Temnosides A and B, Two New Glycosphingolipids from the Sea Urchin *Temnopleurus toreumaticus* of the Indian Coast[1]

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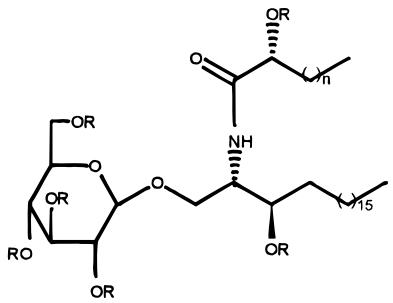
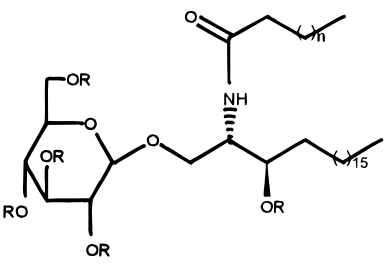
Two new glycosphingolipids, temnosides A (**1**) and B (**2**), have been isolated from a MeOH extract of the sea urchin *Temnopleurus toreumaticus*. The proposed structures 1-*O*-(*β*-D-glucopyranosyl)-D-(+)-(2*S*,3*R*)-2-[(2′(*R*)-hydroxytricosanoyl]amido]-1,3-eicosanediol (**1**) and 1-*O*-(*β*-D-glucopyranosyl)-D-(+)-(2*S*,3*R*)-2-(docosanoylamido)-1,3-eicosanediol (**2**) were established by FABMS, 2D NMR, and chemical degradation studies.

Because of increasing interest in sphingolipids due to their promising biological activities, we started a systematic study of the sphingolipid composition of marine flora and fauna collected from the Indian coast. During the course of these investigations, two ceramides showing antiviral activity from the green alga *Ulva fasciata*[2,3] and a novel Δ^6 phytosphingosine-type ceramide and its glycosyl derivatives from the sponge *Sphirastrella inconstans*[4,5] have been reported. Recently, a novel sulfated ceramide from *Zoanthus*[6] species and phytosphingosine monohexosides from the starfish *Pentaceraster regulus*[7] have been isolated. We now describe the isolation and structure elucidation of two new cerebrosides, temnosides A and B, from the antivirally active[8] MeOH extract of *Temnopleurus toreumaticus* (Leske).

An Me_2CO-insoluble fraction was obtained through bioassay-guided fractionation of the MeOH extract. Chromatography over Si gel with elution by $CHCl_3$−MeOH (9:1→1:1), followed by flash chromatography over Si gel eluting with $CHCl_3$−MeOH (8:2), afforded compounds **1** and **2** as colorless amorphous powders.



1 R=H, n=20
1a R=Ac, n=20



2 R=H, n=19

Table 1. ^{1}H- and ^{13}C-NMR Data (δ/ppm) for Compound **1** in C_5D_5N

position	^{1}H (mult)/(Hz)	^{13}C (mult)[a]	^{1}H−^{1}H COSY
long chain base			
1a	4.75 (dd, J = 6.1, 12.5)	70.60(t)	H-1b, H-2
1b	4.35 (dd, J = 6.0,12.8)		H-1a, H-2
2	5.29 (m)	53.80 (d)	H-1a,H-1b, NH,H-3
3	4.65 (m)	72.69(d)	H-2, H-4
4	1.95 (m)	35.62 (t)	H-3
5−19	1.21 (br s)	22.9−32.1(t)	
20	0.85 (t, J = 8.7)	14.27 (q)	
NH	8.60 (d, J = 9.1)		H-2
N-acyl moiety			
1′		175.75 (s)	
2′	4.28 (m)	75.80 (d)	H-3′
3′	1.82 (m)	32.16 (d)	H-2′
4′-22′	1.21 (brs)	22.9−32.1 (t)	
23′	0.85 (t, J = 8.7)	14.27 (q)	
sugar moiety			
1″	4.95 (d, J = 8.1)	105.45 (s)	H-2″
2″	4.05 (dd, J = 8.4, 9.7)	75.15 (d)	H-1″, H-3″
3″	4.25 (m)	78.47 (d)	H-4″, H-2″
4″	4.36 (m)	71.64 (d)	H-5″, H-3″
5″	3.90 (m)	78.47 (d)	H-4″,H-6a″ H-6b″
6a″	4.52 (dd, J = 5.2, 11.6)	62.73 (t)	H-5″,H-6b″
6b″	4.20 (dd, J = 2.2, 11.8)		H-5″,H-6a″

[a] Assignments were made by DEPT and HMQC studies.

Compound **1** showed strong IR absorption bands at 3400, 1040 cm^{-1} (hydroxy), 1635 cm^{-1} (amide carbonyl), and 2950, 1450 cm^{-1} (aliphatic), suggesting the presence of a fatty-acid amide. The positive FABMS of **1** displayed a molecular ion peak at m/z 866 $(M + Na)^+$ consistent with the molecular formula $C_{49}H_{97}NO_9$. The ^{1}H-NMR spectrum (Table 1) of **1** exhibited a doublet at δ 8.60 (J = 9.1 Hz) due to an NH proton, which was exchangeable with D_2O; a broad singlet at δ 1.21 (methylene protons); a triplet at δ 0.85 (two terminal methyls); an anomeric proton at δ 4.95 (J = 8.1 Hz), and carbinol protons appearing as multiplets between δ 3.90 and 4.75, suggesting it to be a cerebroside. The ^{13}C-NMR spectrum of **1** showed carbon signals at δ 175.7 (s), 53.8 (d), 35.6−22.9 (methylene carbons), and 14.3 (q), further supporting the fatty-acid amide nature of **1**. In the ^{13}C-NMR spectrum the carbon resonances appeared at δ 62.7 (t), 71.6 (d), 75.1 (d), 78.5 (d) (two carbons), and 105.4 revealing the presence of β-glucopyranoside[9] as the sugar residue in **1**. The anomeric proton at δ 4.95 (d, J = 8.1 Hz) correlated to the carbon signal at δ 105.4 in the HMQC spectrum, further confirming the β-configuration of the sugar residue.

Compound **1** on acetylation with Py−Ac_2O gave a peracetyl derivative (**1a**) that showed a molecular ion

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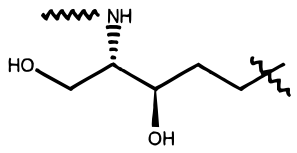
peak at m/z 1118 $(M + Na)^+$ in its FABMS, consistent with the composition $C_{61}H_{109}NO_{15}$ for **1a**. The presence of a fragment ion peak at m/z 764 $[M - 331]^+$ confirmed hexose as the sugar residue. The ^{1}H-NMR spectrum of **1a** showed six acetyl groups resonating at δ 1.99−2.09. The placement of hydroxyl groups in the ceramide group and the assignment of individual sugar protons was established by ^{1}H−^{1}H COSY and HMQC spectral data.

In the ^{1}H−^{1}H COSY spectrum (Table 1), the NH doublet at δ 8.60 showed a cross peak at δ 5.29 (m) attributed to the H-2 proton. The latter proton showed coupling with two double doublets at δ 4.35, 4.75 and a multiplet at δ 4.65, assigned to protons H-1 and H-3, respectively. The H-3 proton also showed coupling with the multiplet at δ 1.95 (2H) assigned to the H-4 protons. The other carbinol proton appearing as a multiplet at δ 4.28 showed only one cross peak to δ 1.82 (m). The above ^{1}H−^{1}H correlation studies suggested the placement of one hydroxyl group in the *N*-acyl moiety.

The HMQC spectrum of **1** showed correlation for H-1a (4.35), H-1b (4.75), H-2 (5.29), and H-3 (4.65) protons with the carbon signals at δ 70.60 (C-1), 53.80 (C-2), and 72.69 (C-3) respectively. The carbinol proton resonating at δ 4.28 (m) was also correlated with the carbon signal at δ 75.80. These correlations support the presence of a 2-amino-1,3-alkane diol as the long-chain base (LCB) and a 2-hydroxy fatty acid as the *N*-acyl moiety. The above 2D NMR spectroscopic evidence (^{1}H−^{1}H COSY and HMQC) established the partial structure A.



Partial Structure A

Compound **1**, on methanolysis[10] using aqueous MeOH−0.9 N HCl and extraction with *n*-hexane, yielded a mixture of three fatty-acid methyl esters (FAME). The GC−MS of FAME showed three peaks in the ratio of 8:1:1. The major peak [m/z 384 (M^+), $[\alpha]^{27}_D + 3.1°$] was identified as methyl 2(*R*)-hydroxytricosanoate by comparing optical rotation[11] and spectral data[12] to those reported. The aqueous layer from the hydrolysis was neutralized with Ag_2CO_3 and subjected to column chromatography over Si gel [$CHCl_3$−MeOH−H_2O 12:7:1] to yield methyl glucoside, m/z 194 $(M)^+$, $[\alpha]^{27}_D$ +76.1° (*c* 0.01, MeOH), which was close to that of an authentic sample, $[\alpha]^{25}_D$ +77.3°,[13] indicating that glucose was present as the D-isomer.

Because the major *N*-acyl moiety and the sugar residue were respectively identified as 2(*R*)-hydroxytricosanoic acid and β-glucopyranoside, the LCB of **1** must be assigned as 2-amino-1,3-dihydroxyeicosane. This was confirmed by the fragment ion peak at m/z 328 constituting the molecular formula $C_{20}H_{42}NO_2$ (LCB) after substracting the *N*-acyl moiety and sugar residue from **1**.

The relative stereochemistry at C-2 and C-3 in **1** were found to be same as that of D-sphingosine (D-erythro), as evidenced by ^{13}C-NMR chemical shifts of C-2 (δ 53.80) and C-3 (δ 72.69), which are consistent with those reported for synthetic glycosyl D(+)-(2*S*,3*R*)-sphingosine (δ 53.8, 72.6).[14] Thus, the structure of **1** was unambiguously assigned as 1-*O*-(β-D-glucopyranosyl)-D-(+)-(2*S*,3*R*)-2-[[2′(*R*)-hydroxytricosanoyl]amido]-1,3-eicosanediol.

Compound **2** was obtained as a colorless, amorphous powder. Its IR spectrum showed characteristic absorption bands at 3400, 1040 cm^{-1} (hydroxy) 1630 cm^{-1} (amide), and 2940, 1435 cm^{-1} (aliphatic) due to a cerebroside unit. The positive FABMS showed a molecular ion peak at m/z 836 $[M + Na]^+$, consistent with the composition $C_{48}H_{95}NO_8$. The ^{1}H-NMR spectrum of **2** exhibited characteristic features due to a cerebroside unit at δ 8.52 (d, $J = 9.0$ Hz) for an NH proton; a doublet at δ 4.95 ($J = 8.4$ Hz) for an anomeric proton, a broad singlet at δ 1.24 (aliphatic protons), and a triplet at δ 0.85 (two terminal methyls). The carbinol proton region at δ 3.90−4.75 was very similar to that of **1** (see Experimental Section). The presence of a triplet at δ 2.42 ($J = 7.2$ Hz) due to carboxy methylene protons indicated that the *N*-acyl moiety in **2** was a non-hydroxy fatty acid. This was further confirmed when **2** was subjected to methanolysis followed by usual work up, which gave FAME. The GC−MS of the FAME showed three peaks in the approximate ratio of 8:2:1, and the major peak was identified as methyl docosanoate, m/z 354 (M^+). The sugar residue obtained from the aqueous layer was identified as methyl-β-D-glucopyranoside by comparing the spectral data and optical rotation with those reported for an authentic sample.[13]

The relative stereochemistry at C-2, C-3 was taken as 2*S*,3*R* (erythro) based on ^{1}H-NMR chemical shifts and optical rotation reported for synthetic D(+)-erythro sphingosine.[15] Thus, the structure **2** was assigned as 1-*O*-(β-D-glucopyranosyl)-D-(+)-(2*S*,3*R*)-2-(docosanoylamido)-1,3-eicosanediol.

The natural occurrence of the dihydrosphingosine-type ceramide symbiromide was earlier reported from the dinoflagellate *Symbodium* species.[16] To the best of our knowledge, cerebrosides containing 2-amino-1,3-eicosanediol as the LCB, an analogue of dihydro sphingosine, have not previously been reported from natural sources.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Perkin-Elmer 881 instrument; FABMS on a JEOL-Sx-120/DA-6000 mass spectrometer using a beam of Argon/Xenon (2−8 kev) and *m*-nitrobenzyl alcohol as the matrix. EIMS spectra were recorded on a JEOL-JMS-D-300 at 70 eV. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker WM (400 MHz) instrument. ^{1}H−^{1}H COSY and HMQC experiments were performed on Bruker DRX at 300 MHz equipped with an Aspect 2000 computer using TMS as internal reference. Optical rotations were taken on an Autopol-III automatic polarimeter. GC was carried out using a steel column (2 m × 4 mm) packed with 3% OV-25 on Gas chrom Q; FID temperature 80 °C; N_2 at 50 mL/min as carrier gas. Flash chromatography was performed on E.E 10 (EYELA) A.S.C. equipment with fraction collector using Si gel (230−400 mesh).

Animal Material. The fresh animal material (15 kg) was collected from Mandapam coast, Tamilnadu, India, and brought to the laboratory submerged in *n*-BuOH. A voucher specimen has been preserved in the museum of the Institute (voucher no. CDR-50).

Extraction and Isolation. The shade-dried material was crushed to pieces and extracted with MeOH (2 × 3 L) to yield 400 g of MeOH extract, which was further desalted with dry MeOH to give a MeOH-soluble fraction (271 g). This fraction was partitioned between EtOAc and H_2O, and the organic phase was concentrated to give an EtOAc soluble-fraction (21 g). The chemical constituents of this fraction have been reported elsewhere.[17] The aqueous layer was extracted with *n*-BuOH to yield an *n*-BuOH-soluble fraction (15 g) that was treated with CH_3COCH_3 to remove nonpolar metabolites. The CH_3COCH_3-insoluble portion (1.15 g) was then chromatographed over Si gel (60−120 mesh) eluting with $CHCl_3$−MeOH (9:1→1:1), and 30 fractions of 100 mL each were collected. Fractions 11−16, eluted with $CHCl_3$−MeOH (3:1), were combined and concentrated *in vacuo* after monitoring by TLC [Si gel plates, solvent system: $CHCl_3$−MeOH (8:2)]. Flash chromatography of this material (120 mg) was carried out over Si gel (230−400 mesh) using $CHCl_3$−MeOH (8:2→7:3) as eluents, and 50 fractions of 25 mL each were collected. The fractions 15−20 and 22−27 afforded **2** (7 mg) and **1** (25 mg), respectively, as colorless, amorphous powders.

Temnoside A (1): amorphous powder; mp 205−207 °C; $[\alpha]^{27}_D$ +8.6° (*c* 0.01, pyridine); IR (KBr) ν_{max} 3400 (OH), 2950, 1635 (C=O), 1535, 1450, and 1040 cm^{-1}, ^1H- and ^{13}C-NMR data, see Table 1; FABMS, *m*/*z* 866 $(M + Na)^+$, 844 $(M + H)^+$, 681 $(M - 162)^+$, 328 ($C_{20}H_{42}NO_2$).

Temnoside B (2): amorphous powder; mp 216−17 °C; $[\alpha]^{27}_D$ +8.1° (*c* 0.01, pyridine) IR (KBr) ν_{max} 3400 (OH), 2940, 1630 (C=O), 1530, 1435, and 1040 cm^{-1}; FABMS 836 $(M + Na)^+$, 651 $(M - 162)^+$, ^1H NMR (δ, pyridine-d_5) 0.85 (t, 6H, J = 7.8 Hz, 2 × CH_3), 1.24 (br s, 68H), 1.77−1.85 (m, 2H, H-3′), 1.94 (m, 2H, H-4), 2.42 (t, 2H, J = 7.2 Hz, H-2′), 4.55 (m, 1H, H-6″), 4.05 (t, 1H, J = 8.4 Hz, H-2″), 4.20 (m, 1H, H-6″), 4.25 (m, 1H, H-3″), 4.35 (dd, 1H, J = 6.1, 12.9 Hz, H-1a), 4.36 (m, 1H, H-4″), 3.90 (m, 1H, H-5″), 4.66 (m, 1H, H-3), 4.75 (dd, 1H, J = 5.7, 12.8, H-1b), 4.95 (d, 1H, J = 8.4 Hz, H-1″), 5.29 (m, 1H, H-2), 8.52 (d, 1H, J = 9.0 Hz, N*H*).

Acetylation of 1. Compound **1** (4 mg) was added to dry pyridine (0.25 mL) and Ac_2O (0.5 mL) and left overnight. After usual workup, the reaction mixture was chromatographed over Si gel [hexane−EtOAc (8:2)] to yield peracetyl derivative **1a** (6.2 mg) as a colorless syrup: FABMS, 1118 $(M + Na)^+$ 764 $(M - 331)^+$, 331 (base peak); ^1H NMR (δ, $CDCl_3$), 0.87 (t, 6H, J = 7.2 Hz), 1.25 (br s, 68H), 1.59 (m, 2H), 1.85 (m, 2H), 1.99−2.09 (s, 6 × $COCH_3$), 3.68 (m, 1H, H-1a), 3.84 (m, 1H, H-1b), 4.14 (m, 1H, H-5″), 4.23 (m, 1H, H-2), 4.48 (d, 1H, J = 8.1 Hz, H-1″), 4.86−5.21 (m, 7H, carbinol protons), 6.79 (d, 1H, J = 9.0 Hz, N*H*).

Methanolysis of 1. Compound **1** (6 mg) was refluxed with 1 mL of 82% aqueous MeOH and 0.1 mL of 0.9 N HCl for 18 h. The reaction mixture was extracted with *n*-hexane to give FAME. The GC−MS of the FAME showed three peaks in the ratio of 8:1:1 [M^+ 384 (t_R 31.60), M^+, 370 (t_R 30.10), M^+, 398 (t_R 34.01)]. The preparative TLC of FAME on HPTLC (Whatman) plates [solvent system: MeOH−H_2O (4%)] afforded methyl tricosanoate as major component, *m*/*z* 384 (M^+); $([\alpha]^{27}_D$ +3.1° (*c* 0.01, $CHCl_3$); ^1H NMR (δ, $CDCl_3$), 0.85 (t), 1.25 (br s), 3.85 (s, OCH_3), 4.22 (m, 1H). The aqueous layer was treated with $AgCO_3$ to neutral pH and concentrated *in vacuo*. The column chromatography of crude product over Si gel afforded a mixture of α and β-anomer of methyl glucosides [R_f 0.65 (β), 0.62 (α)], [solvent system, $CHCl_3$−MeOH−H_2O (12:7:1)] $[\alpha]^{27}_D$ +76.1° (*c* 0.01, MeOH); EIMS *m*/*z* 194 (M^+), 163 $(M - 31)^+$; ^1H NMR (δ pyridine d_5) 4.70 (H-1, α anomer, d, J = 3.6 Hz), 4.27 (H-1, β-anomer,d, J = 7.8 Hz), 3.60 (OCH_3, β-anomer), 3.46 (OCH_3, α-anomer). The LCB could not be isolated due to paucity of the sample.

Methanolysis of 2. Compound **2** (4 mg) on methanolysis using 82% aqueous MeOH and 0.9 N HCl followed by usual workup and column chromatography afforded FAME and methyl glucoside. The GC−MS of the FAME showed three peaks in the ratio of 8:2:1 [M^+ 354 (t_R 37.15), M^+ 340 (t_R 36.95), M^+ 368 (t_R 38.20)]. The methyl glucoside was identified as methyl β-glucopyranoside by comparing spectral data and optical rotation, and by CO-TLC with an authentic sample.

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