New lipids from the soft corals of the Andaman Islands

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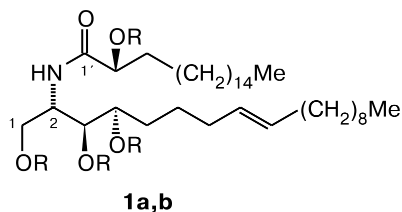
Sphingolipids and glycolipids including previously unknown (2*S*,3*S*,4*R*)-1,3,4-trihydroxy-2-(2-(*R*)-hydroxyoctadecanoylamino)octadec-8*E*-ene, (2*S*,3*R*)-1,3-dihydroxy-2-octadecanoylamino-4*E*,8*E*-hexadecadiene, and (2-hydroxy-3-hexadecyloxypropyl)-α-L-fucopyranoside were isolated from soft corals collected on the shelf near the Andaman Islands (Indian Ocean). The structures of all compounds were established by spectroscopic methods and chemical analyses. The lipids possessed antibacterial activity against *Bacillus subtilis*, *Bacillus pumilus*, *Escherichia coli*, and *Pseudomonas aeruginosa* and antifungal activity against *Aspergillus niger*, *Rhizopus oryzae*, and *Candida albicans*.

Key words: soft corals, *Alcyonaceae*, *Sinularia*, sphingolipids, glycolipids, ^{1}H and ^{13}C NMR spectra.

Marine invertebrates provide a rich source of new secondary metabolites possessing various kinds of biological activity.[1] In a continuation of our studies of natural compounds present in marine organisms from the Andaman Islands,[2] in this work we studied specimens of soft corals collected in this region, isolated some new natural compounds, and structurally characterized them.

Compound **1a** was found in a soft coral (the class *Anthozoa*, the order *Alcyonaceae*) identified as *Sinularia grandilobata* Verseveldt and was isolated by repeated column chromatography on silica gel.



R = H (**a**), Ac (**b**)

The NMR spectra of a solution of **1a** in a $CDCl_3$—CD_3OD mixture suggest the ceramide nature of the compound isolated. In the ^{13}C NMR spectrum, the polymethylene fragments give an intense signal at δ 29.8—30.4, while the methyl groups give a signal at δ 14.3 (q). The signal of the CONH group at δ 176.5 (s) and the signal of the <u>C</u>H—NHCO fragment at δ 52.4 (d) indicate the presence of the acylated amino group. The spectrum exhibits signals of carbon atoms of the CH_2O group at δ 61.7 (t) and three CHO groups at δ 72.6, 72.9, and 76.0. Finally, the presence of the disubstituted double bond in the compound under study is confirmed by two doublets at δ 130.8 and 131.3.

The ^{1}H NMR spectrum confirms the presence of all the above-mentioned groups and fragments in compound **1a**. In particular, this spectrum exhibits signals of the methyl groups at δ 0.89 (t), polymethylene fragments at δ 1.27 (m), allylic methylene groups at δ 1.95 (m), protons of the CHO and CH_2O groups at δ 3.50—4.20, and of the olefinic protons at δ 5.40. The spectral pattern indicates no branching at the termini of the aliphatic chains.

Methanolysis of the ceramide afforded methyl ester of a fatty acid as the major component, which was identified by TLC and GLC-MS as methyl 2-hydroxyoctadecanoate ($[M]^+$ 314). Based on the specific rotation, the methyl ester obtained ($[\alpha]_D^{25}$ −5.3 (*c* 1.00, $CHCl_3$)) can be assigned to the D-series.[3]

Treatment of compound **1a** with acetic anhydride in Py resulted in peracetate **1b**, which was studied in more detail because of better solubility in organic solvents. The MALDI-TOF mass spectrum of compound **1b** exhibits a peak of pseudomolecular ion $[M + Na]^+$ with *m/z* 788.532 (calc. 788.5647), which, along with the ^{1}H and ^{13}C NMR spectral data, makes it possible to propose the following molecular formula $C_{44}H_{79}NO_9$.

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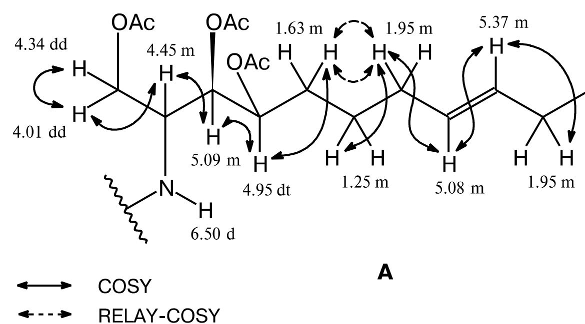
The signals in the NMR spectra were assigned based on the results of ^1H—^1H COSY, HMQC, TOCSY, and RELAY-COSY experiments. Analysis of the COSY spectrum revealed the presence of the 2-*N*-acyl-1,3,4-triacetoxy fragment **A**. Though this technique allowed the determination of the chemical shifts of the H(5) protons (δ 1.63, multiplet) and allylic protons (δ 1.95), we failed to establish the position of the double bond, since the signals of the C(6)H_2 group overlapped with the signal of the $(CH_2)_n$ fragment at δ 1.25. The 2D RELAY-COSY spectra are known to exhibit not only conventional COSY signals but also cross-peaks of the protons separated by two C—C bonds. The appearance of a cross-peak between the H(5) protons (δ 1.63) and the allylic protons at δ 1.95 proved that the double bond connects C(8) and C(9).

The type of the double bonds in olefins can be determined from the chemical shift of allylic carbon atoms (δ ~27 for the *cis*-isomer and ~32 for the *trans*-isomer).[4] The corresponding methylene signals in the ^{13}C NMR spectrum of acetate **1b** were observed at δ 32.6 and 32.2, thus indicating the *trans*-geometry of the double bond (Table 1). The absolute configuration of **1b** was determined based on the similarity of the corresponding chemi-



cal shifts, spin-spin coupling constants, and optical rotation ($[\alpha]_D^{25}$ +14.6 (*c* 3.00, $CHCl_3$)) with the corresponding values for the known peracetate of the (2*S*,3*S*,4*R*)-ceramide from the soft coral *Sinularia leptoclados*[5] ($[\alpha]_D^{25}$ +8.0 (*c* 0.10, $CHCl_3$)) and synthetic ceramide (2*S*,3*S*,4*R*)-*N*-[2-(*R*)-acetoxytetracosanoyl]-2-amino-1,3,4-heptadecanetriol[6] ($[\alpha]_D^{25}$ +6.0 (*c* 0.10, $CHCl_3$)). This allowed identification of the sphingosine base in **1a** as (2*S*,3*S*,4*R*)-1,3,4-trihydroxy-2-aminooctadec-8*E*-ene.

Thus, based on these data and on the results of analysis of fatty acid composition, we established that the ceramide isolated is (2*S*,3*S*,4*R*)-1,3,4-trihydroxy-2-

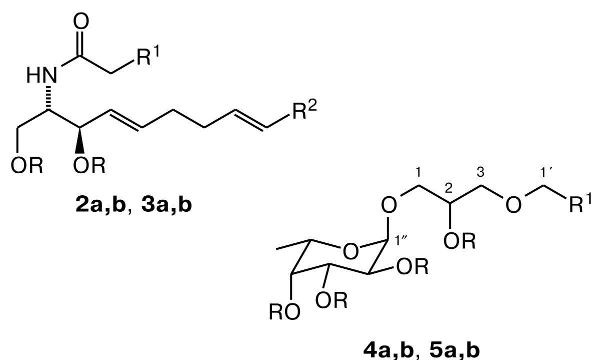
Table 1. Parameters of ^1H (500 MHz) and ^{13}C (75 MHz) NMR spectra of lipids **1b**, **3b** ($CDCl_3$), and **4a**+**5a** (C_5D_5N)

Atom	**1b**		**3b**	
	δ_H (*J*/Hz)	δ_C	δ_H (*J*/Hz)	δ_C
C(1)	4.01 (dd, *J* = 11.5, *J* = 3.2); 4.34 (dd, *J* = 11.5, *J* = 6.2)	62.4	4.03 (dd, *J* = 11.6, *J* = 3.8); 4.30 (dd, *J* = 11.6, *J* = 6.2)	62.6
C(2)	4.45 (m)	47.9	4.46 (dtd, *J* = 9.3, *J* = 5.8, *J* = 3.7)	50.4
C(3)	5.09 (m)	72.4	5.27 (dd, *J* = 7.2, *J* = 6.0)	73.7
C(4)	4.95 (dt, *J* = 9.6, *J* = 3.4)	72.6	5.40 (m)	124.5
C(5)	1.63 (m)	28.3	5.80 (m)	136.6
C(6)	1.25 (m)	29.9	2.10 (m)	29.7
C(7)	1.95 (m)	32.6	2.10 (m)	32.6
C(8)	5.08 (m)	131.2	5.08 (m)	131.4
C(9)	5.37 (m)	129.3	5.37 (m)	128.8
C(10)	1.95 (m)	32.2	1.95 (m)	32.2
C(11)	1.28 (m)	29.9	1.28 (m)	29.9
CH_2	1.25 (m)		1.25 (m)	
C(ω´)	0.87 (t, *J* = 7.0)	14.1	0.87 (t, *J* = 7.0)	14.1
C(ω´-1)	1.25 (m)	22.7	1.25 (m)	22.7
C(ω´-2)	1.25 (m)	31.9	1.25 (m)	31.9
C(1´)	—	171.1	—	172.8
C(2´)	5.11 (m)	74.0	2.16 (t, *J* = 7.9)	36.9
C(3´)	1.82 (m)	31.8	1.58 (m)	25.7
NH	6.58 (d, *J* = 9.0)	—	5.6 (d, *J* = 9.2)	—
OAc	2.03, 2.06, 2.09, 2.11 (all s)	173.0, 173.1, 173.3 (2C)	2.07, 2.06 (both s)	171.1, 173.5

Atom	**4a+5a**	
	δ_H (*J*/Hz)	δ_C
C(1)	3.98 (dd, *J* = 10.2, *J* = 4.7); 4.24 (dd, *J* = 10.2, *J* = 5.8)	71.1
C(2)	4.40 (m)	69.9
C(3)	3.86 (dd, *J* = 10.0, *J* = 5.0); 3.80 (dd, *J* = 10.0, *J* = 5.8)	73.5
C(1´)	3.50 (t, *J* = 6.7)	71.7
C(2´)	1.63 (m)	30.2
C(3´)	1.28 (m)	26.6
C(4´)	1.28 (m)	29.9
C(5´)—C(13´)	1.28 (m)	29.9
C(14´)	1.28 (m)	32.1
C(15')	1.28 (m)	23.0
C(16')	0.87 (t, *J* = 6.8)	14.3
C(1″)	5.41 (d, *J* = 3.7)	101.3
C(2″)	4.62 (dd, *J* = 3.7, *J* = 9.9)	70.4
C(3″)	4.51 (dd, *J* = 9.9, *J* = 3.0)	71.7
C(4″)	4.15 (dd, *J* = 3.0, *J* = 1.2)	73.3
C(5″)	4.43 (dq, *J* = 1.2, *J* = 6.4)	67.3
C(6″)	1.51 (d, *J* = 6.4)	17.2

[2′-(*R*)-hydroxyoctadecanoylamino]octadec-8*E*-ene. No ceramides containing this base have been reported to date. A ceramide with 9*Z*-geometry of the double bond was isolated from the starfish *Acanthaster planci*.[7] This compound is a derivative of D-2-hydroxypalmitic acid and C_{22}-sphingosine base. Its spectral data were similar to those of ceramide **1a**; however, signals of the allylic carbon atoms were observed at δ 27.6 and 27.8, which corresponded to *Z*-geometry of the double bond.

Compounds **2a—5a** were found in the specimen of the soft coral *Sinularia* sp. (the class *Anthozoa*, the order *Alcyonaceae*) and isolated by repeated column chromatography on silica gel.

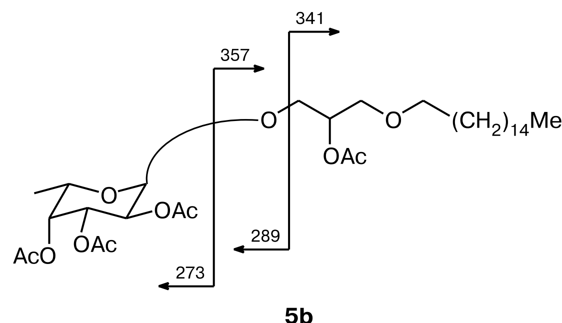


R = H (**a**), Ac (**b**);
R^1 = $(CH_2)_{13}Me$ (**2**), $(CH_2)_{15}Me$ (**3**), $(CH_2)_{16}Me$ (**4**); $(CH_2)_{14}Me$ (**5**);
R^2 = $(CH_2)_8Me$ (**2**), $(CH_2)_6Me$ (**3**)

Based on the MALDI-TOF mass spectrometric (*m/z* 558.52 $[M + Na]^+$) and optical rotation data, the results of analysis of the ^1H and ^{13}C NMR spectra, and GLC and GLC-MS data for methyl esters of fatty acids obtained by methanolysis (major component, *m/z* 270 $[M]^+$), compound **2a** was unambiguously identified as (2*S*,3*R*)-1,3-dihydroxy-2-palmitoylaminooctadeca-4*E*,8*E*-diene. Earlier,[8] this compound was isolated from the gorgonian *Acabaria undulata*.

The NMR spectra, mass spectra, and the optical rotation of compound **3a** were similar to those of ceramide **2a**. However, methanolysis of ceramide **3a** resulted in methyl octadecanoate (identified by GLC and GLC-MS, *m/z* 298 $[M]^+$) as the major component. Hence, ceramide **3a** differs from **2a** in the length of the acyl chain (C_{18}) and the sphingosine base (C_{16}). The structure of (2*S*,3*R*)-1,3-dihydroxy-2-octadecanaminohexadeca-4*E*,8*E*-diene can be proposed for this compound, which has not been reported to date. At the same time, some other derivatives of the C_{16}-sphingosine bases were isolated earlier[7] from, *e.g.*, the starfish *Acanthaster planci*.

GLC and GLC-MS analyses of peracetates from another fraction of the ethanolic extract from the soft coral *Sinularia* sp. (a fraction of higher polarity) revealed the presence of a mixture of two structurally similar compounds, **4a** and **5a**, differing from each other in two methylene units. We failed to separate these compounds by HPLC. The MALDI-TOF mass spectrum of a mixture of peracetates of these compounds, **4b** and **5b**, exhibited peaks with *m/z* 681.45 and 653.476 $[M + Na]^+$. Analysis of the ^1H and ^{13}C NMR spectra of the mixture **4a**+**5a** revealed that these compounds belong to disubstituted glycerol fucosides. Based on the results of detailed two-dimensional NMR (in particular, ^1H—^1H COSY and HMQC) study of the structure of the peracetates, the structure of α-fucopyranoside of batyl alcohol was assigned to compound **4a**, while the structure of (2-hydroxy-3-hexadecyloxypropyl)-α-fucopyranoside was assigned to compound **5a**. The optical rotation of the monosaccharide ($[\alpha]_D^{25}$ −76.5 (*c* 0.92, H_2O)) obtained upon acid hydrolysis of a mixture of compounds **4a** and **5a** indicates that fucopyranose is present in these substances in the form of the L-isomer. The structures of compounds **4a** and **5a** were also confirmed by EI mass spectra of their peracetates. For instance, chromato-mass spectrometric analysis showed that the mass spectrum of compound **4b** exhibits peaks with *m/z* 495 $[M - 2\ AcOH - 43]^+$ (1%), 478 $[M - 3\ AcOH]^+$ (1%), 369 $[M - C_{12}H_{17}O_8]^+$ (100%), 289 $[C_{12}H_{17}O_8]^+$ (1%), and 273 $[C_{12}H_{17}O_7]^+$ (70%), while the mass spectrum of **5b** exhibits peaks with *m/z* 467 $[M - 2\ AcOH - 43]^+$ (1%), 450 $[M - 3\ AcOH]^+$ (1%), 341 $[M - C_{12}H_{17}O_8]^+$ (100%), 289 $[C_{12}H_{17}O_8]^+$ (1%), and 273 $[C_{12}H_{17}O_7]^+$ (70%).



Earlier,[9] compound **4a** was isolated from the soft coral *Sinularia* sp.; however, the assignment of signals in the NMR spectra was incomplete. From the soft coral *Sinularia gravis* Tixier-Durivault, α-D-fucopyranoside of batyl alcohol was isolated[10] and its stereochemistry was determined based on comparison with the published data. Lipid **5a** has not been known hitherto.

The biological activity of compounds **1a** and **3a—5a** against bacteria *Bacillus subtilis*, *Bacillus pumilus*, and *Escherichia coli* and fungi *Pseudomonas aeruginosa* and the *Aspergillus niger*, *Rhizopus oryzae*, and *Candida albicans* was tested *in vitro*. Preliminary tests revealed that these compounds exhibit antibacterial and antifungal activity (Table 2).

Table 2. Antibacterial and antifungal activity of lipids **1a**, **3a**, and **4a+5a**

Bacteria and fungi	Inhibition zone diameter (mm)								
	C/μg mL^{-1}								
	1a			**3a**			**4a+5a**		
	50	100	200	50	100	200	50	100	200
E. coli	11	12	15	11	13	16	13	15	17
P. aeruginosa	11	13	14	12	15	17	10	12	14
B. subtilis	12	13	16	13	15	18	11	12	15
B. pumilus	11	14	16	12	14	16	13	15	17
A. niger	10	13	15	12	15	16	11	13	16
R. oryzae	10	13	14	11	13	15	11	12	14
C. albicans	11	13	16	8	10	11	13	16	17

Experimental

The melting points were determined on a Boetius hot stage. ^{1}H and ^{13}C NMR spectra were recorded on Bruker DPX-300 (300 and 75 MHz, respectively) and DRX-500 (500 and 125 MHz, respectively) spectrometers with Me_4Si as an internal standard. The optical rotation was measured on a Perkin—Elmer 141 polarimeter. The laser desorption mass spectra (MALDI-TOF) were recorded on a Bruker Biflex III mass spectrometer with laser desorption using an N_2 laser (337 nm). Samples of compounds under study were dissolved in MeOH (1 mg mL^{-1}). Analyses were carried out using aliquots (1 μL) with 2,5-dihydroxybenzoic acid as a matrix. TLC Analyses were performed on glass plates with a fixed silica gel L layer (300 mesh, Chemapol). GLC Analyses were performed on a Sigma 2000 Perkin—Elmer gas chromatograph equipped with a capillary column (25 m × 0.2 mm) packed with OV-101 at 100—250 °C (5 deg min^{-1}), with helium as the carrier gas. Chromato-mass spectrometric analyses were carried out on a Hewlett-Packard 5973 instrument equipped with a capillary column (30 m × 0.32 mm) packed with HP-5 MS using helium as the carrier gas at an ionizing voltage of 70 eV.

Biological material. Specimens of soft corals were collected by divers on the shelf near the Andaman Islands (the Hori and Diglipur islands; latitude 13°20´ N, longitude 93°02´ E) in March 1993. A specimen with typical morphological features was placed in a plastic container with EtOH for subsequent identification. The specimen of *Sinularia grandilobata,* Verseveldt, 1980 was identified by Dr. V. Jayasree (National Oceanographic Institute, Goa, India) and was stored at the Museum of the National Oceanographic Institute, Goa, India and at the Department of Organic Chemistry of the Andhra University, Visakhapatnam, India (No. MF-VA/34).

The specimen of soft corals *Sinularia* sp. was identified by B. B. Grebnev (Pacific Institute of Bioorganic Chemistry, Far-Eastern Branch of the Russian Academy of Sciences, Vladivostok) and was stored at the Department of Organic Chemistry of the Andhra University, Visakhapatnam, India (No. MF-VA/44).

Isolation of lipids 1a—5a. Ground soft coral *Sinularia grandilobata* (the weight of the animal was 800 g) were exhaustively extracted with ethanol at ~20 °C. After removal of the solvent *in vacuo*, the extract obtained was reextracted with ethyl acetate. The combined extracts were concentrated *in vacuo* to dryness and the resudie (30 g) was repeatedly chromatographed on a column (120×6 cm) with silica gel G (500 g, Acme, 100—200 mesh) using a hexane—ethyl acetate system (100 : 0 → 0 : 100). The fraction eluted with a 1 : 1 ethyl acetate—hexane mixture contained ceramide **1a**, R_f 0.52, CH_2Cl_2—MeOH (9 : 1). Rechromatography on silica gel and crystallization from the 4.5 : 0.5 $CHCl_3$—MeOH mixture gave ceramide **1a** (9 mg), m.p. 128—130 °C. Treatment of compound **1a** with a 1 : 1 acetic anhydride—pyridine mixture (~20 °C, 24 h) resulted in peracetate **1b**, a colorless amorphous powder, $[\alpha]_D$ −23.8 (*c* 4.40, $CHCl_3$). Mass spectrum (MALDI): found m/z 788.532 [M + Na]$^+$, calculated for the pseudomolecular ion $[C_{44}H_{79}NO_9Na]^+$ m/z 788.5647.

Specimen of soft coral *Sinularia* sp. (the weight of the animal was 900 g) was exhaustively extracted with ethanol at ~20 °C. After evaporation of the solvent *in vacuo* the extract was reextracted with ethyl acetate. The extract thus obtained (50 g) was chromatographed on a column (110×8 cm) packed with silica gel G (500 g, Acme, 100—200 mesh) using a hexane—ethyl acetate system (100 : 0 → 0 : 100). The fraction eluted with a 3 : 1 hexane—ethyl acetate mixture contained ceramide **2a**, R_f 0.54, CH_2Cl_2—MeOH (19 : 1). Rechromatography on silica gel and crystallization from the 4.5 : 0.5 $CHCl_3$—MeOH mixture afforded compound **2a** (20 mg) as colorless crystals, m.p. 96—98 °C. Mass spectrum (MALDI): found m/z 558.52 [M + Na]$^+$, calculated for the pseudomolecular ion $[C_{34}H_{65}NO_3Na]^+$ m/z 558.4857. Peracetate **2b** was obtained as a colorless amorphous powder after acetylation.

The fraction eluted with a 1 : 1 hexane—ethyl acetate mixture contained ceramide **3a**, R_f 0.38, CH_2Cl_2—MeOH (19 : 1). Rechromatography and crystallization from a 4.5 : 0.5 $CHCl_3$—MeOH mixture resulted in compound **3a** (50 mg) as colorless crystals, m.p. 88—90 °C. Mass spectrum (MALDI): found m/z 558.468 [M + Na]$^+$, calculated for the pseudomolecular ion $[C_{34}H_{65}NO_3Na]^+$ m/z 558.4857. Peracetate **3b** was obtained as a colorless amorphous powder after acetylation.

The fraction eluted with a 1 : 9 hexane—ethyl acetate mixture contained a mixture of lipids **4a** and **5a**, R_f 0.41, CH_2Cl_2—MeOH (19 : 1). Rechromatography and crystallization from a 9 : 1 $CHCl_3$—MeOH mixture gave a mixture of compounds **4a** and **5a** (15 mg) as colorless amorphous powder, m.p. 125—127 °C, $[\alpha]_D^{25}$ −35.0 (*c* 0.40, $CHCl_3$—MeOH (1 : 1)). Subsequent acetylation resulted in a mixture of peracetates **4b** and **5b** as a colorless amorphous powder. Mass spectrum (EI, 70 eV), m/z (I_{rel} (%)): **4b** — 495 [M – 2 AcOH – 43]$^+$ (1), 478 [M – 3 AcOH]$^+$ (1), 453 (1), 435 [M – 3 AcOH – 43]$^+$ (1), 415 (3), 385(1), 369 [M – $C_{12}H_{17}O_8$]$^+$ (100), 351 (2), 325 (3), 309 (1), 289 (1), 273 $[C_{12}H_{17}O_7]^+$ (70), 250 (1), 231 (1), 213 (15), 153 (80); **5b** — 467 [M – 2 AcOH – 43]$^+$ (1), 450 [M – 3 AcOH]$^+$ (1), 407 (1), 435 [M – 3 AcOH – 43]$^+$ (1), 387 (8), 357 (3), 341 [M – $C_{12}H_{17}O_8$]$^+$ (100), 323 (1), 297 (3), 289 (1), 273 $[C_{12}H_{17}O_7]^+$ (50), 253 (5), 231 (1), 213 (8), 153 (80). Mass spectrum (MALDI): found for **4b** — m/z 681.45 [M + Na]$^+$, calculated for $[C_{35}H_{62}O_{11}Na]^+$ pseudomolecular ion — m/z 681.4185; found for **5b** — m/z 653.476 [M + Na]$^+$, calculated for $[C_{33}H_{58}O_{11}Na]^+$ pseudomolecular ion — m/z 653.3872.

Determination of the fatty-acid composition of ceramides 1a—3a. Ceramide (1 mg) was heated in a sealed tube with

1 *M* HCl in 82% MeOH (1 mL) at 100 °C for 7 h. The reaction mixture was extracted with hexane (3×1 mL). The hexane layer was separated and passed through a small column (0.2×2 cm) with silica gel. Then the column was eluted with a 1 : 1 hexane—ethyl acetate mixture (10 mL). The eluates were combined, concentrated *in vacuo*, and analyzed by GLC and TLC. TLC analysis of compound **1a** on Sorbfil plates (1 : 1 hexane—chloroform) revealed a spot with R_f 0.12 (*cf.* R_f 0.75 for methyl octadecanoate). Based on the results of GLC and GLC-MS analyses, the major components of the hexane extracts of the methanolysis products were identified as methyl 2-hydroxyoctadecanoate (compound **1a**), mass spectrum (EI, 70 eV), *m/z* (I_{rel} (%)): 314 $[M]^+$ (40), 296 (1), 282 (3), 269 (3), 255 (95), 145 (10), 127 (20), 111 (50), 97 (100), 83 (95), 69 (85), 57 (100); methyl palmitate (compound **2a**), mass spectrum (EI, 70 eV), *m/z* (I_{rel} (%)): 270 $[M]^+$ (20), 243 (5), 227 (25), 208 (5), 185 (12), 163 (5), 143 (25), 115 (1), 97 (5), 74 (100), 55 (30); and methyl stearate (compound **3a**), mass spectrum (EI, 70 eV), *m/z* (I_{rel} (%)): 298 $[M]^+$ (20), 271 (5), 255 (20), 227 (1), 199 (10), 171 (1), 143 (25), 115 (1), 97 (5), 74 (100), 55 (30).

Hydrolysis of lipids 4 and 5. A mixture of glycolipids (3 mg) was heated in a sealed tube with 1 *M* HCl at 100 °C for 7 h. The solution was concentrated, the resudue was dissolved in water, and the hydrophobic fraction was extracted with chloroform. After evaporation of the aqueous layer, the resudie was dissolved in water, $[\alpha]_D$ −76.5 (*c* 0.92, H_2O, 3 h).

Determination of antibacterial and antifungal activity of lipids. Preliminary tests for antibacterial activity of lipids were performed using the following bacterial cultures: *Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 1688, *Bacillus subtilis* MTCC 441, and *Bacillus pumilus* NCIM 2327. A conventional solid agar technique[11] was employed. The inhibition zones were measured after 24 to 72 h. The lipid concentrations used were as follows: 50, 100, and 200 μg mL^{-1}.

Preliminary tests for antifungal activity of lipids were performed using the following cultures: *Aspergillus niger* MTCC 1344, *Rhizopus oryzae* MTCC 1987, and *Candida albicans* MTCC 183 and a conventional method of diffusion in agar.[12] The lipid concentrations used were as follows: 50, 100, and 200 μg mL^{-1}. The inhibition zones were measured after 24 to 96 h.

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