New Sphingosines from the Marine Sponge *Grayella cyatophora*

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The marine sponge *Grayella cyatophora* furnished several new homologous sphingosines having the same acyl substituent. Structure elucidations were achieved by spectroscopic methods and chemical transformations.

Sphingosines are constituents of sphingolipids found in animal tissues of vertebrates and invertebrates, but also occur in plants. They are known to be constituents of biomembranes and play an important role in biological systems.[1] Sphingolipids have received increasing attention in the last years, after the discovery that sphingolipid-derived products may function as second messengers.[2] Sphingolipids and their metabolites, ceramide, sphingosine, and sphingosine-1-phosphate, are involved in a variety of cellular processes including differentiation, cellular senescence, apoptosis, and proliferation,[3,4] and sphingolipid consumption suppresses colon carcinogenesis in rats.[5] Marine organisms have led to the isolation of a number of new sphyngosines and glycosphingosines, most of them exhibiting biological activity.[6] We report here the isolation and structural elucidation of a new series of sphingosines (**1**) isolated from the sponge *Grayella cyatophora*. To our knowledge, it is the first report of a series of sphingosines acylated by the same fatty acid.

Specimens of *Grayella cyatophora* (Carter, 1869)[7] were collected in the Red Sea and preserved in methanol until extraction successively with methanol and methylene chloride. Column chromatography on silica gel of the methanolic extract eluted with 9:1 CH_2Cl_2/CH_3OH afforded mixture **1** as an amorphous solid, $[\alpha]^{25}_D$ +24° (*c* 0.05). The CIMS spectrum of **1** showed a series of pseudomolecular ion peaks $(M + H)^+$ at *m*/*z* 612, 626, 640, 654, 668, 682, 696, indicative of a mixture of homologous compounds. HRMS furnished the formula $C_{40}H_{82}O_4N$ (M + H) for the peak at *m*/*z* 640.6244 (calcd 640.6248).

The infrared spectrum (KBr disk) showed absorptions at 3200−3400 and 1680 cm^{-1}, a peak attributed to the carbonyl of an amide. The ^1H NMR spectrum exhibited a doublet signal at δ 6.35 ppm exchangeable by D_2O, due to an amide proton, along with signals at δ 4.15, 3.90, 3.72, 3.65, 3.45, 2.22, 1.6, 1.25, and δ 0.82 ppm (t, 6H, $2CH_3$), characteristic of sphingosines (Table 1). This was confirmed by the ^{13}C NMR spectrum of **1** (J_{MOD}) exhibiting signals at δ 173.9 ppm for the carbonyl of an amide, two secondary alcohol groups at δ 76.5 and 71.9, one primary alcohol at δ 61.2, and a CHNH at δ 52.7 in addition to signals for several methylene groups (29−30 ppm) and methyl groups at 13.4 ppm. Assignment of all carbon and proton signals was made by $^1H-^1H$ COSY and heteronuclear correlation (HMBC, HSQC) experiments, which led us to propose structure **1**.

The fatty acid and sphingosine chain lengths of **1** were determined through acid methanolysis according to Gaver−Sweeley.[8] Usual workup furnished the methyl ester of the long-chain acid, which corresponded to the methyl ester of palmitic acid (*m*/*z* 270). Acetylation of the sphingamine moiety, followed by filtration on LH 20, gave the tetraacetyl-sphingamines **2**. The ^1H NMR spectrum exhibited, in addition to characteristic signals at δ 6.09 (d, CH−NH), 5.10 (dd, H-3), 5.05 (1H, dd, H-1a), 4.93 (1H, dd, H-1b), 4.90 (ddd, H-4), and 4.49 (dt, H-2), four singlets at δ 2.22−2.00 ppm, methylene groups at δ 2.19, 1.65, and 1.48 ppm, a large signal at δ 1.25 ppm, and a triplet at δ 0.82 ppm. EIMS of the mixture of acetylated sphingamines showed ions at *m*/*z* 481, 495, 509, 523, 537, 551, and 565 for $[M - AcO]^+$, corresponding to the 1,3,4-triacetyl-2-acetaminoalkane mixture **2** (Figure 1). By comparison with literature data of natural[9] and synthetic sphingamines,[10] the optical rotation $[\alpha]^{25}_D$ +20 (*c* 0.8, $CHCl_3$) supports the 2*S*,3*S*,4*R* configuration.

Table 1. ^1H (300 MHz) and ^{13}C (75 MHz) NMR Data of Compound **1** ($CDCl_3$)

assignment	^1H δ ppm (m, *J* Hz)	^{13}C δ ppm
1	3.90 (dd, 1H, 2, 11.5)	61.9
	3.72 (dd, 1H, 5, 11.5)	
2	4.15 (m, 1H)	52.7
3	3.45 (dd, 1H, 2, 7)	76.5
4	3.65 (m, 1H)	71.9
5	1.75 (m, 1H); 1.40 (m, 1H)	33.2
$(CH_2)_n$	1.20−1.35	29−30
CH₂−CH₂−CH₃	1.25	32.7
CH₂−*CH₂*−CH₃	1.3	29.5
CH_3	0.82 (t, 6H, 7)	13.4
NH	6.35 (d, 1H, 7)	
1′		175.2
2′	2.22 (t, 2H, 7)	35.9
3′	1.62 (m, 2H),	24.7
$(CH_2)_{3'-14'}$	1.25	29−30
$CH_{3'}$	0.85 (t, 3H)	14.3

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Perkin-Elmer 141 polarimeter with a sodium lamp (l 589 nm) in a 10 cm microcell. UV spectra were obtained in MeOH, using a Kontron type Uvikon 930 spectrophotometer, and IR spectra (KBr pellets) were recorded on a Nicolet (Impact 400D) FTIR spectrophotometer. ^1H NMR and ^{13}C NMR spectra were obtained on a Bruker Advance 400 spectrometer with standard pulse sequences operating at 400 MHz for ^1H and 100 MHz for ^{13}C, respectively. The chemical shift values are reported as ppm units and the coupling constants in Hz. The programs used for J_{MOD}, HMQC, and HMBC (J = 7 Hz) experiments were those of the Bruker manual (1991). FAB-HRMS (positive mode) was measured on a Jeol 700 spectrometer at the Service de Spectrometrie de

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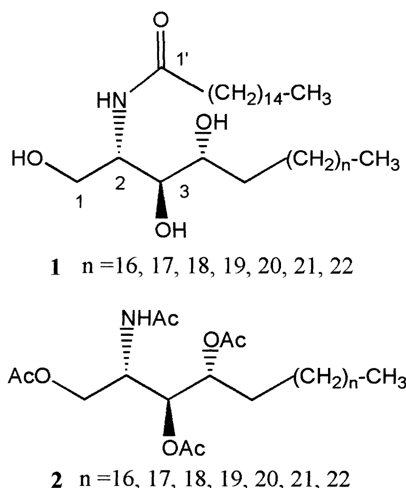

Figure 1. Structure of compounds **1** and **2**.

Masse de l'Ecole Normale Supérieure de Paris and EIMS on a Nermag R 10-10. Silica gel column chromatography was carried out using Kieselgel 60 (230–400 mesh, E. Merck). Gel filtration was carried out using LH20 (Sephadex LH20 17-0090-01 Pharmacia Biotech). Fractionations were monitored by TLC using aluminum-backed sheets (silica gel 60 F-254, 0.25 mm thick) with visualization under UV (254 and 366 nm) and Dragendorff spray reagent. All the solvents were distilled prior to their use.

Animal Material. Specimens of the sponge *Grayella cyatophora,* Carter, 1869, were collected in the Red Sea, near Djibouti, and preserved in methanol until extraction. The animal material (800 g wet wt) was ground and successively extracted with methanol and dichloromethane at room temperature. After filtration, each extract was concentrated under reduced pressure to obtain dichloromethane extract (250 mg) and methanolic extract (360 mg).

Extraction and Isolation. The methanolic extract of the sponge *Grayella cyatophora* was separated on a silica gel column eluting with dichloromethane with increasing amounts of methanol. Fractions eluted with 10% and 20% MeOH were pooled and fractionated on a silica gel column (CH_2Cl_2/MeOH, 9:1) to yield compound **1** (5 mg).

Sphingosines 1: amorphous powder; $[\alpha]^{25}_D$ +24° (*c* 0.05, MeOH); IR (KBr) λ_{max} 1640, 3400–3200 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1 and text; FABMS (positive mode) $[M + H]^+$ *m/z* 612, 626, 640, 654, 668, 682, 696; FAB-HRMS *m/z* 640.6244 (calcd for $C_{40}H_{82}NO_4$, 640.6248).

Hydrolysis of Sphingosines 1. A mixture of 5 mL of 1 N HCl in 15 mL of MeOH and 4 mg of **1** was refluxed for 15 h with magnetic stirring. Water was added and extraction with *n*-hexane yielded, after drying ($MgSO_4$) and purification on silica gel (dichloromethane), the long-chain methyl ester. The methanol/water phase was evaporated and the residue acetylated as follows: a mixture of 2.5 mg of the residue, Ac_2O (1.5 mL), and pyridine (0.15 mL) was allowed to stand at 20 °C overnight, then diluted with 2 mL of H_2O, and extracted with CH_2Cl_2. Purification by filtration over a Sephadex LH20 column (CH_2Cl_2/MeOH, 1:1) gave the tetraacetyl-sphingamine mixture **2**.

Compound 2: $[\alpha]^{25}_D$ +20° (*c* 0.05, CH_3OH); ^1H NMR ($CDCl_3$, 400 MHz, δ ppm) 6.49 (1H, d, NH), 5.95 (1H, dd, H-3), 5.80 (1H, dd, H-1a), 5.5 (1H, dd, H-1b), 4.90 (1H, m, H-4), 4.34 (1H, ddd, H-2), 2.20 (12H, s, 4 OAc), 1.25 (32 H, CH_2), 0.85 (3H, CH_3); EIMS *m/z* 481, 495, 509, 523, 537, 551, 565 (M – OAc).

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