Cytotoxic Sphingosine 4-Sulfates from the Sponge *Spirastrella abata*

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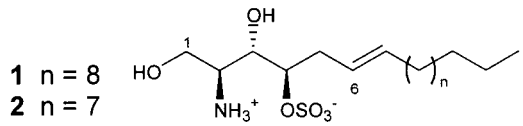
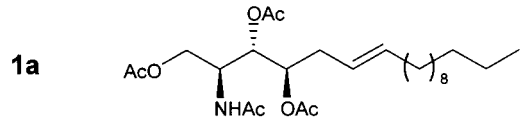
Two new sphingosine 4-sulfates (**1** and **2**) have been isolated from the sponge *Spirastrella abata* as cytotoxic constituents. The gross structures of **1** and **2** were determined by spectroscopic analysis, and their stereochemistry was established by chemical conversion. The compounds exhibited significant cytotoxicity against a small panel of five human tumor cell lines.

In a continuation of our search for bioactive metabolites from the sponge *Spirastrella abata* (Tetraxonida, Spirastrellidae),[1,2] we have isolated sphingosine sulfates from a brine shrimp active fraction of the same sponge. Although ceramide 1-sulfates and ceramide 1-sulfonates are reported from a bryozoan[3,4] and a marine bacterium,[5] respectively, this is the first report of sphingosine 4-sulfates isolated from a marine source. Here we describe the isolation, structure elucidation, and biological activity of these compounds.

The methanolic extract of the frozen sponge was partitioned between CH_2Cl_2 and H_2O, and the brine shrimp active CH_2Cl_2 extract was again partitioned between 90% aqueous MeOH and *n*-hexane. The 90% aqueous MeOH layer was chromatographed on a reversed-phase flash column followed by RP-HPLC separation to yield two sphingosine sulfates (**1** and **2**) as white amorphous solids.





The ^{1}H NMR spectrum of compound **1** displayed signals at δ 4.30 (dt), 4.01 (dd), 3.79 (m), and 3.75 (m) for four protons attached to oxygenated carbons. A doublet of triplets for one proton at δ 3.48, which was correlated to a carbon at δ_C 56.7, was assigned to a methine proton bonded to an amino-substituted carbon. This pattern of the ^{1}H NMR spectrum suggested a sphingosine type structure with three oxygen functions. The various ^{1}H−^{13}C connectivities for compound **1** are shown in Table 1. The signals for the methine proton at δ 3.48 showed COSY correlations with methylene proton signals at δ 4.01 and 3.75, which were correlated to a carbon at δ_C 59.3 (C-1). This proton was also coupled with the H-3 proton at δ 3.79, which in turn showed a COSY correlation with a proton resonating at δ 4.30. The ^{1}H NMR spectrum also featured signals for a monounsaturated long aliphatic chain. The COSY spectrum was helpful to determine the position of the double bond since two allylic methylene proton signals (H-5, 8) were well resolved. The proton at δ 4.30 (H-4) was correlated with the methylene protons at δ 2.68 and 2.44 (H-5), which showed clear correlations with the olefinic protons at δ 5.57. An enhanced peak due to allylic cleavage at *m/z* 120 also confirmed the position of the double bond at C-6. The geometry of the double bond was ascertained to be trans according to the chemical shift of the allylic carbons.[6] The protonated molecular ion $[M + H]^+$ of compound **1** appeared at *m/z* 396 in the LRFABMS, which corresponded with the molecular formula $C_{18}H_{37}NO_6S$. A prominent peak at *m/z* 316 $[M + H - SO_3]^+$ also indicated a sulfate group in the compound. An intense IR band at 1213 cm^{-1} further supported the presence of a sulfate function. The location of this group was determined to be at the C-4 position from downfield shifts of the respective proton and carbon signals compared to those of a ceramide from *Spirastrella inconstans*.[7]

Table 1. NMR Spectral Data of **1** in CD_3OD[a,b]

position	δ_C	δ_H (mult, *J* in Hz)
1	59.3	4.01 (dd, 11.7, 3.6)
		3.75 (m)[c]
2	56.7	3.48 (dt, 7.8, 3.9)
3	70.6	3.79 (m)[c]
4	79.1	4.30 (dt, 7.8, 3.9)
5	34.9	2.68 (dt, 14.5, 4.8)
		2.44 (ddd, 14.5, 4.8, 4.2)
6	135.7	5.57 (m)
7	124.9	5.57 (m)
8	33.8	2.01 (q, 6.7)
9−15	30.4−30.8	1.20−1.40 (m)
16	33.1	1.20−1.40 (m)
17	23.7	1.20−1.40 (m)
18	14.4	0.89 (t, 7.8)

[a] The ^{1}H and ^{13}C NMR spectra were measured at 300 and 50 MHz, respectively. [b] Assignments were based on COSY and HMQC data. [c] The signals were overlapped with each other.

The compound was acetylated to determine stereochemistry. The ^{1}H NMR data of the resulting tetraacetate (**1a**, see Experimental Section) was in good correlation with those of the synthetic (2*S*,3*S*,4*R*)-2-acetamido-1,3,4-triacetoxyhexadecane[8] in terms of the chemical shifts and coupling constants of the respective protons, thus suggesting the same relative stereochemistry. The optical rotation of **1a** ($[\alpha]^{25}_D$ + 26°, $CHCl_3$) was close to the reported values of (2*S*,3*S*,4*R*)-tetraacetylsphingosines.[8−11] Thus the structure of **1** was deduced as (*E*)-(2*S*,3*S*,4*R*)-2-amino-1,3-dihydroxyoctadec-6-ene-4-sulfate.

Compound **2**, also a white amorphous solid, was considered to be a homologue of **1** by comparison of the NMR and

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Table 2. In Vitro Cytotoxicities (ED_{50} μg/mL) of **1** and **2** against Human Solid Tumor Cells[a,b]

compound	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	9.9	8.2	4.0	4.6	3.7
2	0.3	0.3	0.3	0.3	0.3
cisplatin	0.6	0.9	0.7	0.6	0.6

[a] A549: human lung cancer; SK-OV-3: human ovarian cancer; SK-MEL-2: human skin cancer; XF498: human CNS cancer; HCT15: human colon cancer. [b] Compounds **1** and **2** were assayed in separate batches.

IR spectra (see Experimental Section). It showed a $[M + H]^+$ peak at *m/z* 382 in the FABMS and a pattern of fragmentation almost similar to that of **1**, indicating that it had one methylene group less than **1**. It was assumed that **1** and **2** share the same stereochemistry at their three chiral centers. Thus the structure of compound **2** was proposed as (*E*)-(2*S*,3*S*,4*R*)-2-amino-1,3-dihydroxyheptadec-6-ene-4-sulfate.

These compounds were tested for their cytotoxicity against human solid tumor cell lines to exhibit significant activity. Compound **2** was shown to be more potent than cisplatin (Table 2). Certain lipids are known to bind to and inhibit human DNA topoisomerase.[12,13] Ceramide 1-sulfates have also been reported to possess potent DNA topoisomerase I inhibitory activity[3] and neuraminidase inhibitory activity.[4] Ceramide 1-sulfonates were reported to inhibit DNA polymerase α.[5] Considering the level of cytotoxicity of compound **2**, further biochemical studies to investigate its mechanism of action may be of interest.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-1000 digital polarimeter. ^1H and HMQC spectra were recorded on a Varian Unity Plus 300 spectrometer, while ^{13}C NMR were measured on a Bruker AC200 spectrometer. Chemical shifts were reported in reference to the respective residual solvent peaks (δ 3.3 and δ_C 49.0 for CD_3OD). FABMS data were obtained using a JEOL JMS-HX110/110A (four-sector instrument with a E1B1E2B2 configuration).

Animal Material. The sponge S*pirastrella abata* was collected by hand using scuba at a 13 m depth in November 1996, off Cheju Island, Korea, and was described elsewhere.[1]

Extraction and Isolation. The frozen sponge (5.25 kg) was extracted with MeOH at room temperature. The MeOH-soluble fraction was partitioned between H_2O and CH_2Cl_2. The CH_2Cl_2-soluble portion was further partitioned between 90% aqueous MeOH and *n*-hexane to yield 19.8 and 10.9 g of residues, respectively. The 90% aqueous MeOH fraction was subjected to reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 500/400 mesh) eluting with 33−100% MeOH−H_2O followed by EtOAc to yield seven fractions (F1−F7). Fraction F3 was eluted with 85% MeOH−H_2O and was very active in brine shrimp cytotoxicity assay.[14] This fraction was further chromatographed on a reversed-phase HPLC (YMC-Pack CN, 250 × 10 mm, S-5 μm, 120 Å) using CH_3CN−H_2O (1:1) to afford compounds **1** and **2** in semipurified form. Re-HPLC using the same conditions yielded compounds **1** (5 mg) and **2** (1.2 mg).

Compound 1: white amorphous solid; IR (film) 3398, 2924, 2853, 1622, 1213 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; LRFABMS *m/z* 396 $[M + H]^+$ (100), 316 $[M + H - SO_3]^+$ (1.3), 298 $[316 - H_2O]^+$ (2.4), 280 $[298 - H_2O]^+$ (1.0), 255 (0.5), 120 (1.0), 60 (2.2).

Compound 2: white amorphous solid; $[\alpha]^{19}_D$ +11.6° (*c* 0.07, $CHCl_3$); IR (film) 3375, 2924, 2853, 1593, 1225 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 5.58 (2H, m, H-6, H-7), 4.30 (1H, dt, J = 7.8, 3.9 Hz, H-4), 4.00 (1H, dd, J = 11.7, 3.6 Hz, H-1), 3.77 (1H, m, H-3), 3.74 (1H, m, H-1), 3.45 (1H, dt, J = 7.8, 3.9 Hz, H-2), 2.66 (1H, dt, J = 13.9, 4.8 Hz, H-5), 2.45 (1H, ddd, J = 13.9, 4.8, 3.9 Hz, H-5), 2.02 (1H, q, J = 6.7, H-8), 1.20−1.40 (16H, m, H-9−H-16), 0.89 (3H, t, J = 7.8 Hz, H-17); ^{13}C NMR (CD_3OD, 50 MHz) δ 135.6 (C-6), 125.0 (C-7), 79.1 (C-4), 70.8 (C-3), 59.7 (C-1), 56.0 (C-2), 34.8 (C-5), 33.9 (C-8), 33.1 (C-15), 30.4−30.8 (C-9−C-14), 23.7 (C-16), 14.4 (C-17); LRFABMS *m/z* 382 $[M + H]^+$ (100), 302 $[M + H - SO_3]^+$ (1.0), 284 $[302 - H_2O]^+$ (1.2), 266 $[284 - H_2O]^+$ (0.6), 255 (0.3), 120 (0.4), 60 (3.5).

Acetylation of 1. Compound **1** (2.5 mg) was mixed with a mixture of Ac_2O−CH_3SO_3H (40:1 v/v)[15] and heated to 100 °C for 30 min and was then allowed to cool. About 1 g of ice was added to the reaction mixture and extracted three times with an equal volume of benzene. The combined extracts were purified by reversed-phase HPLC (YMC-Pack CN, 250 × 10 mm, S-5 μm, 120 Å) using CH_3CN−H_2O (6:4) to afford **1a** (0.5 mg): $[\alpha]^{25}_D$ +26° (*c* 0.17, $CHCl_3$); ^1H NMR data (200 MHz, $CDCl_3$) δ 5.99 (d, J = 9.3 Hz, −N*H*-Ac), 5.44 (dt, J = 15.6, 7.3 Hz, H-7), 5.27(dt, J = 15.6, 6.8 Hz, H-6), 5.08 (dd, J = 7.3, 3.9 Hz, H-3), 4.94 (dt, J = 7.3, 3.9 Hz, H-4), 4.48 (m, H-2), 4.25 (dd, J = 11.7, 4.9 Hz, H-1), 3.99 (dd, J = 11.7, 2.5 Hz, H-1), 2.08, 2.04, 2.03, 2.01 (4 × s, 3 × OAc, 1 × NHAc), 1.58−1.23 (m, H-9−H-17), 0.87 (t, J = 7.0 Hz, H-18).

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