

## Structure Elucidation of a New Antifungal Sterol Sulfate, Sch 575867, from a Deep-Water Marine Sponge (Family: Astroscleridae)

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In the course of our continuing search for novel antimicrobial agents, a new sterol sulfate, Sch 575867 (**1**), was isolated and identified from a marine sponge (Family: Astroscleridae). In this paper, we describe the isolation and structure elucidation of **1** using high resolution ESMS and extensive NMR spectroscopic analysis.

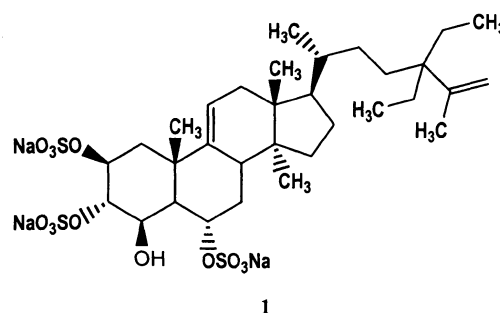
The sponge was collected using the Johnson-Sea-Link I manned submersible at a depth of 375 ft off Andros Island, Bahamas near Stafford creek. (Latitude: 24°55.0'N; Longitude 77°53.3'W, 375 feet). The sponge can not be identified to genus, but has been identified as belonging to the Family Astroscleridae (Demospongiae, Sclerospongiae, Ceratoporellidae).<sup>1)</sup> It is described as a red-orange, rock hard, 15 cm spherical sponge, with a thin veneer of living tissue over an aragonite skeleton. The surface is porous and smooth, with 2 cm diameter mounds. A reference sample preserved in ethanol has been deposited in the Harbor Branch Oceanographic Museum (catalog number 003:00989, DBMR number 24-VIII-85-1-053) and is available for taxonomic evaluation.

Crude sponge sample (~100 g) was diced and ground for three minutes in ethanol (40 ml) three times. After the filtration and solvent removal in vacuum, the organic extract was fractionated on a column of polymeric resin, CG161 (~60 ml) to generate 6 fractions using stepwise gradient of aqueous acetonitrile (ACN) (120 ml of each 10%, 25%, 50%, 75%, 100% ACN, and 1:1 methanol-EtOAc wash). All fractions were dried and yielded a total of 118 mg of enriched material. Antifungal activity was observed in fraction 3 (45 mg, 50% ACN elution). Fraction

3 was further fractionated on an HPLC semi-preparative ODS-A column (YMC, 120 A, S-7, 2 cm×25 cm). The column was eluted with a gradient of ACN-H<sub>2</sub>O (3% to 40% ACN in 40 minutes, and then 40% to 100% ACN in another 40 minutes, 15 ml/minute). A total of 100 fractions were collected (13 ml/fraction). Pure **1** (5 mg) was obtained at 48~49 minutes as colorless gum.

From high-resolution negative ESMS, the molecular formula of **1** was established as C<sub>32</sub>H<sub>53</sub>O<sub>13</sub>S<sub>3</sub> (Found 741.2648; calcd. 741.2653 for [M-3Na+2H]<sup>-</sup>, Δ 0.78 ppm) suggesting three sulfate groups in the molecule (performed on a PE Sciex QSTAR mass spectrometer; two internal standards, taurocholic acid and nitrazepan, were used in the negative ion ESI-HR-MS measurements). The structure of **1** was elucidated by extensive NMR data analysis. Thirty two carbons were observed in the <sup>13</sup>C NMR spectrum, suggesting a triterpene skeleton with two additional carbons. Highly overlapped proton signals in <sup>1</sup>H NMR can be interpreted and assigned to their adjacent carbons by HSQC analysis. The connectivity of protonated fragments were determined primarily by HSQC-TOCSY data (Table 1). Thus the fragments A, B, C, and D were established through two or three H-C bond correlations (Figure 1). With the information of fragments A, C, and D, the entire ring skeleton was further determined by analysis of HMBC data with the following H-C correlations: H<sub>3</sub>-19 (δ 1.41) to C-1 (δ 38.0), C-5 (δ 48.3), C-9 (δ 146.4), and C-10 (δ 39.5); H<sub>3</sub>-18 (δ 0.70) to C-12 (δ 38.4), C-13 (δ 45.7), C-14 (δ 48.2), and C-17 (δ 52.3); H<sub>3</sub>-32 (δ 0.81) to C-8 (δ 41.5), C-13 (δ 45.7), C-14 (δ 48.2), and C-15 (δ 35.0) (see Figure 2).

Excluding the ring system and the fragments B and C, a vinyl group (δ 113.0 t, δ 150.9 s), a methyl group (δ 20.0 q) and a quaternary carbon (δ 45.7 s) were to be assigned to the side chain. These groups can be connected based on



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Table 1. NMR spectral data for compound 1 in CD<sub>3</sub>OD<sup>a</sup>.

C/H no.	<sup>1</sup> H (δ)	<sup>13</sup> C (δ)	HSQC- TOCSY C No.	HMBC
1α	1.85, dd, <i>J</i> = 14.5, 4.0	38.0 t	2	
1β	2.28, , brd, <i>J</i> = 14.5			
2	4.93, brs	76.1 d	1, 3	
3	4.83, brs	76.8 d	2, 4	
4	4.44, brs	68.6 d	3, 5	C-3, C-10
5	1.42, m	48.3 d	4, 6	
6	4.83, m	76.3 d	5, 7	
7α	1.55, ddd, <i>J</i> = 12, 12, 12	35.6 t	6, 8	
7β	2.22, dt, <i>J</i> = 11, 4.5			
8	2.48, brd, <i>J</i> = 13	41.5 d	7	
9		146.4 s		
10		39.5 s		
11	5.35, d, <i>J</i> = 5.5	118.0 d	12	C-8, C-10, C-12, C-13
12α	2.11, d, <i>J</i> = 18	38.4 t	11	
12β	1.96, dd, <i>J</i> = 18, 6			
13		45.7 s		
14		48.2 s		
15	1.37, m 1.46, m	35.0 t	16	
16	1.30, m 1.91, m	29.2 t	15, 17	
17	1.66, m	52.3 d	16, 20	
18	0.70, s	15.1 q		C-12, C-13, C-14, C-17
19	1.41, s	25.5 q		C-1, C-5, C-9, C-10
20	1.34, m	38.3 d	17, 21, 22	
21	0.92, d, <i>J</i> = 6.5	19.4 q	20	C-17, C-20, C-22
22	0.83, m 1.24, m	31.0 t	20, 23	
23	1.16, m 1.44, m	31.4 t	22	
24		45.7 s		
25		150.9 s		
26	4.67, d, <i>J</i> = 1.5 4.90, t, <i>J</i> = 1.5	113.0 t		C-24, C-25, C-27
27	1.64, s	20.0 q		C-24, C-25, C-26
28	1.37, m	27.2 t	30	C-23, C-24, C-25, C-29
29	1.37, m	27.0 t	31	C-23, C-24, C-25, C-28
30	0.68, t, <i>J</i> = 7.5	8.4 q	28	C-24, C-28
31	0.69, t, <i>J</i> = 7.5	8.4 q	29	C-24, C-29
32	0.81, s	19.0 q		C-8, C-13, C-14, C-15

<sup>a</sup>Recorded on a Varian Unity 500 NMR instrument at 500 MHz for <sup>1</sup>H and 125

MHz for <sup>13</sup>C, using standard Varian pulse sequence programs (VNMR Version

6.1 Software). δ in ppm; *J* in Hz.

Fig. 1. Fragments A, B, C, and D determined by HSQC-TOCSY.

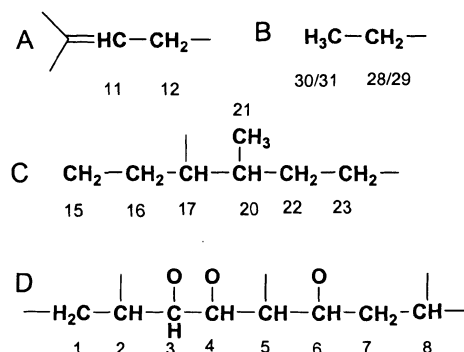
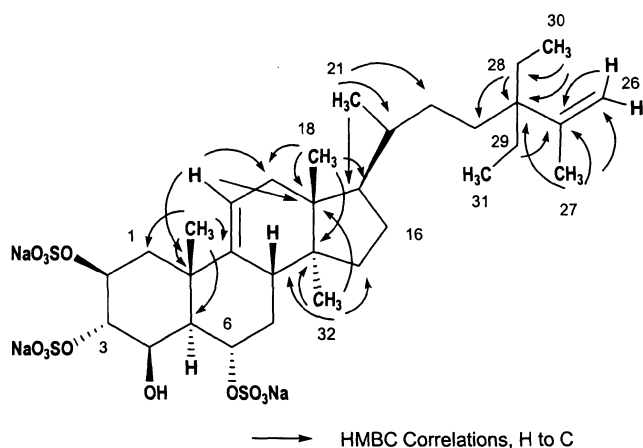


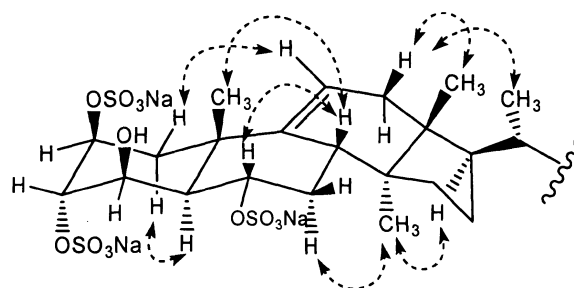
Fig. 2. Major HMBC correlations of **1**.



H-C long range correlations from a HMBC spectrum. Observation of long range  $^1\text{H}$ - $^{13}\text{C}$  correlations of  $\text{H}_3$ -27 ( $\delta$  1.64) to C-24 ( $\delta$  45.7), C-25 ( $\delta$  150.9), and C-26 ( $\delta$  113.0) allowed us to determine that the vinyl group was in between the methyl group and the quaternary carbon. The two remaining ethyl groups (fragment B) could only be located on the quaternary carbon (C-24). This was confirmed by the long range correlations of  $\text{H}_2$ -28,  $\text{H}_2$ -29 ( $\delta$  1.37),  $\text{H}_3$ -30 ( $\delta$  0.68) and  $\text{H}_3$ -31 ( $\delta$  0.69) to C-24 ( $\delta$  45.7). Eventually, the connection of C-23 and C-24 was determined based on the correlations of  $\text{H}_2$ -28,  $\text{H}_2$ -29 to C-23 ( $\delta$  31.4). Therefore the side chain terminal was determined to be 24,24-di-ethyl-25-methyl-25,26-ene.

Three sulfate groups were assigned to C-2 ( $\delta$  76.1), C-3 ( $\delta$  76.8), and C-6 ( $\delta$  76.3) due to their downfield chemical

Fig. 3. Key NOESY correlations of **1**.



shifts, and these assignments were also supported by  $^{13}\text{C}$  data in comparison with those of reported analogues, such as topsentiasterol sulfates.<sup>2)</sup> Stereochemistry of **1** was determined by the  $^1\text{H}$ - $^1\text{H}$  coupling pattern and NOE data. Key NOE Correlations are shown in Figure 3. These data were virtually identical to those of topsentiasterol sulfates.<sup>2)</sup> Thus, the structure elucidation of **1** was completed and shown in Figure 2.

Compound **1** was determined as a new sterol sulfate having a rare side chain which has di-ethyl substitution on C-24. To the best of our knowledge, this is the first marine sterol to possess such a side chain. In the plant kingdom, only laureolol and its 3-*O*-methyl analogue possess this side chain.<sup>3,4)</sup>

Compound **1** exhibited antifungal activity with MIC 15  $\mu\text{g}/\text{ml}$  against supersensitive *Saccharomyces cerevisiae* PM503, a modification strain of wild-type *S. cerevisiae* in which two efflux pumps, a gene involved in sterol metabolism and a chitin synthetase gene have been disrupted. Standard compound posaconazole gave MIC of 4 ng/ml when tested under the same conditions. No activity was observed at 60  $\mu\text{g}/\text{ml}$  against *S. aureus*, *C. albicans*, and *E. coli*.

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