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Syriacin, a novel unusual sulfated ceramide glycoside from the freshwater sponge *Ephydatia syriaca* (Porifera, Demospongiae, Spongillidae)

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Abstract—Syriacin, a novel unusual sulfated ceramide glycoside with branched very-long-chain fatty acid, i.e., (all *Z*)-34*S*-methylhexatriaconta-5,9,12,15,18,21-hexaenoic acid, has been isolated from the freshwater sponge *Ephydatia syriaca*. Its structure was identified by means of extensive spectroscopic analysis (IR, UV, 2D NMR, MS, CD) and chemical degradation. Syriacin showed antifeeding activity against goldfish at natural concentration (~10 μg/ml).

1. Introduction

Sponges, an ancient and highly successful group of animals, common inhabitants of the benthos, have been living in the waters of the world for more than 600 million years, and can be found in all marine and many freshwater habitats.[1] In addition to many marine forms, the freshwater sponges belonging to the genus *Ephydatia* are typical representatives of Spongillidae and occur in different rivers and lakes around the world. A few natural compounds have been isolated from this genus.[2] *Ephydatia fluviatilis* contains almost exclusively Δ^5-sterols. In addition, *E. fluviatilis* contains sterols with the 24β configuration, which predominate over the 24α-epimers.[3] Phospholipids and fatty acids were also studied in *E. fluviatilis*.[4] Multibranched, polyunsaturated and very-long-chain fatty acids have been isolated from *Ephydatia syriaca*.[5,6]

This report is part of our investigation of marine and freshwater sponges[2,4,5] in the framework of a comprehensive program on the chemistry and biotoxicity of natural compounds. We isolated a novel unusual sulfated ceramide, named syriacin, with very-long-chain branched fatty acid having six double bonds.

2. Results and discussion

The extract of the freshwater sponge *E. syriaca*, which was collected in August 2003, in the Jordan River, was subjected to gel filtration chromatography on Sephadex LH-20. The fractions were further purified by gradient RP-HPLC to give glycoside (**1**, see Fig. 1), which was identified by its IR, MS, UV, CD, and ^{1}H and ^{13}C NMR spectroscopic data and by chemical degradation.

The IR spectrum of compound **1** has absorption bands at 3300–3400 (hydroxy groups), 1540, 1640, 3250 (amide),

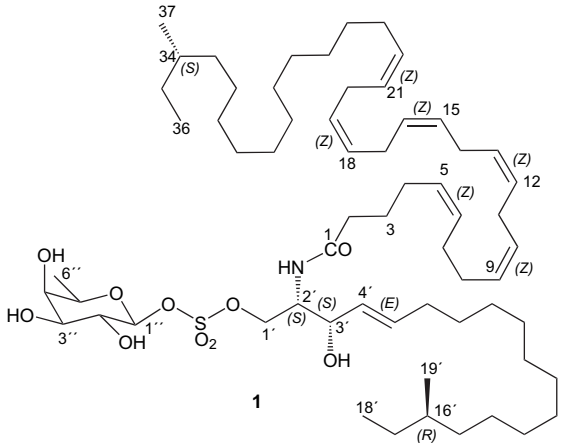

Figure 1. Structure of syriacin (**1**), a sulfated ceramide glycoside from the freshwater sponge *E. syriaca*.

Keywords: Ceramide; Glycoside; Sulfate; Freshwater sponge; *Ephydatia syriaca*; Antifeeding activity.
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1450, 2850, 2900 (the aliphatic chain), and 1070, 1230 (sulfate group) cm^{-1}.

The high-resolution mass spectrum of **1** shows a pseudomolecular ion peak $[M+Na]^+$ at *m/z* 1082.7666, which corresponds to the molecular formula $C_{62}H_{109}NO_{10}S$. The presence of a sulfate group was supported by the ion peaks at *m/z* 96 (SO_4) and 80 (SO_3) in FABMS.

The sulfate group was located at the C-1′ position from low-field shifts of the methylene signals of H-1′ (δ 4.28 and 4.18) and C-1′ (δ 74.2) (chemical shifts of typical ceramide are δ 62.6 and δ 3.94 and/or 3.68, respectively).[7]

The presence of the ester linked sulfo group was further confirmed enzymatically. β-D-Galactosidase from *E. coli* (EC 3.2.1.23) was used to hydrolyze compound **1** to afford fucose (**3**) and sulfoceramide (**1a**). The reaction mixture was lyophilized and then analyzed by FABMS; the corresponding ion at *m/z* 936.7090 $C_{56}H_{99}NO_6SNa$ $[M+Na]^+$ was identified. Although the enzyme used for the hydrolysis was not β-D-fucosidase (EC 3.2.1.38), β-D-galactosidase is known to be insensitive to substitution in position 6 of the substrate[8] and can be used for the purpose.

Another enzyme used for splitting **1** was β-D-glucuronidase (EC 3.2.1.31) from the keyhole limpet, which exhibited a high sulfatase (EC 3.1.6.1) activity. FABMS analysis of the reaction mixture showed that the spectrum contains sulfofucose (**3a**) ion at *m/z* 267.0151 $C_6H_{12}O_8SNa$ $[M+Na]^+$. The two enzymatic reactions thus clearly show that the sulfate group forms a bridge between fucose and the ceramide.

The ^1H NMR spectrum revealed the presence of two secondary methyls at δ 0.82 and 0.83, respectively, six heteroatom-bearing methines, four of them in hexose, and an oxygenated methylene protons at δ 4.18 and 4.28, two trans olefinic protons at δ 5.57 and 5.69, 12 cis olefinic protons (δ 5.38–5.45), and a huge methylene envelope at δ 1.25–1.40 (Table 1). The ^1H NMR spectrum also showed, in the methyl region, two triplets at δ 0.85 and δ 0.86, respectively (ethyl termini). Interpretation of the ^1H–^1H COSY and HMQC spectra resulted in three partial structures, two of which were connected to long aliphatic chains.

The amide broad singlet at δ 6.51 allowed us to assign all the protons of the polar part of the sphingosine through the COSY spectrum. The lack of substitution of the fatty acid residue in α position was revealed by the presence of a characteristic triplet at δ ~2.2 of the α-protons of acyl in the ^1H NMR spectrum of **1**, and an intense correlation peak with the amide NH signal in the ROESY spectrum. In addition, both H_2-2′ and NH were shown to be coupled with the amidic carbon atom at δ 172.4 (C-1) in the HMBC spectrum. This correlation between H-2′ and C-1 not only confirmed the position of the nitrogen atom but also connected the two partial structures through an amide bond. Two olefinic protons at 5.57 and 5.69, respectively were found to be trans configuration, because the coupling constant was 15 Hz.

The ^{13}C NMR spectrum of **1** showed 14 olefinic carbons at δ 126–135, one anomeric carbon at δ 101.6, methylene carbons at δ 29.0–31.5, and two secondary methyls (δ 20.7 and 21.2, respectively; both quartets), further supporting the nature of **1**. Additionally, the carbon resonances at δ 74.2, 51.6, 78.3, 131.2, and 134.8 revealed the presence of a dihydroxyaminoene system in **1**.

Table 1. ^1H and ^{13}C NMR data of syriacin (**1**) (measured in $CDCl_3$)

No.	^1H NMR	^{13}C NMR
1	—	172.4
2	2.18 (2H, t, *J*=6.3 Hz)	36.9
3	1.61 (2H, m)	25.6
4, 23	1.90 (4H, m)	27.2–27.9
5, 6, 9, 10, 12, 13, 15, 16, 18, 19, 21, 22	5.38–5.45 (12H, m)	126.0–135.0
7, 8	2.00–2.10 (4H, m)	31.2
11, 14, 17, 20	2.60–2.75 (8H, m)	25.0–26.0
24–32, 35	1.25–1.40 (20H, m)	29.0–31.5
33	1.24 (2H, m)	37.1
34	1.61 (1H, m)	35.4
36	0.85 (3H, t, *J*=7.1 Hz)	12.4
37	0.83 (3H, d, *J*=6.8 Hz)	21.2
1a′	4.18 (1H, dd, *J*=10.5, 5.2 Hz)	74.2
1b′	4.28 (1H, dd, *J*=10.5, 8.4 Hz)	
2′	4.01 (1H, ddd, *J*=8.6, 8.4, 5.2 Hz)	51.6
3′	4.14 (1H, dd, *J*=8.6, 7.2 Hz)	78.3
4′	5.57 (1H, dd, *J*=7.2, 15.0 Hz)	131.2
5′	5.69 (1H, dt, *J*=15.0, 6.9 Hz)	134.8
6′	1.96 (2H, m)	34.3
7′–15′, 17′	1.25–1.40 (20H, m)	29.0–31.5
16′	1.65 (1H, m)	35.4
18′	0.86 (3H, t, *J*=7.2 Hz)	11.6
19′	0.82 (3H, d, *J*=6.7 Hz)	20.7
NH	6.51 (1H, br s)	—
1″	5.28 (1H, d, *J*=7.5 Hz)	101.6
2″	4.26 (1H, dd, *J*=7.5, 8.5 Hz)	73.6
3″	4.39 (1H, dd, *J*=3.4, 8.5 Hz)	75.1
4″	4.15 (1H, dd, *J*=3.4, 1.4 Hz)	74.4
5″	4.42 (1H, dq, *J*=1.4, 6.7 Hz)	72.5
6″	1.52 (1H, d, *J*=6.7 Hz)	16.6

In the saccharide unit, the coupling system in the ^1H–^1H COSY spectrum of **1** started from the anomeric proton signal. This was established by tracing and joining the coupling points among C-1″–C-6″; this revealed contiguous coupling between H-1″ and H-2″, H-2″ and H-3″, H-3″ and H-4″, and H-5″ and 5″-Me. The coupling constants of the anomeric protons H-1″ ($J_{1''-2''}$=7.5 Hz) and H-2″ ($J_{2''-3''}$=8.5 Hz) of **1** suggest its axial orientation. The double-doublet signal of H-4″ suggested that H-4″ was equatorial. Selected NOE difference experiments were used to confirm the configuration of the monosaccharide residues in **1**, irradiation at H-5″ resulted in NOE enhancement at H-3″, confirming that both hydrogens were axial and that 5″-Me was equatorial. Finally, based on these spectral data, it was concluded that the monosaccharide unit of **1** was β-fucopyranose (6-deoxy-galactose). The presence of a β-fucopyranose moiety in **1** was confirmed by comparing the ^{13}C NMR chemical shifts of the monosaccharide unit with those of known monosaccharides.[9]

The relative stereochemistry of **1** was elucidated by chemical derivatization (Scheme 1). The sulfate group of **1** was easily removed by treatment with 2,2-dimethoxypropane under acidic conditions to furnish acetonide **2**. The *anti*-relationship of H-2′ and H-3′ in **2** was inferred from the coupling constant between H-2′ and H-3′ (*J*=8.6 Hz) as well as