New Sphingolipids and a Sterol from a *Lobophytum* Species of the Indian Ocean

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Chemical investigation of a soft coral species of the genus *Lobophytum* has resulted in the isolation of three new sphingolipids—(2*S*,3*S*,4*R*)-2-nonadecanoylamino-octadecane-1,3,4-triol (1), (2*S*,3*R*,4*E*,8*E*)-[(2′*R*)-2′-hydroxy-heptadecanoylamino]-4,8-octadecadiene-1,3-diol (2), 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*R*,4*E*,8*E*)-2-[(2′*R*)-2′-hydroxynonadecanoylamino]-9-methyl-4,8-octadecadiene-1,3-diol (3) and a sterol—(24*S*)-ergost-5-en-3β,7β-diol (4) along with the known sphingolipid—(2*S*,3*R*,4*E*,8*E*)-2-hexadecanoylamino-4,8-octadecadien-1,3-diol (5) which showed cytotoxicity against human peripheral blood mononuclear cells (PBMC).

Key words *Lobophytum* species; marine soft coral; sphingolipids; sterol

As part of our continuous search for bioactive metabolites from marine organisms,[2—6)] we have investigated a soft coral species belonging to the genus *Lobophytum* collected from the Indian Ocean. Earlier studies of the genus *Lobophytum* led to the isolation of several sesquiterpenoids, cembrane and lobane diterpenes, sterols and sphingolipids.[7,8)] Several compounds from this genus showed cytotoxic,[9—11)] anti-HIV,[12)] antibacterial,[13)] and ichthyotoxic[14—17)] activities. In this paper we report the isolation and structural elucidation of three new sphingolipids and a sterol along with cytotoxic activity of a known sphingolipid isolated from the organism.

Sphingolipids exist widely in the eukaryotic cell membranes of both terrestrial and marine organisms.[18)] Sphingolipids attracted much attention in recent years due to their involvement in various cellular processes including cell growth, survival, differentiation, and adhesion.[19,20)] Sphingolipids were reported from various marine organisms including star fishes, soft corals, sponges, dinoflagellates, algae and tunicates.[21)] Some of them exhibited biological activities such as cytotoxic, antitumour, immunomodulatory, antiviral, antifungal and Ca^{2+}-ATPase activities.[21)] KRN 7000, a glycosphingolipid from *Agelas mauritianus*, is under Phase I clinical trials for cancer treatment.[22)]

Results and Discussion

Repeated column chromatography of EtOAc solubles of the methanolic extract of the organism followed by purification by medium pressure liquid chromatography (MPLC) afforded compounds **1**—**5** as colourless solids. The IR spectra of **1**—**3** suggested the presence of hydroxyl and amide groups while the UV spectra had no characteristic signals above 210 nm. Close examination of ^{1}H- and ^{13}C-NMR spectra of **1**—**3** (Table 1) revealed the presence of NH, carbonyl, end methyls and long aliphatic chain suggesting sphingolipid skeleton.[4,5)]

Compound **1**, analyzing for $C_{37}H_{75}NO_4$ by positive ion FAB-MS ($[M+H]^+$, *m*/*z* 598) and elemental analysis, formed triacetate (Ac2O/Py) and showed the presence of three hydroxylated carbons at δ 76.5, 72.9 and 62.0. A proton triplet at δ 2.23 (2H, J=7.8 Hz) suggested the presence of a $-CH_2-CO-NH-$ system.[4)] The positions of hydroxylated carbons were determined as C-1, C-3 and C-4 by their connectivities from ^{1}H–^{1}H COSY and HMQC spectra.

The chain lengths of the acyl and alkyl chains were determined as C_{19} and C_{18} respectively by methanolysis[23)] of **1** followed by GC-MS analysis of the methyl ester and acetyl sphingamine base. The configurations at C-2, C-3 and C-4 are given as 2*S*, 3*S* and 4*R* by comparing spectral and optical rotation data of natural and synthetic sphingolipids.[4,24—26)] Thus compound **1** is described as (2*S*,3*S*,4*R*)-2-nonadecanoylamino-octadecane-1,3,4-triol.

Compound **2**, analyzing for $C_{35}H_{67}NO_4$ by positive ion FAB-MS ($[M+H]^+$, *m*/*z* 566) and elemental analysis, formed triacetate (Ac_2O/Py) and showed three hydroxylated carbons at δ 62.1, 72.5, 74.2 and carbonyl carbon at δ 175.2. The ^{1}H–^{1}H COSY and HMQC spectral data and the absence of a triplet signal at δ 2.2—2.5, that is assigned to 2′-H of compound **1**, suggested the presence of hydroxyls at C-1, C-3 and C-2′. The presence of four olefinic protons at δ 5.78, 5.52, 5.41 and four carbons at δ 133.8, 131.4, 129.3, 129.0 and absence of proton singlet signals at δ 1.5—1.6 indicated the presence of two disubstituted double bonds. The correlations of olefinic proton at δ 5.52 with H-3 proton at δ 4.28 and H-5 proton at δ 5.78 and the coupling of H-5 proton with the olefinic proton at δ 5.41 (H-8) through two methylenes at δ 2.09 (4H, m) observed in COSY spectrum indicated a $\Delta^{4,8}$ system.

Methanolysis[23)] of **2** gave methyl ester and base which upon GC-MS analysis, after acetylation, yielded methyl-2-acetoxy heptadecanoate (*m*/*z* 342) and 1,3-diacetoxy-2-acetamino-4,8-octadecadiene (*m*/*z* 423). The stereochemistries at C-2, C-3 and C-2′ were assigned 2*S*, 3*R* and 2′*R* by comparing the NMR and optical rotation data with those of natural and synthetic sphingolipids.[24—27)] The geometry of the double bonds was taken as *trans* (4*E*,8*E*) on the basis of large coupling constants (15.3, 15.0 Hz).[28)] Thus the compound **2** is described as (2*S*,3*R*,4*E*,8*E*)-[(2′*R*)-2′-hydroxyheptadecanoylamino]-4,8-octadecadiene-1,3-diol.

The molecular formula of compound **3** was deduced as $C_{44}H_{83}NO_9$ on the basis of positive ion FAB-MS of its acetate ($[M+H-OAc]^+$, *m*/*z* 963) and elemental analysis. The presence of anomeric carbon at δ 105.1, several oxygenated carbons at δ 62.4—78.5 and many oxymethine protons at δ 3.80—4.95 indicated its glycosidic nature.[24)] Due to overlap-

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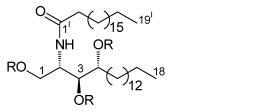
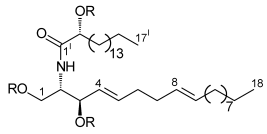
ping of signals, **3** was acetylated and spectra were recorded again on the peracetate (**3a**). The ^{1}H-NMR spectrum of **3a** indicated six acetoxy methyls while the fragment ions in FAB-MS at *m/z* 691 $[M+H-C_{14}H_{19}O_9$ (acetylated glucose)$]^+$, 632 $[691-OAc]^+$, 352 $[691-(COCH(OAc)-(CH_2)_{16}CH_3)]^+$ and 293 $[352-OAc]^+$ suggested the presence of two acetoxyls on sphingolipid moiety in addition to four in the sugar moiety. The absence of a triplet signal at δ 2.2—2.5 and ^{1}H–^{1}H COSY spectral data suggested the presence of a –CH(OAc)–CONH– moiety. The COSY and HMQC spectral data showed the presence of the other acetoxyl at C-3 and a glycosidic linkage to C-1 (δ_C 67.0). Three olefinic protons at δ 5.40, 5.82, 5.11, carbons at δ 124.4, 136.6, 122.8, 136.0, an olefinic methyl (δ_H 1.57, δ_C 15.8) and 2D NMR data suggested a $-CH=CH-CH_2-CH_2-CH=C(CH_3)-CH_2-$ moiety. The large coupling constant between H-4 and H-5 (15.0 Hz) and typical upfield shift of olefinic methyl group (C-19) confirmed '*E*' configuration for the double bonds.[28] The chemical shifts of the anomeric proton with large coupling constant (J=7.8 Hz) at δ 4.48 and other ring protons derived from COSY data suggested the presence of β-D-glucopyranose.[30,31]

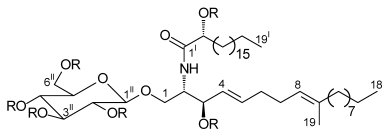
Methanolysis of **3** yielded a fatty acid methyl ester, a long chain base and methylated sugar. The acetylated methyl ester and the base were subjected to GC-MS analysis and identified as methyl-2-acetoxy nonadecanoate (*m/z* 370) and 1,3-diacetoxy-2-acetamino-9-methyl-4,8-octadecadiene (*m/z* 437). The configurations at C-2, C-3 and C-2′ were assigned as 2*S*, 3*R*, 2′*R* by comparing spectral and optical rotation data with the literature data.[30—32] The sugar from the aqueous layer was identified as methyl-β-D-glucopyranose by



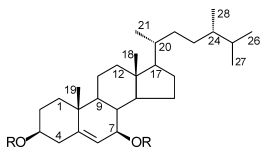
1 R = H; **1a** R = Ac



2 R = H; **2a** R = Ac



3 R = H; **3a** R = Ac



4 R = H; **4a** R = Ac

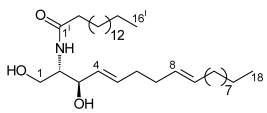


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Table 1. NMR Data of Compounds **1**, **2** and **3a**

Position	**1** ($CDCl_3$)		**2** ($CDCl_3$)		**3a** ($CDCl_3$)	
	δ_H (int., multi., *J* in Hz)	δ_C	δ_H (int., multi., *J* in Hz)	δ_C	δ_H (int., multi., *J* in Hz)	δ_C
1a	3.94 (1H, dd, 11.1, 3.3)	62.0	3.87 (1H, dd, 10.3, 3.3)	62.1	3.61 (1H, dd, 4.2, 10.2)	67.0
1b	3.73 (1H, dd, 10.9, 4.2)	—	3.76 (1H, dd, 10.3, 4.8)	—	3.93 (1H, dd, 3.9, 10.2)	—
2	4.14 (1H, m)	53.5	3.90 (1H, m)	54.6	4.30 (1H, m)	50.5
3	3.62 (1H, dd, 4.0, 3.4)	76.5	4.28 (1H, dd, 7.2, 6.0)	72.5	5.31 (1H, dd, 6.9, 6.6)	72.9
4	3.78 (1H, m)	72.9	5.52 (1H, dd, 15.3, 6.0)	129.3	5.40 (1H, dd, 15.0, 7.5)	124.4
5	1.77 (2H, m)	33.6	5.78 (1H, dt, 15.3, 6.0)	133.8	5.82 (1H, dt, 15.0, 7.2)	136.6
6			2.09 (2H, m)	32.4[a]	2.06 (2H, m)	32.4[a]
7			2.09 (2H, m)	32.1[a]	2.06 (2H, m)	31.7[a]
8			5.41 (1H, dd, 15.0, 6.0)	131.4[b]	5.11 (1H, t, 6.0)	122.8
9			5.41 (1H, dd, 15.0, 6.0)	129.0[b]	—	136.0
10			1.97 (2H, m)	32.6	1.95 (2H, br t, 7.5)	31.8
$-CH_2-$	1.26 (54H, br s)	30.1—22.8	1.26 (40H, br s)	31.9—22.7	1.25 (44H, br s)	22.5—29.5
$-CH_3$	0.88 (6H, t, 7.8)	14.1	0.88 (6H, t, 6.9)	14.1	0.88 (6H, t, 6.9)	14.0
NH	6.31 (1H, d, 7.2)	—	7.19 (1H, d, 7.2)	—	6.33 (1H, d, 9.0)	—
19-CH_3					1.57 (3H, s)	15.8
1′	—	171.3	—	175.2	—	170.4 (s)
2′	2.23 (2H, t, 7.8)	36.4	4.11 (1H, dd, 7.2, 4.5)	74.2	5.15 (1H, dd, 4.5, 5.4)	73.8
3′	1.65 (2H, m)	30.1	1.79 (2H, m)	34.8	1.78 (2H, m)	39.6
1″					4.48 (1H, d, 7.8)	100.4
2″					4.96 (1H, dd, 9.0, 8.4)	71.1
3″					5.19 (1H, m)	72.5
4″					5.05 (1H, m)	68.1
5″					3.69 (1H, m)	71.8
6a″					4.15 (1H, dd, 12.3, 2.6)	61.6
6b″					4.23 (1H, dd, 12.6, 4.5)	—
$-COCH_3$					1.99—2.17 (18H, s)	20.4—20.8, 169.1—170.0

a), *b*), *c*) Values with the same letter are interchangeable.

comparing optical rotation and spectral data with authentic sample.[4,24] Thus compound **3** is 1-*O*-(β-D-glucopyranosyl)-(2*S*,3*R*,4*E*,8*E*)-2-[(2′*R*)-2′-hydroxynonadecanoylamino]-9-methyl-4,8-octadecadiene-1,3-diol. The compounds **1**—**3** are reported for the first time.

Compound **4** was analyzed for $C_{28}H_{48}O_2$ by EI-MS (M^+-H_2O, *m/z* 398) and elemental analysis. Close examination of NMR spectra revealed 3β-hydroxy-Δ^5 sterol skeleton. The oxygenated carbons at δ 73.3, 71.4 and formation of diacetate suggested the presence of two hydroxyls. The ^{1}H-NMR spectrum indicated characteristic 3α-H at δ 3.59 (m) and another hydroxylated methine at δ 3.86 (br d). The prominent fragment ions at *m/z* 253 and 211 indicated lack of hydroxyls on either side chain or ring D and presence of conventional 24-methyl cholestane side chain.[33] The trisubstituted olefinic proton signal appeared at δ 5.29, instead of δ 5.36 characteristic of 3β-hydroxy-Δ^5 sterols, suggested the presence of the allylic hydroxyl at C-7.[34] It is reported that in 3β,7α-diol Δ^5 sterols, the H-6 proton resonates at *ca.* δ 5.60,[35] whereas in the 7β isomer it resonates at *ca.* δ 5.29.[36] Since **4** showed H-6 proton at δ 5.29, β orientation was assigned for the C-7 allylic hydroxyl. The stereochemistry of the C-24 methyl was assigned as *S*, based on the ^{1}H-NMR shifts of 26 and 27 methyls.[37,38] Thus the structure of the sterol **4** was established as (24*S*)-ergost-5-en-3β,7β-diol. The 7α- and 24*R*-isomers were reported earlier.[39,40]

Compound **5** was identified as (2*S*,3*R*,4*E*,8*E*)-2-hexadecanoylamino-4,8-octadecadien-1,3-diol, reported earlier from marine sources,[41,42] by comparing NMR and mass spectral data.

All these sphingolipids were tested for cytotoxicity against human Peripheral Blood Mononuclear Cells (PBMC) following the standard procedure.[43] Compound **5** showed cytotoxicity with an ED_{50} of 20 μg/ml whereas compound **1**—**3** are nontoxic up to 160 μg/ml.[43] The sphingolipids which are reported as cytotoxic in literature[21] are all glycosides containing one or more sugar residues. The cytotoxicity of the non-glycosidic sphingolipid, compound **5**, suggested that glycosidation may not be necessary for the activity.

Experimental

General Optical rotations were determined on JASCO DIP-370 polarimeter and are given in 10^{-1} deg cm^2 g^{-1}. UV and IR spectra were recorded on Shimadzu UV-150 double beam spectrophotometer and Perkin-Elmer FTIR 881 spectrophotometer, respectively. Melting points were determined on Boitus melting point apparatus and were uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker FT DRX-300 at 300 and 75 MHz respectively using TMS as internal standard. Elemental analysis was carried out on Carlo Erba 1108 instrument. FAB-MS (positive mode) were measured on Jeol Sx-120/DA-6000 using a beam of Argon/Xenon (2—8 KeV) using *m*-nitrobenzyl alcohol as the matrix and EI-MS were recorded on Jeol D-300 at 70 eV mass spectrometers. GC-MS analyses were performed on Shimadzu QP-5050A GC-MS instrument. Si gel column chromatography was carried out using silica gel (finer than 200#, ACME), gel filtration was carried out using LH20 (Sephadex LH20, Pharmacia Biotech) and MPLC was performed on Buchi B-688 MPLC system.

Animal Material The soft coral was collected from the Gulf of Mannar (9°17′N, 79°22′E) of the Indian Ocean at 10—15 ft depth during March 2001. It was identified as a *Lobophytum* species (Alcyoniidae). A voucher specimen was deposited in Marine organisms museum of the Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam with code AU2-179.

Extraction and Isolation The organism (wet weight *ca.* 4 kg) was extracted with MeOH and concentrated under reduced pressure. The extract was partitioned between EtOAc and water, the EtOAc layer was concentrated to give EtOAc extract (*ca.* 15 g). The extract was repeatedly chromatographed over MPLC using Si gel to afford compounds **1** (15 mg), **2** (20 mg), **3** (27 mg), **4** (10 mg) and **5** (32 mg).

Compound **1**: Colourless solid. mp 122—124 °C. IR (KBr) cm^{-1}: 3400, 3240, 2940, 2850, 1645, 1040, 470. FAB-MS *m/z*: 598 $[M+H]^+$, 318, 300, 282, 264. $[\alpha]_D^{28}$ +19.2° (*c*=0.5, $CHCl_3$). *Anal.* Calcd for $C_{37}H_{75}NO_4$: C, 74.3; H, 12.6; N, 2.3. Found: C, 74.3; H, 12.6; N, 2.35.

Compound **2**: Colourless solid. mp 111—112 °C. IR (KBr) cm^{-1}: 3400, 3250, 2920, 2850, 1625, 970. FAB-MS *m/z*: 566 $[M+H]^+$, 298, 280, 262. $[\alpha]_D^{28}$ −11.0° (*c*=0.5, $CHCl_3$). *Anal.* Calcd for $C_{35}H_{67}NO_4$: C, 74.3; H, 11.9; N, 2.45. Found: C, 74.25; H, 11.9; N, 2.5.

Compound **3**: Colourless solid. mp 218—220 °C. ^{1}H-NMR (pyridine-d_5) δ: 8.50 (1H, d, *J*=8.7 Hz, NH), 1.28 (br s), 0.89 (6H, t, *J*=6.6 Hz, end methyls), 3.80—4.95 (14H), 2.09 (m), 1.95 (m), 1.83 (m); ^{13}C-NMR (pyridine-d_5) δ: 175.2, 136.4, 134.1, 126.0, 124.1, 105.1, 78.7, 78.5, 76.0, 73.0, 72.6, 70.5, 62.4, 52.2, 34.9, 32.2, 31.9, 29.5—23.0, 16.2, 14.3. IR (KBr) cm^{-1}: 3400, 3300, 2940, 2850, 1650, 1450, 970. FAB-MS: 770 $[M+H]^+$, 752, 734, 589, 571, 276, 109. $[\alpha]_D^{28}$ −13.2° (*c*=0.05, MeOH). *Anal.* Calcd for $C_{44}H_{83}NO_9$: C, 68.6; H, 10.9; N, 1.8. Found: C, 68.6; H, 10.9; N, 1.8.

Compound **4**: Colourless crystals. mp 211—213 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.59 (1H, m, H-3α), 5.29 (1H, br s, H-6), 3.86 (1H, br d, *J*=7.5 Hz, H-7), 0.68 (3H, s, H_3-18), 1.05 (3H, s, H_3-19), 0.94 (3H, d, *J*=6.0 Hz, H_3-21), 0.85 (3H, d, *J*=6.7 Hz, H_3-26), 0.79 (3H, d, *J*=6.6 Hz, H_3-27), 0.77 (3H, d, *J*=6.6 Hz, H_3-28). ^{13}C-NMR ($CDCl_3$) δ: 37.0 (C-1), 31.4 (C-2), 71.4 (C-3), 41.7 (C-4), 143.5 (C-5), 125.4 (C-6), 73.3 (C-7), 40.9 (C-8), 48.3 (C-9), 36.9 (C-10), 21.1 (C-11), 39.5 (C-12), 42.0 (C-13), 55.6 (C-14), 26.4 (C-15), 28.4 (C-16), 55.9 (C-17), 11.8 (C-18), 19.1 (C-19), 36.1 (C-20), 18.2 (C-21), 33.7 (C-22), 29.6 (C-23), 39.1 (C-24), 31.4 (C-25), 17.6 (C-26), 20.7 (C-27), 15.4 (C-28). IR (KBr) cm^{-1}: 3400—3300, 1640, 1370, 1050, 970. EI-MS *m/z*: 398 $[M^+-H_2O]$, 383 $[M^+-H_2O-CH_3]$, 380 $[M^+-2H_2O]$, 365 $[M^+-2H_2O-CH_3]$, 253 [M^+−side chain (C_9H_{19})−$2H_2O$], 211 [M^+−side chain−ring D (C_3H_5)−$2H_2O$−H], 43. $[\alpha]_D^{28}$ −64.1° (*c*=0.05, $CHCl_3$). *Anal.* Calcd for $C_{28}H_{48}O_2$: C, 80.6; H, 11.6. Found: C, 80.7; H, 11.6.

Compound **5**: Colourless solid. mp 98—100 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.88 (1H, m, H-1a), 3.68 (1H, m, H-1b), 3.91 (1H, m, H-2), 4.27 (1H, m, H-3), 5.52 (1H, dd, *J*=15.0, 6.0 Hz, H-4), 5.80 (1H, dt, *J*=15.0, 6.0 Hz, H-5), 2.08 (4H, m, H_2-6,7), 5.40 (2H, dd, *J*=15.0, 6.0 Hz, H-8, 9), 1.97 (2H, m, H_2-10), 2.21 (2H, t, *J*=7.5 Hz, H_2-2′), 1.62 (2H, m, H_2-3′), 1.26 (38H, br s, H_2-11 to 17, 4′ to 15′), 0.88 (6H, t, *J*=6.9 Hz, H_3-18, 16′), 6.42 (1H, d, *J*=6.9 Hz, NH). ^{13}C-NMR ($CDCl_3$) δ: 62.2 (C-1), 54.6 (C-2), 74.1 (C-3), 129.1 (C-4), 133.3 (C-5), 32.3 (C-6), 32.1 (C-7), 131.2 (C-8), 129.0 (C-9), 32.6 (C-10), 31.9—22.6 (C-11 to 17, C-3′ to 15′), 14.1 (C-18, 16′), 174.2 (C-1′), 36.8 (C-2′). FAB-MS *m/z*: 536 $[M+H]^+$, 518, 298, 280, 262. $[\alpha]_D^{28}$ +12.1° (*c*=0.05, $CHCl_3$). *Anal.* Calcd for $C_{34}H_{65}NO_3$: C, 76.1; H, 12.1; N, 2.7. Found: C, 76.2; H, 12.2; N, 2.6.

Acetylation Compounds **1**—**4** were acetylated using Ac_2O and pyridine to yield corresponding acetate derivatives **1a**—**4a** as colourless crystals.

Compound **1a**: mp 64—65 °C. ^{1}H-NMR ($CDCl_3$) δ: 6.72 (1H, d, *J*=9.0 Hz, NH), 4.23 (1H, m, H-2), 1.99 (3H, s, $COCH_3$), 2.01 (3H, s, $COCH_3$), 2.03 (3H, s, $COCH_3$), 1.25 (br s, $-CH_2-$), 0.86 (6H, t, *J*=6.9 Hz, end methyls).

Compound **2a**: mp 59 °C. ^{1}H-NMR ($CDCl_3$) δ: 6.81 (1H, d, *J*=7.2 Hz, NH), 1.99—2.05 (9H, s, 3 $COCH_3$), 1.26 (br s, $-CH_2-$), 0.85 (6H, t, *J*=6.9 Hz, end methyls).

Compound **3a**: mp 65—67 °C. FAB-MS *m/z*: 963, 904 $[M+H-2OAc]^+$, 691 [M+H−$C_{14}H_{19}O_9$ (acetylated glucose)]$^+$, 632 $[691-OAc]^+$, 352 $[691-(COCH(OAc)(CH_2)_{16}CH_3)]^+$, 331 $[C_{14}H_{19}O_9]^+$, 293 $[352-OAc]^+$, 276 $[CH_2C(NH_2)=CHCH=CH(CH_2)_2CH=C(CH_3)-(CH_2)_8CH_3]^+$, 229, 169, 109. $[\alpha]_D^{28}$ −18.3° (*c*=0.05, $CHCl_3$).

Compound **4a**: ^{1}H-NMR ($CDCl_3$) δ: 1.99 (3H, s, $COCH_3$), 2.01 (3H, s, $COCH_3$), 4.52 (1H, m), 4.12 (1H, br d), 5.27 (1H, br s), 0.68 (3H, s), 0.85 (6H, d, *J*=6.0 Hz), 0.98 (3H, d, *J*=6.6 Hz), 0.78 (3H, d, *J*=6.6 Hz), 1.05 (3H, s).

Methanolysis Compounds **1**—**3** were refluxed separately with 3 ml of 1 N HCl and 10 ml of MeOH for 15 h. The reaction mixtures were then extracted with *n*-hexane to give corresponding fatty acid methyl esters which were analyzed by GC-MS after acetylation with Ac_2O-Py. In each case a single compound was obtained. The aqueous layers from **1** and **2** were evaporated and the residues were acetylated. Purification over Sephadex LH-20 column (CH_2Cl_2/MeOH, 1 : 1) gave the corresponding acetylated sphingamines which were analyzed by GC-MS. The aqueous layer from **3** was evaporated to dryness and the residue was separated by TLC as sphingosine base and methylated sugar. The base was acetylated and analyzed by GC-MS. The sugar was identified as methyl β-D-glucopyranose by comparing NMR and optical rotation data with literature data, $[\alpha]_D^{28}$ +74° (*c*=0.02,

MeOH), *Rf* 0.46 (EtOAc/MeOH/H_2O, 5 : 2 : 0.5).

Methyl Ester from **1**: ^{1}H-NMR ($CDCl_3$) δ: 2.23 (2H, t, J=7.2 Hz, H_2-2), 3.55 (3H, s, OCH_3), 1.26 (br s, $-CH_2-$), 0.85 (3H, t, J=7.2 Hz, H_3-19). GC-MS m/z: 312 $[M]^+$.

Methyl Ester from **2**: ^{1}H-NMR ($CDCl_3$) δ: 5.10 (1H, dd, J=6.6, 4.2 Hz, H-2), 3.57 (3H, s, OCH_3), 1.99 (3H, s, $COCH_3$), 1.26 (br s, $-CH_2-$), 0.86 (3H, t, J=7.2 Hz, H_3-17). GC-MS m/z: 342 $[M]^+$.

Methyl Ester from **3**: ^{1}H-NMR ($CDCl_3$) δ: 5.12 (1H, dd, J=6.6, 3.9 Hz, H-2), 3.55 (3H, s, OCH_3), 1.99 (3H, s, $COCH_3$), 1.25 (br s, $-CH_2-$), 0.86 (3H, t, J=7.2 Hz, H_3-19). GC-MS m/z: 370 $[M]^+$.

Acetyl Sphingamine from **1**: ^{1}H-NMR ($CDCl_3$) δ: 6.62 (1H, d, J=8.7 Hz, NH), 5.10 (1H, dd, J=7.2, 3.9 Hz, H-3), 4.97 (1H, m, H-4), 4.41 (1H, m, H-2), 4.35 (1H, dd, J=6.2, 10.8 Hz, H-1a), 4.26 (1H, dd, J=4.2, 10.5 Hz, H-1b), 1.26 (br s, $-CH_2-$), 0.85 (3H, t, J=7.2 Hz, H_3-18), 1.99—2.03 (12H, s, 4×$COCH_3$). GC-MS m/z: 485 $[M]^+$.

Acetyl Sphingamine from **2**: ^{1}H-NMR ($CDCl_3$) δ: 6.65 (1H, d, J=8.4 Hz, NH), 5.12 (1H, dd, J=7.2, 4.2 Hz, H-3), 5.81 (1H, dt, J=6.9, 15.4 Hz, H-5), 5.42 (1H, dd, J=6.4, 15.7 Hz, H-4), 5.31 (2H, dd, J=15.3, 6.0 Hz, H-8, 9), 2.06 (4H, m, H_2-6, 7), 1.96 (2H, m, H_2-10), 1.26 (br s, $-CH_2-$), 0.85 (3H, t, J=7.2 Hz, H_3-18), 1.99—2.05 (9H, s, 3×$COCH_3$). GC-MS m/z: 423 $[M]^+$.

Acetyl Sphingamine from **3**: ^{1}H-NMR ($CDCl_3$) δ: 6.62 (1H, d, J=7.5 Hz, NH), 3.60 (1H, dd, J=10.2, 4.2 Hz, H-1a), 3.97 (1H, dd, J=10.2, 3.9 Hz, H-1b), 4.32 (1H, m, H-2), 5.32 (1H, dd, J=7.2, 6.3 Hz, H-3), 5.40 (1H, dd, J=15.0, 7.2 Hz, H-4), 5.80 (1H, dt, J=15.3, 7.2 Hz, H-5), 2.05 (4H, m, H_2-6, 7), 5.09 (1H, t, J=6.3 Hz, H-8), 1.95 (2H, t, J=7.5 Hz, H_2-10), 1.62 (3H, s, H_3-19), 2.01—2.03 (9H, s, 3×$COCH_3$), 1.26 (br s, $-CH_2-$), 0.85 (3H, t, J=7.2 Hz, H_3-18). GC-MS m/z: 437 $[M]^+$.

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