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## A SULFATED NORMONOTERPENOID FROM THE ASCIDIAN *POLYCITOR ADRIATICUS*

A. CRISPINO, A. DE GIULIO, S. DE ROSA, S. DE STEFANO, A. MILONE,\*

ICMIB-CNR, Via Toiano 6, 80072 Arco Felice, Naples, Italy

and N. ZAVODNIK

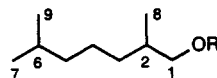
Center for Marine Research, R. Bosković Institute, 52210 Rovinj, Croatia

**ABSTRACT.**—Sodium (or potassium) 2,6-dimethylheptyl sulfate has been isolated from the ascidian *Polycitor adriaticus*, collected in the northern Adriatic Sea. The structure was proposed by means of spectroscopic data and by chemical correlation.

Sulfuric acid esters with alcoholic or phenolic hydroxy groups occur in microorganisms, plants, and animals (1). In animals, sulfate esters are of significance in the elimination of secondary products, and high levels of sulfate esters are associated with diseases, for example, X-linked ichthyosis shows a high level of sulphated steroids (2). Within marine organisms, the echinoderms are a rich source of sulfated saponins, having both triterpenoid (sea cucumbers) and steroidal (starfish) aglycones (3), sterols, and polyhydroxysteroids (ophiuroids) (3). Recently, Findlay and coworkers have reported the isolation of two sulfated hydrocarbons from a starfish (4), and from a sea cucumber (5).

In the course of our search for polar metabolites from marine organisms we have investigated the tunicate *Polycitor adriaticus* (= *Distoma adriaticum*) Drasche (class Ascidiacea, order Aplousobranchiata, family Polycitoridae), collected near Rovinj, Croatia. From the aqueous extract of whole *P. adriaticus* animals, after reversed-phase and Si gel chromatography, a sulfated hydrocarbon [**1**] was recovered.

The <sup>13</sup>C-nmr spectrum and DEPT analysis of **1** showed eight signals comprising three methyls, four methylenes, and two methines. The presence of a methylene signal at δ 73.9 and an ABX system at δ 3.90 (1H, dd, *J*=9.4 and 5.8 Hz) and 3.81 (1H, dd, *J*=9.4 and 6.7 Hz) in the <sup>13</sup>C- and <sup>1</sup>H-nmr spectra, respec-



- 1 R=SO<sub>3</sub>Na(K)  
2 R=H

tively, suggested the presence of a primary alcohol near a chiral center in **1**. The high polarity of **1** suggested that the hydroxyl group was esterified with an inorganic acid. The eims showed no molecular ion, with the highest value in the mass spectrum at *m/z* 127 due to cleavage of a carbon oxygen bond. The eims resembled that of the corresponding olefin. The negative fabms of **1** exhibited a quasi-molecular anion peak at *m/z* 223 ([M-H]<sup>-</sup>), corresponding to a monosulfated derivative of a nonanol, while the positive fabms in glycerol (G) matrix exhibited two prominent ions at *m/z* 355 and 339 corresponding to [M(K)+G+H]<sup>+</sup> and [M(Na)+G+H]<sup>+</sup>, respectively, suggesting that the compound was a mixture of salts of potassium and sodium sulfate. This was further substantiated by its ir spectrum with bands at 1242, 1215, and 1110 cm<sup>-1</sup>. Moreover, the <sup>1</sup>H-nmr spectrum showed two doublets at δ 0.91 (6H, *J*=6.6 Hz) and 0.98 (3H, *J*=6.7 Hz), with the first doublet being part of an isopropyl group that was coupled with a methine at δ 1.58, which in turn was coupled with a methylene at δ 1.22 (from the COSY spectrum). The loss of 43 mass units (C<sub>3</sub>H<sub>7</sub>) in the eims also helped to confirm the pres-

ence of an isopropyl group in the molecule. The second methyl doublet was coupled with a methine at  $\delta$  1.81 that is the X part of the ABX system described above, and was coupled with a methylene at  $\delta$  1.45–1.18. The last signal observed in the  $^1\text{H}$ -nmr spectrum was a methylene at  $\delta$  1.41–1.35 which was coupled with the two methylenes at  $\delta$  1.22 and 1.18–1.45. These data are in accordance with structure **1** (2,6-dimethylheptyl sulfate). The  $^{13}\text{C}$ -nmr chemical shifts of **1** are in excellent agreement with those calculated using the  $^{13}\text{C}$ -nmr correlation table by Strothers (6).

Solvolysis of **1** with pyridine-dioxane (1:1) gave, in low yield (part of the alcohol was lost during the evaporation of solvent), the corresponding alcohol **2**, which showed an upfield shift ( $\delta$  3.51 and 3.42) for H-1 in the  $^1\text{H}$ -nmr spectrum, and displayed a molecular ion at  $m/z$  144 in the eims.

The finding of **1** from *P. adriaticus* is the first report of a sulfated hydrocarbon from a tunicate.

## EXPERIMENTAL

**GENERAL EXPERIMENTAL PROCEDURES.**—Ir spectra were recorded on a Bio-Rad FTS-7 Ft-ir spectrometer. IR cards 3M (KC-0061) were purchased from Carlo Erba Reagenti, Italy. Optical rotations were measured on a Jasco DIP 370 polarimeter, using a 10-cm microcell. Eims were recorded on an Fisons TRIO 2000 spectrometer, coupled with an Intel computer; fab ms were recorded on a VG analytical ZAB2SE double-focusing mass spectrometer, equipped with a cesium gun operating at 25 keV (2  $\mu\text{A}$ ) using glycerol (G) as matrix.  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr spectra were recorded at 500 and 125 MHz, respectively, with TMS as internal standard on a Bruker AM 500 instrument, under Aspect X32 control. The 2D nmr spectra were obtained using Bruker's microprograms. Tlc and cc were performed using precoated Merck F<sub>254</sub> plates, Kieselgel 60 powder and Lichroprep RP-18 (40–63  $\mu\text{m}$ ).

**EXTRACTION AND ISOLATION OF COMPOUNDS.**—*Polycitor adriaticus* was collected by dredging (–15 m) at Rovinj, Croatia, and was frozen at –20° until extracted. It was identified by Dr. D. Zavodnik, "R. Bosković" Institute, Rovinj, Croatia. A voucher specimen is maintained in Italian institute collection (voucher No. T03/92).

The frozen animals (50 g dry wt after extraction) were extracted with 4×500 ml of H<sub>2</sub>O-Me<sub>2</sub>CO (1:1), and after elimination of the solvent *in vacuo*, the aqueous residue was introduced onto a Lichroprep RP-18 (30 g) column, and eluted with H<sub>2</sub>O, H<sub>2</sub>O-MeOH (1:1), and MeOH (300 ml for each solvent). The MeOH fraction gave, after evaporation, a mixture which was treated with MeOH. The MeOH-soluble portion was chromatographed on a Si gel column eluting with CHCl<sub>3</sub>-MeOH (4:1) to give **1** (20 mg).

**Sodium (or potassium) 2,6-dimethylheptyl sulfate [1].**—Amorphous; [ $\alpha$ ]<sub>D</sub> +4.7° ( $c=0.01$  MeOH); ir (dry film)  $\nu$  max 2950–2925 (CH, aliphatic), 1242, 1215, 1110  $\text{cm}^{-1}$ ;  $^1\text{H}$  nmr (CD<sub>3</sub>OD)  $\delta$  3.90 (1H, dd,  $J=9.4$  and 5.8 Hz, H-1a), 3.81 (1H, dd,  $J=9.4$  and 6.7 Hz, H-1b), 1.81 (1H, m, H-2), 1.58 (1H, m, H-6), 1.45–1.41 (2H, m, overlapped, H-3a, H-4a), 1.37 (1H, m, H-4b), 1.22 (2H, m, H-5), 1.18 (1H, m, H-3b), 0.98 (3H, d,  $J=6.7$  Hz, H-8), 0.91 (6H, d,  $J=6.6$  Hz, H-7, H-9);  $^{13}\text{C}$  nmr (CD<sub>3</sub>OD)  $\delta$  73.9 (t, C-1), 40.4 (t, C-5), 34.6 (t, C-3), 34.4 (d, C-2), 29.1 (d, C-6), 25.7 (t, C-4), 23.1 (q, C-7 or C-9), 23.0 (q, C-7 or C-9), 17.2 (q, C-8); eims (70 eV)  $m/z$  [ $\text{M}-\text{SO}_4\text{Na(K)}^+$ ] 127 (25), [ $\text{M}-\text{HSO}_4\text{Na(K)}^+$ ] 126 (54), 111 (45), 85 (68), 83 (75), 69 (100); negative fabms  $m/z$  [ $\text{M}-\text{Na(K)}^-$ ] 223 (100); positive fabms [ $\text{M(K)+G+H}^+$ ] 355 (100), [ $\text{M(Na)+G+H}^+$ ] 339 (50), [ $\text{M(H)+G+H}^+$ ] 225 (8).

**SOLVOLYSIS OF 1.**—Compound **1** (15 mg) in dioxane (3 ml) and pyridine (3 ml) was heated at 110° overnight. The cooled solution was neutralized with 2 N HCl and extracted with CHCl<sub>3</sub> (3×5 ml). The combined extract was evaporated *in vacuo* to give the alcohol **2** (2 mg); colorless oil;  $^1\text{H}$  nmr (CDCl<sub>3</sub>)  $\delta$  3.51 (1H, dd,  $J=9.4$  and 5.8 Hz, H-1a), 3.42 (1H, dd,  $J=9.4$  and 6.7 Hz, H-1b); eims  $m/z$  [ $\text{M}^+$ ] 144.

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