Hariamide, a Novel Sulfated Sphingolipid from a *Zoanthus* sp. of the Indian Coast†

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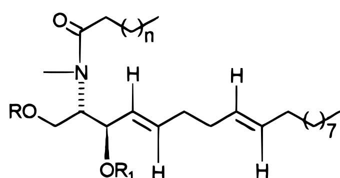
A novel sulfated sphingolipid, hariamide (**1**), and a known ceramide (**4**) have been isolated from the methanolic extractive of a *Zoanthus* sp. of the Indian coast. The proposed structure for hariamide as *N*-methyl-*N*-octadecanoyl-D-(+)-(2*S*,3*R*,4*E*,8*E*)-1-*O*-sulfonosphinga-4,8-dienine (**1**) was established by FABMS, 2D NMR, and chemical degradation studies.

In continuation of our studies on bioactive secondary metabolites from marine organisms of the Indian coast and nearby islands, the methanolic extractive of an unidentified zoanthid showing moderate hypotensive activity[1] was chemically investigated. We previously reported a rare Δ^{20} cholesterol, zoanthamine, and fatty constituents from this zoanthid.[2] We herein report the isolation and structure elucidation of a new sulfated sphingolipid, hariamide (**1**), and a known ceramide (**4**), previously reported from *Ulva fasciata*.[3]



1	R = SO_3H,	R_1 = H,	n = 15
2	R = SO_3H,	R_1 = Ac,	n = 15
3	R = H,	R_1 = H,	n = 15
4	R = H,	R_1 = H,	n = 13

Repeated column chromatography of an acetone-insoluble fraction from the chloroform−methanol (1:1) extract followed by reversed-phase flash chromatography afforded hariamide (**1**) as a colorless crystalline compound and the known ceramide **4** as an amorphous powder.

The IR spectrum of **1** exhibited strong absorption bands at 3400, 1040 (hydroxy), 2950 (aliphatic), 1640, 1545 (amide), and 970 cm^{-1} (trans double bond), suggesting it to be a fatty acid amide. The FAB mass spectrum of **1** showed molecular ion peaks at *m/z* 680 (M + Na)$^+$ and 658 (M + H)$^+$ consistent with the molecular formula $C_{37}H_{71}NSO_6$. The presence of a sulfate group in **1** was evidenced by strong IR absorption bands[4] at 1200 and 1060 cm^{-1} and also a fragment ion peak at *m/z* 559 (M − 98 (H_2SO_4))$^+$ in its FABMS.

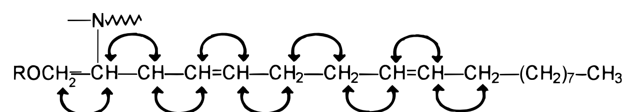
The ^{1}H NMR spectrum of **1** displayed a broad singlet at δ 1.20 (42H), two triplets at δ 0.85 (3H) and 2.20 (2H), two multiplets at δ 3.95 (2H) and 4.10 (1H), and a double doublet at δ 4.00 (1H), further suggesting it to be a ceramide. The absence of an NH proton signal, a characteristic feature of sphingolipids, and the sharp singlet observed at δ 2.80 (3H) in the ^{1}H NMR spectrum and its corresponding carbon signal at δ 33.60 (HMQC) supported the presence of an NCH_3 group. The ^{1}H NMR spectrum of **1** further showed three envelopes of olefinic protons at δ 5.45 (m, 2H), 5.50 (dd, 1H), and 5.70 (dt, 1H) due to two double bonds. Three multiplets appeared at δ 1.55, 1.95, and 2.05 due to methylene groups adjacent to double bonds.

The ^{13}C NMR spectrum of **1** showed carbon signals at δ 176.0 (s), 55.45 (d), 23.66−30.41 (methylene carbons), and 14.39 (q), further supporting the fatty acid amide nature of **1**. In the ^{13}C NMR spectrum, the carbon resonances at δ 64.81 (t), 72.68 (d), 130.62 (d), 131.16 (d), 131.97 (d), and 134.97 (d) revealed the presence of a dihydroxydiene system in **1**.

Acetylation of **1** with dry py/Ac_2O gave monoacetate **2**, whose FAB mass spectrum showed a molecular ion peak at *m/z* 601 [M − 98(H_2SO_4)]$^+$, further indicating that one of the hydroxyl groups was sulfated. The ^{1}H NMR spectrum of **2** in $CDCl_3$ provided further evidence that the $-SO_3H$ group must be on the C-1 hydroxy group since the chemical shift of H-1 was not shifted while that of the H-3 proton was shifted downfield by 0.4 ppm due to acetylation.

The presence of the $-SO_3H$ group was further confirmed when compound **1** was subjected to solvolysis[5] using py/dioxan to afford desulfated ceramide **3** [FABMS *m/z* 577 (M$^+$)]. The ^{1}H NMR spectral data of **3** was very similar to that of **4**.

The ^{1}H−^{1}H COSY spectrum of **1** showed cross peaks between δ 2.22 (t) and δ 1.55 (m), which in turn connected with the broad singlet at δ 1.20 and thus assigned H-2′ and H-3′, respectively. The olefinic proton



^{1}H-^{1}H correlations of **1**

appearing as a double doublet at δ 5.50 (H-4) showed cross peaks at δ 4.0 to the H-3 proton and at δ 5.70 (dt), assigned to the H-5 proton. The latter proton (H-5) also showed coupling with the multiplet at δ 2.05 attributed to the H-6 protons. The above evidence fully supported the placement of one olefinic bond at C-4. The other olefinic protons appearing at δ 5.45 did not show coupling with the H-5 proton, revealing that the other double bond must be isolated from C-4, but did show

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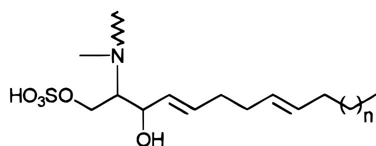
Table 1. ^{1}H and ^{13}C NMR Data (δ/ppm) for Compound 1 in CD_3OD

position	^{1}H (mult, *J* (Hz))	^{13}C (mult)[a]	^{1}H−^{1}H COSY
long chain Base (LCB)			
1	3.95 (m)	64.81 (t)	H-2
2	4.10 (m)	55.45 (d)	H-1, H-3
3	4.00 (dd, 6.2, 11.5)	72.68 (d)	H-2, H-4
4	5.50 (dd, 6.1, 15.1)	130.62 (d)	H-3, H-5
5	5.70 (dt, 6.0, 15.1)	131.16 (d)	H-4, H-6
6	2.05 (m)	33.03 (t)	H-5, H-7
7	1.95 (m)	33.42 (t)	H-6, H-8
8	5.45 (m)	131.97 (d)	H-7, H-9
9	5.45 (m)	134.29 (d)	H-8, H-10
10	2.05 (m)	33.60 (t)	
11−17	1.20 (brs)	23.66−30.41 (t)	
18	0.85 (t, 8.0)	14.39 (q)	
N-CH_3	2.80 (s)	33.66 (q)	
N-acyl moiety			
1′		176.00 (s)	
2′	2.22 (t, 8.2)	37.41 (t)	H-3′
3′	1.55 (m)	30.41 (t)	H-2′
4′−17′	1.20 (brs)	23.66−30.41 (t)	
18′	0.85 (t, 8.0)	14.39 (q)	

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

coupling with the multiplet at δ 1.95. The latter protons showed cross peaks at δ 2.05 (H-6) and thus were assigned to the H-7 protons. The above correlation studies have thus unambiguously assigned the position of the second double bond at C-8. The above spectral and chemical studies established the partial structure A.



Partial Structure A

Methanolysis of **1** with aqueous MeOH/H_2SO_4 afforded methyl octadecanoate [EIMS *m*/*z* 267 (M − $OCH_3)^+$], and thus, the tentative structure of **1** was assigned as *N*-methyl-*N*-octadecanoyl-1-*O*-sulfonosphinga-4,8-dienine. The geometry of the double bonds was assigned as trans 4*E*,8*E* on the basis of the coupling constant between the H-4 and H-5 ($J = 15.1$ Hz) protons and also on the ^{13}C NMR chemical shifts of the methylene groups adjacent to the (C-8) trans isomer (δ 32−33).[6] The relative stereochemistry at C-2 and C-3 was established as 2*S*,3*R* on the basis of ^{13}C NMR spectral data (C-2 = δ 55.4, C-3 = 72.6), which were very similar to those reported for synthetic D-(+)-*erythro*-sphingosine (δ 54.7, 73.1).[7] Thus, the structure of **1** was assigned as *N*-methyl-*N*-octadecanoyl-D-(+)-*erythro*-(2*S*,3*R*,4*E*,8*E*)-1-*O*-sulfonosphinga-4,8-dienine.

The occurrence of sulfated sphingolipids has not been previously reported from marine invertebrates, although a dihydroceramide sulfonic acid was previously reported from the marine diatom *Nitschia alba*.[8] To the best of our knowledge, this is the first report of a sulfated ceramide containing an *N*-methyl-*N*-sphinga-4,8-dienine as a long-chain base (LCB) from marine sources.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a Perkin-Elmer 881 instrument, FAB mass spectra on a JEOL-Sx-120/DA-6000 mass spectrometer using a beam of xenon (2−8 KeV) and *m*-nitrobenzyl alcohol as the matrix, and EIMS spectra 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 a Bruker DRX at 300 MHz equipped with an Aspect 2000 computer using TMS as internal reference. Flash chromatography was performed on EE 10 (EYELA) A.S.C. equipment with a fraction collector using RP-18 Si gel.

Animal Material. The fresh animal material (25 kg) was collected from Veraval, Gujrat, on the western coast of India in 1993. The specimens are colonial, epizoic, small anemone-like polyps united by basal stolons, which resemble the characteristic features of the order *Zoanthidae* of phylum coelenterata. Numerous pairs of mesenteries mostly composed of one complete and one incomplete mesentry and zooids were not sand encrusted, which are characteristic features of the genus *Zoanthus*. A voucher specimen has been deposited at National repository, NIO, Goa for preservation and Identification (voucher no. CDR-83).

Extraction and Isolation. The shade-dried animal material was pulverized and extracted with MeOH (3 × 5 L) to yield 800 g of methanolic extract. The residual animal material was further extracted with $CHCl_3$−MeOH (1:1) (2 × 3 L) to give 750 g of extract. About 250 g of methanolic extract was fractionated into hexane-, $CHCl_3$-, and MeOH-soluble fractions. The chemical constituents of the hexane- and $CHCl_3$-soluble fractions have been reported elsewhere.[2] The $CHCl_3$−MeOH (1:1) extract (100 g) was treated with acetone to give acetone-soluble (97 g) and -insoluble (1.8g) fractions. Column chromatography of the acetone-insoluble fraction over silica gel (60−120 mesh) using gradient elution of $CHCl_3$:MeOH resulted in the collection of 50 fractions of 100 mL each which were combined after monitoring by TLC to give two subfractions A and B. The subfraction A obtained as a dark green material (500 mg) was subjected to rechromatography on a Chromatotron using Kielsel gel G254 as adsorbent. Elution with a hexane:EtOAc gradient gave the known ceramide **4** (10 mg) as an amorphous powder. Subfraction B (350 mg) was subjected to flash chromatography over RP-18 silica gel using MeOH−H_2O (98:2) as eluent,

and 77 fractions of 2 mL each were collected. Similar fractions 55−65 were combined and concentrated under vacuo to give hariamide (**1**) (40 mg) as a colorless crystalline compound.

Hariamide (1): crystalline compound; mp 165 °C; $[\alpha]^{27}{}_D$ +11.3° (c = 0.01, methanol); IR (KBr) ν_{max} 3400, 3350, 2950, 1640, 1545, 1200, 1060, 1040, 970 cm^{-1}; ^1H and ^{13}C NMR data (pyridine-d_5) given in Table 1; FABMS *m/z* 680 $(M + Na)^+$, 658 $(M + H)^+$, 559 $(M - H_2SO_4)^+$, 262, 163, 140, 81.

Acetylation of 1. To 10 mg of **1** were added 0.5 mL of dry pyridine and 0.8 mL of Ac_2O and the mixture was left overnight. After usual workup, the crude product was purified over Si gel column using hexane−$CHCl_3$ as eluent to give monoacetate **2** (8 mg) as a colorless semisolid: IR (KBr) ν_{max} 2940, 1720, 1640, 1200, 1060, 965 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (t, 6H, J = 6.9 Hz), 1.25 (brs), 1.57 (m), 1.96 (m), 2.05 (s, COCH_3), 2.17 (m), 3.0 (s, 3H, NCH_3), 3.49 (m, 2H, H-1), 3.90 (m, 1H, H-2), 4.32 (m, 1H, H-3), 5.26 (m, 1H), 5.38 (m, 2H), 5.76 (m, 1H); FABMS 601 $(M - H_2SO_4)^+$.

Methanolysis of 1. Compound **1** (5 mg) was treated with 1 mL of 82% aqueous MeOH and 0.2 mL of concd H_2SO_4 and refluxed for 10 h. The reaction mixture was then extracted with *n*-hexane to give a fatty acid methyl ester. GC−MS of the FAME showed a single peak [M^+, 267 $(M - OCH_3)^+$, t_R 29.63 min] that was identified as methyl octadecanoate: ^1H NMR ($CDCl_3$) δ 0.88 (t), 1.26 (brs), 1.57 (m), 2.20 (t), 3.65 (OCH_3).

Desulfonation of 1. Compound **1** (5 mg) was treated with 0.5 mL of dry pyridine and 0.5 mL of dioxane and heated at 120 °C for 18 h. The mixture was cooled to room temperature and then partitioned between $CHCl_3$ and H_2O. The organic layer afforded desulfated product **3** as a colorless solid, with an ^1H NMR spectrum similar to that of **4**: FABMS *m/z* 557 (M^+), 539 $(M - H_2O)^+$, 526 $(M - CH_2 = OH)^+$.

***N*-Palmitoyl-D-(+)-*erythro*-octadecasphinga-4(*E*),-8(*E*)-dienine (4):** amorphous powder; mp 86 °C; $[\alpha]^{27}{}_D$ +20.1° (c = 0.01, $CHCl_3$); IR (KBr) ν_{max} 3340 (OH), 3300, 1630, 1545, 1030, 985, 920 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.90 (t, 6H, J = 9.0 Hz), 1.28 (brs), 1.65 (2H, m), 1.95 (2H, m), 2.10 (4H, dt), 2.23 (t, 2H, J = 8.5 Hz), 2.70 (2H, brs, OH), 3.71 (1H, dd, J = 12, 7 Hz, H-1a), 3.90 (1H, m, H-2), 3.98 (1H, dd, J = 12, 7 Hz, H-1b), 4.32 (1H, brt, J = 7 Hz, H-3), 5.40 (2H, m, H-8 and H-9), 5.55 (1H, dd, J = 15.0, 6 Hz, H-4), 5.75 (1H, dt, J = 15.6 Hz, H-5), 6.25 (1H, d, J = 8.5 Hz, N*H*); ^{13}C NMR ($CDCl_3$) δ 14.07 (q), 22.66−29.67 (methylene carbons), 31.89 (t), 32.78 (t), 34.09 (t), 36.83 (t), 54.58 (d), 62.36 (t), 73.37 (d), 128.97 (d), 129.19 (d), 131.30 (d), 133.31 (d), 174.03 (s); FABMS *m/z* 538 $(M + H)^+$, 518 $(M - H_2O)^+$, 298, 280, 262, 239.

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