New Sphingosines from a Gorgonian, *Pseudopterogorgia australiensis* Ridley, of the Indian Ocean†

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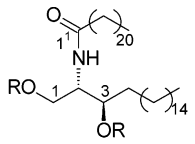
Two new sphingosines, (2*S*,3*R*)-2-(docosanoyl amino)nonadecane-1,3-diol (**1**) and (2*S*,3*S*,4*R*)-2-[(2′*R*)-2′-hydroxynonadecanoylamino]nonadecane-1,3,4-triol (**3**), along with the known (2*S*,3*R*,4*E*)-2-(heptadecanoylamino)octadec-4-ene-1,3-diol, have been isolated from *Pseudopterogorgia australiensis*, of the Indian Ocean. The structures were deduced from spectral and chemical methods. Compounds **1**−**3** showed moderate antibacterial activity.

In continuation of our studies on marine organisms,[1−5] we have examined the gorgonian *Pseudopterogorgia australiensis* collected from the Tuticorin coast of the Indian Ocean. A number of terpenoids, sterols,[6] and a sphingolipid[7] were isolated from various *Pseudopterogorgia* species. Cytotoxic, ichthyotoxic, neurotoxic, anti-inflammatory, analgesic, and antibacterial activities were reported for some of the compounds isolated from this genus.[6,7] A derivative of the terpenoid glycoside, pseudopterosin E, isolated from *P. elisabethae*, is undergoing phase I clinical trials as an anti-inflammatory agent.[8] Herein we report the isolation of sphingolipids from the organism.

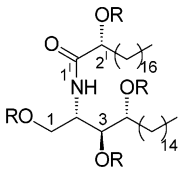
Sphingolipids exist widely in the membranes of eukaryotic cells and play important roles in many physiological processes.[9] Sphingolipids have been isolated from a number of marine organisms including sea stars, sea anemones, gorgonians, sponges, tunicates, dinoflagellates, and algae.[10] Some of the sphingolipids are reported to have cytotoxic, antitumor, immunostimulatory, antifungal, antimicrobial, antiviral, and Ca^{2+}-ATPase activities.[10] KRN 7000, a glycosphingolipid isolated from *Agelas* sp., is under phase I clinical trials as an anticancer agent.[11]

The EtOAc fraction of the methanol extract of the shade-dried organism, upon chromatography over MPLC, yielded compounds **1**−**3** as colorless crystals. These compounds had IR absorptions for the hydroxyl and amide NH (3500−3200 cm^{-1}), amide carbonyl (1640 cm^{-1}), and a long aliphatic chain (2910−2850 cm^{-1}) and had no significant UV absorptions above 200 nm. The presence of carbons and protons corresponding to an amide, a long methylene chain, and a terminal methyl in ^{13}C and ^{1}H NMR spectra and a close scrutiny of the spectra suggested a sphingolipid frame for the compounds.[5]

Compound **1**, analyzing for $C_{41}H_{83}NO_3$ by positive ion FABMS ([M + H]$^+$, *m*/*z* 638) and elemental analysis, showed the presence of two oxygenated carbons at δ 62.5 and 72.5 in the ^{13}C NMR spectrum and formed a diacetate (Ac_2O/Py) suggestive of two hydroxyls. A triplet at δ 2.25 suggested a $-CH_2-CO-NH-$ skeleton. The hydroxyls on C-1 and C-3 were ascertained from the $^{1}H-^{1}H$ COSY and HMQC spectra. The lengths of the acyl and alkyl chains were determined by employing methanolysis,[12] and the resultant methyl ester was identified as methyl docosanoate (GC−MS, *m*/*z* 354) and the basic moiety on acetylation gave 1,3-diacetoxy-2-acetaminononadecane (GC−MS, *m*/*z* 441). The relative stereochemistries at C-2 and C-3 were assigned as 2*S*, 3*R* on the basis of ^{13}C NMR chemical shifts of C-2 (δ 53.8) and C-3 (δ 72.5), which are consistent with those reported for glycosyl D(+)-(2*S*,3*R*)-sphingosine (δ 53.8, 72.6).[13,14] Thus compound **1** could be described as (2*S*,3*R*)-2-(docosanoylamino)nonadecane-1,3-diol.



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



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

Compound **3** analyzed for $C_{38}H_{77}NO_5$ by positive ion FABMS ([M + H]$^+$, *m*/*z* 628) and elemental analysis. The presence of four hydroxyls was suggested by the presence of three oxymethine carbons at δ 76.7, 73.1, and 72.7 and an oxymethylene carbon at δ 62.1 and the formation of tetraacetate. The absence of a triplet at δ 2.2−2.5 and careful study of $^{1}H-^{1}H$ COSY and HMQC spectral data revealed the positions of hydroxyls on C-1, C-3, C-4, and C-2′. The fatty acyl and alkyl chains were ascertained by the method employed earlier vide infra. The relative stereochemistries at C-2, C-3, and C-4 were assigned as 2*S*, 3*S*, 4*R* based on comparison of optical rotation[15] and ^{13}C NMR chemical shifts of C-2 (δ 53.1), C-3 (δ 76.7), and C-4 (δ 73.1) with literature data of natural[15−17] and synthetic sphingamines.[18] Thus compound **3** is described as (2*S*,3*S*,4*R*)-2-[(2′*R*)-2′-hydroxynonadecanoylamino]nonadecane-1,3,4-triol.

Compound **2** analyzed for $C_{35}H_{69}NO_3$ by positive ion FABMS ([M + H]$^+$, *m*/*z* 552) and elemental analysis. The ^{1}H and ^{13}C NMR spectra of the **2** were found to be identical with those of (2*S*,3*R*,4*E*)-2-(heptadecanoylamino)octadec-

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4-ene-1,3-diol reported earlier from the red algae *Amansia glomerata*.[19]

Compounds **1**−**3** showed moderate antibacterial activity, at 1 mg/mL concentration, against Gram-positive bacteria *Bacillus pumilis*, *B. subtilis*, and *Staphylococcus aureus* and Gram-negative bacteria *Escherichia coli*, *Proteus vulgaris*, and *Pseudomonas aeruginosa*, by cup-diffusion method.[20] None of the compounds showed antifungal activity against *Candida albicans* and *Aspergillus niger*.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX spectrometer operating at 300 and 75 MHz, respectively. The chemical shift values were reported in parts per million units, and the coupling constants were in Hz. Positive ion FABMS was recorded on a JEOL-Sx-120/DA-6000 mass spectrometer using a beam of argon/xenon (2−8 keV) and *m*-nitrobenzoyl alcohol as the matrix. Optical rotations were taken on a JASCO DIP-370 polarimeter. Elemental analysis was carried out on a Carlo Erba 1108 analyzer. IR spectra were recorded on a Perkin-Elmer 881 instrument. Melting points were recorded on a Boitus melting point apparatus and were uncorrected. Silica 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 a Buchi B-688 MPLC system. GC−MS experiments were carried out on a Shimadzu GCMS-QP 5050A system.

Animal Material. The gorgonian *Pseudopterogorgia australiensis* was collected from the Tuticorin Coast, India (8°45′ N, 78°12′ E) at a depth of about 10 m. A voucher specimen was deposited in the Marine Organisms Museum in the Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India (Voucher No. AU2-183).

Extraction and Isolation. The organism was shade dried and powdered (dry wt ca. 1 kg), and the powder was extracted with MeOH eight times at room temperature. The combined alcoholic extract was concentrated under reduced pressure, and the EtOAc-soluble fractions were pooled and concentrated under reduced pressure after drying on anhydrous $MgSO_4$ to yield a crude residue (25 g). It was chromatographed over MPLC (silica gel, finer than 200#, ACME) using eluants with increasing polarity starting from *n*-hexane through EtOAc to MeOH. Fractions eluted with 30% and 50% EtOAc in hexane and EtOAc yielded, after repeated purification over MPLC, compounds **1** (50 mg), **2** (30 mg), and **3** (25 mg), respectively.

Compound 1: colorless crystals ($CHCl_3$); mp 131−133 °C; $[\alpha]_D^{25}$ +28.5° (*c* 0.1, $CHCl_3$); IR (KBr) ν_{max} 3520, 3460, 1640, 2910−2850 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.36 (1H, d, J = 7.4 Hz, N*H*), 3.70 (1H, d, J = 4.0 Hz, H-1a), 3.44 (1H, br s, H-1b), 3.91 (1H, m, H-2), 4.31 (1H, br s, H-3), 2.01 (1H, m, H-4), 2.25 (2H, t, J = 7.5 Hz, H-2′), 1.52 (2H, m, H− 3′), 1.25 (br s, −C*H*$_2$−), 0.88 (6H, t, J = 6.4 Hz, CH$_2$C*H*$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 62.5 (CH_2, C-1), 53.8 (CH, C-2), 72.5 (CH, C-3), 34.5 (CH_2, C-4), 173.6 (C, C-1′), 36.8 (CH_2, C-2′), 25.8 (CH_2, C-3′), 31.9−29.2 (CH_2, −*C*H$_2$−), 14.1 (CH_3, CH$_2$*C*H$_3$); positive ion FABMS *m*/*z* 638 [M +H]$^+$, 620, 314, 286; *anal.* C 77.19%, H 13.09%, N 2.26%, calcd for $C_{41}H_{83}NO_3$, C 77.16%, H 13.12%, N 2.20%.

Compound 3: colorless crystals (MeOH); mp 101−103 °C; $[\alpha]_D^{25}$ +8.5° (*c* 0.1, pyridine); IR (KBr) ν_{max} 3500−3200, 2910−2850, 1680 cm^{-1}; ^{1}H NMR (pyridine-d_5, 300 MHz) δ 8.59 (1H, d, J = 7.2 Hz, N*H*), 4.65 (1H, m, H-1a), 4.51 (1H, m, H-1b), 5.12 (1H, m, H-2), 4.41 (1H, m, H-3), 4.23 (1H, dt, J = 8.8, 4.4 Hz, H-4), 1.91 (4H, m, H-5, H-4′), 1.72 (4H, m, H-6, H-5′), 4.54 (1H, dd, J = 4.8, 7.0 Hz, H-2′), 2.21 (2H, m, H-3′), 1.25 (50H, br s, −C*H*$_2$−), 0.86 (6H, t, J = 6.6 Hz, CH$_2$C*H*$_3$); ^{13}C NMR (pyridine-d_5, 75 MHz) δ 62.1 (CH_2, C-1), 53.1 (CH, C-2), 72.7 (CH, C-3), 73.1 (CH, C-4), 34.8 (CH_2, C-5), 30.5 (CH_2, C-6), 175.8 (C, C-1′), 76.7 (CH, C-2′), 35.8 (CH_2, C-3′), 34.2 (CH_2, C-4′), 32.3 (CH_2, C-5′), 29.9−26.0 (CH_2, −*C*H$_2$−), 14.0 (CH_3, CH$_2$*C*H$_3$); positive ion FABMS *m*/*z* 628, 330, 312, 294; *anal.* C 72.61%, H 12.49%, N 2.19%, calcd for $C_{38}H_{77}NO_5$, C 72.66%, H 12.36%, N 2.23%.

Acetylation. Dry pyridine (0.5 mL) and Ac_2O (1.0 mL) were added to 5 mg of compounds **1** and **3** separately and left overnight. Usual workup and crystallization in each case yielded **1a** and **3a.** Compound **1a**: $[\alpha]_D^{25}$ +13.8° (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.62 (1H, d, J = 8.9 Hz, N*H*), 1.90−2.01 (6H, s, −COC*H*$_3$), 1.25 (br s, −C*H*$_2$−), 0.85 (6H, t, J = 8.9 Hz, CH$_2$C*H*$_3$). Compound **3a**: $[\alpha]_D^{25}$ +11.5° (*c* 0.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.63 (1H, d, J = 8.9 Hz, N*H*), 5.10 (2H, dd, J = 6.9, 10.8 Hz, H-3, H-2′), 4.93 (1H, m, H-4), 4.44 (1H, m, H-2), 4.35 (1H, dd, J = 6.4,10.6 Hz, H-1a), 4.03 (1H, dd, J = 3.1,11.7 Hz, H-1b), 2.03−2.18 (12H, s, −COC*H*$_3$), 1.84 (1H, m, H-3′), 1.66 (4H, m, H-5, H-4′), 1.25 (54H, br s, −C*H*$_2$−), 0.88 (6H, t, J = 8.9 Hz, CH$_2$C*H*$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 62.4 (CH_2, C-1), 47.8 (CH, C-2), 72.7 (CH, C-3), 72.2 (CH, C-4), 31.8 (CH_2, C-5), 171.3 (C, C-1′), 74.0 (CH, C-2′), 31.9 (CH_2, C-3′), 31.8 (CH_2, C-4′), 29.7−22.7 (CH_2, −*C*H$_2$−), 14.1 (CH_3, CH$_2$*C*H$_3$), 20.6−21.0 (CH_3, −CO*C*H$_3$), 169.9−170.9 (C, *C*OCH$_3$).

Methanolysis. Compounds **1** and **3** (10 mg) were separately treated with 3 mL of 1 N HCl in methanol at 90 °C for 15 h with magnetic stirring. The fatty acid methyl ester so obtained in each case was extracted with *n*-hexane and analyzed by GC−MS. In each case a single compound was obtained. The MeOH/H_2O phase was evaporated, and the residue was acetylated. Purification by filtration over a Sephadex LH20 column (CH_2Cl_2/MeOH, 1:1) gave the acetylated sphingamines. The sphingamines were subjected to GC−MS in each case.

Methyl ester from 1: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.22 (2H, t, J = 7.2 Hz, H-2), 3.53 (3H, s, OC*H*$_3$), 1.84 (m, H-3), 1.25 (br s, −C*H*$_2$−), 0.85 (3H, t, J = 6.4 Hz, CH$_2$CH$_3$); GC−MS *m*/*z* 354 [M]$^+$.

Methyl ester from 3: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 5.13 (1H, dd, J = 6.8, 3.8 Hz, H-2), 3.52 (3H, s, OC*H*$_3$), 2.12 (2H, m, H-3), 1.84 (2H, m, H-4), 1.50 (2H, m, H-5), 1.25 (26H, br s, −C*H*$_2$), 0.88 (3H, t, CH$_2$C*H*$_3$); GC−MS *m*/*z* 328 [M]$^+$.

Acetyl sphingamine from 1: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.60 (1H, d, J = 9.4 Hz, N*H*), 4.39 (1H, dd, J = 9.6, 4.6 Hz, H-1a), 4.93 (1H, dd, J = 9.8, 3.7 Hz, H-1b), 5.08 (1H, m, H-2), 4.28 (1H, m, H-3), 1.96 (2H, m, H-4), 1.26 (br s, −C*H*$_2$−), 0.85 (3H, t, J = 6.0 Hz, CH$_2$C*H*$_3$); GC−MS *m*/*z* 441 [M]$^+$.

Acetyl sphingamine from 3: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.63 (1H, d, J = 8.4 Hz, NH), 5.14 (1H, m, H-2), 4.65 (1H, m, H-1a), 4.52 (1H, m, H-3), 4.20 (1H, dt, J = 8.8, 4.4, H-4), 3.83 (1H, m, H-1b), 2.18−2.03 (12H, s, COC*H*$_3$), 1.90 (2H, m, H-5), 1.46 (2H, m, H-6), 1.25 (24H, br s, −C*H*$_2$−), 0.86 (3H, t, J = 6.4 Hz, CH$_2$C*H*$_3$); GC−MS *m*/*z* 499 [M]$^+$.

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