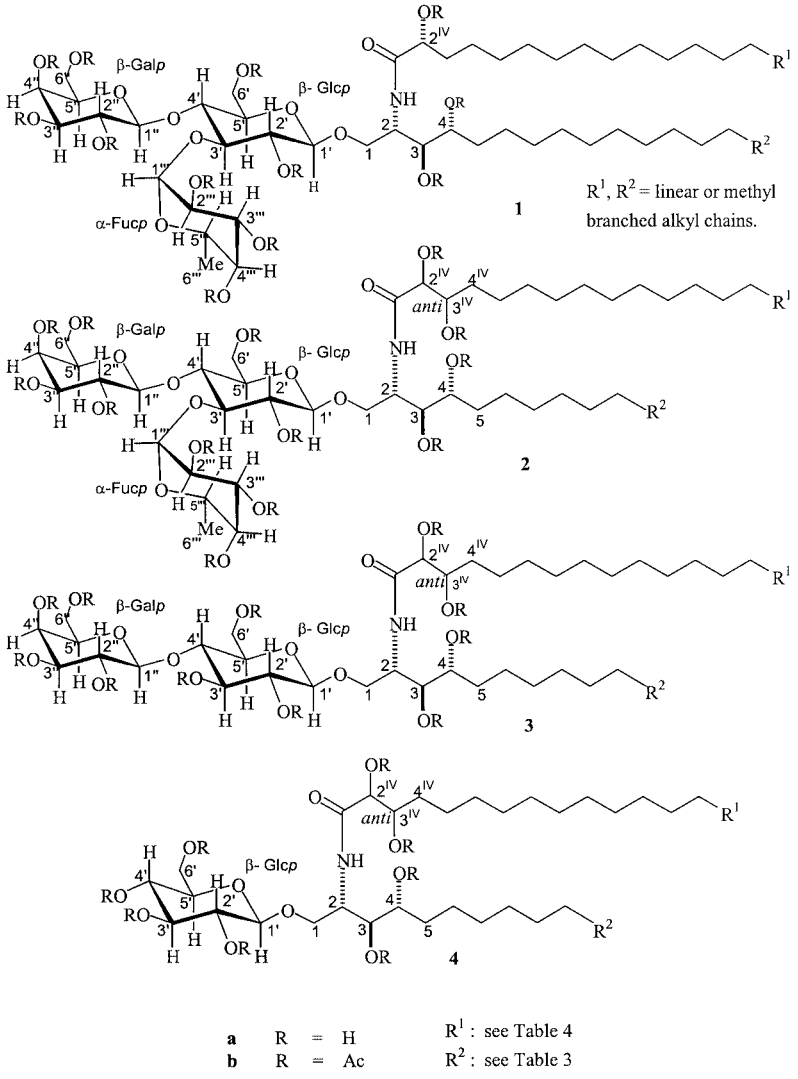


Figure 1. Structures of glycosphingolipids isolated from *M. sulcatus*

hexose unit and established through COSY and HOHAHA analysis show that **2b** possesses the same β-galactopyranosyl-(1→4)-[α-fucopyranosyl-(1→3)]-β-glucopyranosyl sugar chain as sulcaceramide. Therefore, it followed that the further acetylated hydroxy group must lie on the ceramide portion.

A detailed analysis of the COSY and ROESY spectra allowed us to identify the sphingoid base of **2b** as a common trihydroxylated, saturated sphinganine. In fact, starting from the glycosylated oxymethylene protons 1-H^a and 1-H^b, the COSY spectrum (C_6D_6) allowed us to delineate the spin system from 1-$H^{a/b}$ to 5-H_2 (including the amidic proton at C-2 as a doublet at δ = 7.20 ppm) of the long-chain base (see Table 1). Furthermore, the chemical shifts and coupling constants of the sphinganine protons are almost identical to those of the corresponding protons in sulcaceramide peracetate **1b**, thus indicating the same relative stereochemistry. The absolute stereochemistry of the sphingoid base was assumed to be the same as in sulcaceramide since the compounds derive from the same organism.

As a consequence, the extra oxygenated function could only be located on the fatty acyl chain. The presence of a 2,3-diacetoxy fatty acyl chain (instead of the 2-acetoxy acyl chain of sulcaceramide) was deduced from the occurrence of two mutually coupled oxymethine signals at δ = 5.97 (2^{IV}-H) and 5.87 ppm (3^{IV}-H) in the ^{1}H NMR spectrum of **2b**; the former proton shows no further couplings, while the latter is coupled with the methylene protons at δ = 2.06 (4^{IV}-H^a) and 1.92 ppm (4^{IV}-H^b), which, in turn, are coupled with a signal included in the large CH_2 signal at δ = 1.35 ppm. A correlation peak observed in the ROESY spectrum between the amidic proton 2-NH (δ = 7.20 ppm) and 2^{IV}-H (δ = 5.97 ppm) provides evidence that the acyl moiety is linked to the N atom at C-2.

Completion of the structure elucidation of the GSL peracetate **2b** required determination of the nature of the alkyl chains at C-5 and C-4^{IV} in the ceramide portion. This was accomplished by acidic methanolysis of **2b** with 1 M HCl in 90% MeOH. The resulting reaction mixture was separated into fractions of different polarity. Fraction B was a mixture

Table 1. ^{1}H NMR spectroscopic data of compounds **2b**−**4b** (C_6D_6)

Pos.	**2b** δ_H (mult., *J* [Hz])[a]	**3b** δ_H (mult., *J* [Hz])[a]	**4b** δ_H (mult., *J* [Hz])[a]
1 a	3.43 (dd, 10, 2.9)	3.44 (dd, 10, 2.9)	3.56 (dd, 10, 2.9)
1 b	3.73[b]	3.75 (bd, 10)	3.74 (bd, 10)
2	4.63[b]	4.57[b]	4.57[b]
2-NH	7.20 (d, 9.5)	7.19 (d, 9.5)	7.21 (d, 9.5)
3	5.57[b]	5.58[b]	5.54[b]
4	5.33[b]	5.29 (bd, 11)	5.29 (bd, 11)
5 a	1.80[b]	1.80[b]	1.80[b]
5 b	1.92[b]	1.95[b]	1.93[b]
1′	3.75[c] (d, 8)	4.21 (d, 8.1)	4.19 (d, 8.1)
2′	5.05 (dd, 9.5, 8)	5.05 (dd, 9.5, 8.1)	5.08 (dd, 9.5, 8.1)
3′	3.88 (t, 9.5)	5.34 (t, 9.5)	5.35 (t, 9.5)
4′	3.74[b]	3.71 (t, 9.5)	5.23 (t, 9.5)
5′	3.03 (bdd, 9.5, 5)	3.30 (m)	3.26 (m)
6′ a	4.05 (dd, 12, 5)	4.15[b]	4.27 (dd, 11.5, 2.5)
6′ b	4.67 (bd, 12)	4.47 (bd, 11)	4.07 (bd, 11.5)
1″	4.44 (d, 8.5)	4.40 (d, 8.5)	–
2″	5.55[c] (dd, 10, 8.5)	5.52 (dd, 10, 8.5)	–
3″	5.20 (dd, 10, 3)	5.13 (dd, 10, 2.9)	–
4″	5.66[b]	5.48 (bd, 2.9)	–
5″	3.63 (bt, 6.5)	3.49 (bt, 6.6)	–
6″ a	4.76 (dd, 12, 6.5)	4.14[b]	–
6″ b	4.59[b]	4.10 (bd, 9)	–
1‴	5.66[b]	–	–
2‴	5.54[c] (dd, 11, 3.5)	–	–
3‴	5.70 (dd, 11, 2.9)	–	–
4‴	5.81 (bd, 2.9)	–	–
5‴	5.36 (bq, 7.3)	–	–
6‴	1.68 (d, 7.3)	–	–
2^{IV}	5.97 (d, 2.9)	5.82 (d, 2.9)	5.84 (d, 2.9)
3^{IV}	5.87 (ddd, 11, 4.4, 2.9)	5.78 (ddd, 11, 4.5, 2.9)	5.79 (ddd, 11, 4.5, 2.9)
4^{IV} a	2.06[b]	2.05[b]	2.05[b]
4^{IV} b	1.92[b]	1.93[b]	1.93[b]
Ac	1.62−2.27 (13 s)	1.53−2.12 (11 s)	1.64−2.13 (8 s)

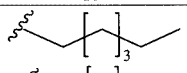
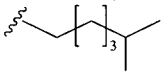
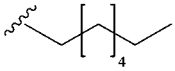
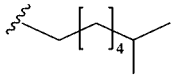
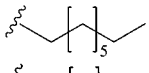
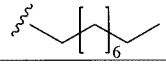
[a] Additional ^{1}H signals: δ = 1.50 ppm [m, C*H*(CH_3)$_2$], 1.35 (br., alkyl chain protons), 0.91 (t, *J* = 7.0 CH_2CH_3), 0.92 [d, *J* = 6.5, CH(CH_3)$_2$]. [b] Submerged by other signals. [c] Submerged by other signals; multiplicity and coupling constants are from the spectrum recorded in $CDCl_3$.

Table 2. ^{13}C NMR spectroscopic data of compounds **2b**−**4b** (C_6D_6)

Pos.	**2b** δ_C (mult.)	**3b** δ_C (mult.)	**4b** δ_C (mult.)
1	66.5 (CH_2)	66.9 (CH_2)	66.9 (CH_2)
2	48.1 (CH)	48.5 (CH)	48.5 (CH)
3	71.8 (CH)	72.0 (CH)	72.0 (CH)
4	73.7 (CH)	73.3 (CH)	73.5 (CH)
5	29.0 (CH_2)	29.0 (CH_2)	29.1 (CH_2)
1′	100.5 (CH)	100.7 (CH)	100.7 (CH)
2′	73.8 (CH)	72.2 (CH)	71.2 (CH)
3′	74.1 (CH)	73.4 (CH)	73.2 (CH)
4′	74.5 (CH)	77.2 (CH)	68.6 (CH)
5′	73.3 (CH)	72.7 (CH)	72.0 (CH)
6′	61.5 (CH_2)	62.5 (CH_2)	61.5 (CH_2)
1″	101.2 (CH)	100.9 (CH)	–
2″	69.0 (CH)	70.0 (CH)	–
3″	71.4 (CH)	71.5 (CH)	–
4″	66.9 (CH)	67.0 (CH)	–
5″	71.2 (CH)	71.5 (CH)	–
6″	60.7 (CH_2)	60.8 (CH_2)	–
1‴	96.0 (CH)	–	–
2‴	69.0 (CH)	–	–
3‴	68.5 (CH)	–	–
4‴	71.8 (CH)	–	–
5‴	64.6 (CH)	–	–
6‴	16.2 (CH_3)	–	–
2^{IV}	73.8 (CH)	73.9 (CH)	73.8 (CH)
3^{IV}	73.4 (CH)	73.5 (CH)	73.5 (CH)
4^{IV}	33.9 (CH_2)	33.9 (CH_2)	34.1 (CH_2)

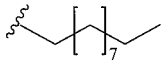
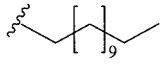
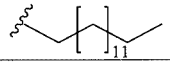
of 4-hydroxysphinganines, and the high-field region of the ^{1}H NMR spectrum indicated it to be composed of homologues with unbranched (triplet at δ = 0.86 ppm) and iso- (doublet at δ = 0.84 ppm) alkyl chains;[7] their nature was ascertained by permanganate/periodate oxidative cleavage of the C-3/C-4 bond, performed according to the procedure reported in ref.[7] The obtained carboxylic acids (possessing two carbon atoms less) were methylated with diazomethane, and the resulting esters were analysed by GC-MS. The results of the analysis are summarized in Table 3, in terms of the original sphinganines.

Table 3. Sphinganine composition of compounds **2b**−**4b**

R^2	**2b**	**3b**	**4b**
	16.9%	17.0%	18.0%
	12.7%	8.5%	9.3%
	20.5%	17.7%	18.1%
	10.2%	7.5%	7.9%
	18.8%	14.5%	16.0%
	20.9%	34.8%	30.7%

The less polar fraction A contained fatty acid methyl esters, which were identified as the *erythro* isomers of methyl 2,3-dihydroxytetracosanoate, 2,3-dihydroxyhexacosanoate, and 2,3-dihydroxyoctacosanoate (see Table 4) as follows. The ESI mass spectrum (positive ion mode) of fraction A shows a peak series at *m/z* = 415, 443, and 471 $[M + H]^+$ while its ^1H NMR spectrum ($CDCl_3$) reveals that all the homologues are unbranched. Furthermore, comparison of the chemical shift and coupling constant values of the signals of fraction A with authentic samples of the *threo* and *erythro* isomers of methyl 2,3-dihydroxypalmitate (see Exp. Sect.) indicate that fraction A contains an *erythro*-diol. Unfortunately, we were unable to establish its absolute stereochemistry due to the very small amount of sample.

Table 4. Fatty acyl composition of compounds **2b**−**4b**

R^1	**2b**	**3b**	**4b**
	38.5%	35.0%	37.6%
	20.3%	22.8%	22.0%
	41.2%	42.2%	40.4%

The FAB mass spectrum (negative ion mode) of the diglycosylceramide **3b** shows several molecular ion peaks at *m/z* = 1456, 1470, 1484, 1498, 1512, 1526, 1540, and 1554 $[M - H]^-$, in accordance with a mixture of homologues with the molecular formula $C_{74}H_{123}NO_{27} + n\ CH_2$ (*n* = 0−7). A comparison of the spectral features of **3b** and **2b** shows that they possess the same aglycon moiety (see Tables 1 and 2) and differ in the nature of the sugar component of the molecule.

The structure of the disaccharide moiety was established on the basis of NMR spectroscopic data. The two anomeric protons (δ = 4.21 and 4.40 ppm) were readily identified from their HMQC correlation with the characteristic anomeric carbon signals at δ = 100.7 and 100.9 ppm. Analysis of COSY and HOHAHA (C_6D_6) data starting from the signal of the anomeric protons at δ = 4.21 ppm allowed us to identify the first sugar unit as a β-glucopyranose. In fact, the large coupling constant between 1′-H and 2′-H, 2′-H and 3′-H, 3′-H and 4′-H, and 4′-H and 5′-H indicated the axial position of these protons.

The second anomeric proton (signal at δ = 4.40 ppm, C_6D_6) was proven to belong to a β-galactopyranose unit in a similar manner. The measurement of the coupling constants of the protons permitted the determination of the axial nature of 1′′-H, 2′′-H, and 3′′-H, showing large axial-axial couplings, as well as the equatorial nature of 4′′-H (see Table 1), but did not allow us to determine the orientation of 5′′-H. This proton was deduced to be axial from a ROESY experiment, which displays distinct correlation peaks of 5′′-H with 1′′-H and 3′′-H, attesting to a 1,3-diaxial relationship with the above protons.

The ROESY correlation peaks of 1′-H with 1-H[a] indicated that the sugar unit linked directly to the ceramide is the β-glucopyranose, while those of 1′′-H with 4′-H and 3′-H, as well as the shielded chemical shift value of 4′-H (δ = 3.73 ppm) suggested C-4′ to be glycosylated by the β-galactopyranose, thus defining the structure of **3b** as a lactosylceramide.

Analysis of the spectroscopic data indicated that the monoglycosylceramide **4b** was a mixture of β-glucopyranosides of phytosphingosine-type ceramides possessing the same 2,3-diacetoxy acyl chain as **2b** and **3b**. In fact, its FAB mass spectrum (negative ion mode) shows several molecular ion peaks at *m/z* = 1168, 1182, 1196, 1210, 1224, 1238, 1252, and 1266 $[M - H]^-$, in accordance with the molecular formula $C_{62}H_{107}NO_{19} + n\ CH_2$ (*n* = 0−7). An HMQC experiment allowed us to identify the sole anomeric proton (δ = 4.19 ppm) through its correlation with the pertinent carbon atom (δ = 100.7 ppm); starting from this signal, all the ^1H-^1H connectivities within the hexose unit were established by a COSY experiment while the analysis of the coupling constants allowed us to determine the axial stereochemistry of all the sugar protons (see Table 1).

Compounds **3b** and **4b** were subjected to acidic methanolysis to determine the nature of the alkyl chains in the ceramide, and the resulting material was analysed under the same experimental conditions as applied to **2b**. The results obtained were similar, as reported in Tables 3 and 4. On the basis of the above-reported data, if the sugars are assumed to belong to the most commonly found D series, the structures and stereochemistry of compounds **2b**−**4b** are as shown in Figure 1.

Conclusion

We have shown that *M. sulcatus* contains three unique glycosphingolipids, which are the first natural glycosphingolipids whose ceramide contains a 2,3-dihydroxy acid. A few remarks on the nature of the long-chain alkyl groups of the ceramide are necessary. As usual for glycosphingolipids from sponges and ascidians, all the three new GSLs **2b**−**4b** have been isolated as a mixture of homologues. They contain considerable amounts of sphingoid bases with an odd number of carbon atoms and/or isoalkyl chains. On the contrary, the fatty acids are all unbranched and even-numbered, with very long chains (up to 28 carbon atoms). Therefore, different biogenetic pathways for the two portions of the molecule are likely. For example, 2,3-dihydroxy acids, until now reported only from plants and fungi, could come from the diet and be incorporated into the molecule by the ascidian.

Experimental Section

General Remarks: FAB MS: VG ZAB mass spectrometer (Xe atoms of energy 2−6 kV). The ESI MS experiment was performed with

an LCQ ion-trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA) equipped with an electrospray ionization source in the positive ion mode. The spectrum was recorded by infusion into the ESI source using MeOH as the solvent. Optical rotation: Perkin−Elmer 192 polarimeter equipped with a sodium lamp (589 nm) and a 10-cm microcell. ^{1}H and ^{13}C NMR: Bruker AMX-500 spectrometer, chemical shifts are referenced to the residual solvent signal (C_6D_6: δ_H = 7.15 ppm, δ_C = 128.5 ppm; $CDCl_3$: δ_H = 7.26 ppm, δ_C = 77.0 ppm); signals of methyl, methylene, and methine carbon atoms were distinguished by DEPT experiments; homonuclear ^{1}H connectivities were determined by using COSY experiments; the 2D HOHAHA experiment was performed in the phase-sensitive mode (TPPI) by using the MLEV-17 (mixing time 125 ms) sequence for mixing; the reverse-detected multiple quantum heteronuclear correlation (HMQC) spectrum was recorded by using a pulse sequence developed by Bax and Subramanian, with a BIRD pulse of 0.5 s before each scan to suppress the signal originating from protons not directly bound to ^{13}C; the interpulse delays were adjusted for an average $^1J_{C,H}$ coupling of 142 Hz. Two- and three-bond ^{1}H-^{13}C connectivities were determined by an HMBC experiment optimized for a $^{2,3}J$ coupling of 10 Hz. Medium-pressure liquid chromatographies (MPLC) were performed with a Büchi 861 apparatus with RP-18 Si gel (particle size 40−63 μm) and SiO_2 (230−400 mesh) packed columns. High performance liquid chromatography (HPLC) separations were achieved with a Waters 501 apparatus equipped with an RI detector. GC-MS analyses were performed with a Hewlett−Packard 5890 gas chromatograph with a mass-selective detector MSD HP 5970 MS, a split/splitless injector, and fused-silica column, 25 m × 0.20 mm HP-5 (cross-linked 25% Ph-Me silicone, 0.33 mm film thickness); the temperature of the column was varied, after a delay if 5 min from the injection, from 150 to 300 °C with a slope of 5 °C min^{-1}. The quantitative determination was based on the area of the GLC peaks.

Extraction and Isolation of GSLs: Specimens of *M. sulcatus* were collected in the Bay of Naples at a depth of 40 m and identified by Prof. Angelo Tursi (university of Bari). They were frozen immediately after collection and kept frozen until extraction. A reference specimen is deposited at the Dipartimento di Chimica delle Sostanze Naturali, University of Naples. For the extraction, the tunic was removed from the animals and the whole bodies (30 g of dry weight after extraction) were homogenized and extracted (4 × 200 mL) twice with methanol and then twice with chloroform. The combined extracts were partitioned between H_2O and *n*BuOH; the organic layer was concentrated in vacuo and afforded 3.5 g of a dark residue which was chromatographed on a column packed with RP-18 silica gel, eluting with $H_2O \rightarrow$ MeOH $\rightarrow CHCl_3$; two fractions containing glycolipids, eluted with MeOH/$CHCl_3$ (9:1) (A, 800 mg) and $CHCl_3$ (B, 500 mg), were obtained. Fraction B was further chromatographed on an SiO_2 column; the fraction (50 mg) eluted with EtOAc/MeOH (7:3) was shown to be a mixture of glycosphingolipids; a portion of this fraction (30 mg) was dissolved in pyridine (500 μL) and allowed to react with Ac_2O (200 μL) for 12 h. The reaction mixture was concentrated and the residue separated by normal-phase HPLC (eluent: *n*-hexane/EtOAc, 1:1), affording compounds **2b**−**4b** (homogenous as far as the polar head is concerned).

Compound 2b: 1.5 mg (0.005% of dry weight). $[\alpha]_D^{25}$ = −15 (*c* = 0.001 in $CHCl_3$). Negative FAB MS: *m*/*z* = 1672, 1686, 1700, 1714, 1728, 1742, 1756, and 1770 $[M - H]^-$ series.

Compound 3b: 2 mg (0.007% of dry weight).$[\alpha]_D^{25}$ = −5 (*c* = 0.002 in $CHCl_3$). Negative FAB MS: *m*/*z* = 1456, 1470, 1484, 1498, 1512, 1526, 1540, and 1554 $[M - H]^-$ series.

Compound 4b: 1.5 mg (0.005% of dry weight). $[\alpha]_D^{25}$ = −6 (*c* = 0.001 in $CHCl_3$). Negative FAB MS: *m*/*z* = 1168, 1182, 1196, 1210, 1224, 1238, 1252, and 1266 $[M - H]^-$ series.

Methanolysis of GSLs: Each GSL (1.5 mg) was dissolved in 1 N HCl in 91% MeOH (500 μL) and the obtained solution was kept for 12 h at 80 °C in a sealed tube. The reaction mixture was dried under nitrogen, dissolved in a small quantity of $CHCl_3$ and passed through an SiO_2 (230−400 mesh) column. Elution with 0.1% pyridine in $CHCl_3$ (10 mL) gave a mixture of α,β-dihydroxy acid methyl esters (fraction A, 0.5 mg), and subsequent elution with 0.1% pyridine in MeOH afforded sphinganines and methyl glycosides. The mixture was partitioned between $CHCl_3$ and H_2O/MeOH (8:2), and the organic layer was separated and concentrated to give a mixture of sphinganines (fraction B, 0.5 mg).

Analysis of Sphinganines: Fraction B ($[\alpha]_D^{25}$ = +7, *c* = 0.0005 in $CHCl_3$) was subjected to oxidative cleavage with $KMnO_4$/$NaIO_4$ as described in ref.[7], and the resulting carboxylic acids were methylated with diazomethane and the obtained esters analysed by GC-MS. The results are compiled in Table 3.

Fatty Acid Methyl Esters from Compounds 2b−4b (Fraction A): ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, *J* = 7 Hz, 3 H), 1.24 (br., alkyl chain protons), 1.49 (m, 2 H), 3.83 (s, 3 H), 3.84 (m, 1 H), 4.22 (d, *J* = 4.0 Hz, 1 H) ppm. ESI MS (positive ion mode): *m*/*z* = 415, 443, and 471 $[M + H]^+$ series. The quantitative estimation, reported in Table 4, is based on the relative intensity of the peaks.

Methyl *erythro*-2,3-Dihydroxypalmitate (Larodan AB, SWEDEN): ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, *J* = 7 Hz, 3 H), 1.26 (br. s), 1.64 (m, 2 H), 1.86 (d, *J* = 8.5 Hz, 1 H), 3.0 (d, *J* = 5.5 Hz, 1 H), 3.83 (s, 3 H), 3.84 (m, 1 H), 4.23 (dd, *J* = 5.5, 4.0 Hz, 1 H) ppm.

Methyl *threo*-2,3-Dihydroxypalmitate (Larodan AB, SWEDEN): ^{1}H NMR ($CDCl_3$): δ = 0.88 (t, *J* = 7 Hz, 3 H), 1.26 (br. s), 1.49 (m, 2 H), 1.86 (d, *J* = 8.5 Hz, 1 H), 3.0 (d, *J* = 5.5 Hz, 1 H), 3.83 (s, 3 H), 3.89 (m, 1 H), 4.11 (dd, *J* = 5.5, 2.0 Hz, 1 H) ppm.

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