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ANTIBACTERIAL AND ANTIFUNGAL SULFATED ALKANE AND ALKENES FROM THE HEPATOPANCREAS OF THE ASCIDIAN *HALOCYNTHIA RORETZI*

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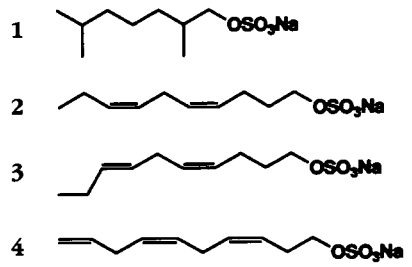
ABSTRACT.—Four new antibacterial and antifungal sulfates, 2,6-dimethylheptyl sulfate [**1**], (4*Z*,7*Z*)-4,7-decadienyl sulfate [**2**], (4*Z*,7*E*)-4,7-decadienyl sulfate [**3**], and (3*Z*,6*Z*)-3,6,9-decatrienyl sulfate [**4**], have been isolated from the hepatopancreas of the ascidian *Halocynthia roretzi*. The structures were determined by spectral analysis.

Only a few examples of sulfated alkanes/alkenes have been reported from marine organisms (1,2), although sulfated terpenoids and steroids are increasingly common groups of marine metabolites (3–5). In the course of our studies on bioactive metabolites from marine organisms (6–9), we found that the MeOH extract from the hepatopancreas of the ascidian *Halocynthia roretzi* Drasche (Pyuridae, Stolidobranchia) showed antibacterial and antifungal activities against *Vibrio alginoliticus* and *Mortierella ramaniana*, respectively. Bioassay-guided isolation afforded a sulfated C_9-alkane [**1**] and three sulfated C_{10}-alkenes [**2**–**4**]. This paper describes the isolation and structure elucidation of these compounds.

The hepatopancreas (1.12 kg, wet wt) was dissected from the ascidian *Halocynthia roretzi* (356 specimens), collected off Mutsu Bay, Aomori, 600 km northeast of Tokyo, and extracted with MeOH. The concentrated aqueous residue was extracted with Et_2O, then *n*-BuOH. The *n*-BuOH layer, which inhibited the growth of *Vibrio alginoliticus* and *Mortierella ramaniana*, was fractionated by Si gel cc (MeOH/$CHCl_3$) and Sephadex LH-20 gel filtration (MeOH) to give a mixture of sulfates, which was further purified by reversed-phase hplc (CH_3CN/0.1 N $NaClO_4$) to afford **1** (yield: 3.8×10^{-3}%, wet wt), **2** (6.5×10^{-3}%), **3** (4.7×10^{-4}%), and **4** (1.9×10^{-3}%).



Compounds **1**–**4** all showed strong ir absorption bands at 1210 cm^{-1} and gave positive sodium rhodizonate tests (10), indicating the presence of a sulfate group. The negative fabms of **1** gave an $[M-Na]^-$ ion peak at *m/z* 223, corresponding to a formula of $C_9H_{19}O_4S$ (Δ −0.6 mmu). The ^{1}H-nmr spectrum exhibited three doublet methyls at δ 0.82 (3H, d, J=6.9 Hz, Me-2) and 0.84 (6H, d, J=6.6 Hz, H_3-7 and Me-6), two methylene protons bearing a sulfate group at δ 3.45 (1H, dd, J=9.5 and 6.8 Hz, H-1) and 3.54 (1H, dd, J=9.5 and 5.9 Hz, H-1), and eight methine/methylene protons at δ 1.0–1.6. Interpretation of the ^{1}H-^{1}H COSY spectrum of **1** led to a gross structure, 2,6-dimethylheptyl sulfate. The specific rotation, $[\alpha]_D^{20}$ 0° (c=0.40, MeOH), indicated that **1** was a racemate, which was confirmed by the cd spectrum, since the cd curve was flat from 200 to 400 nm.

Compounds **2** and **3** have the same molecular formula, $C_{10}H_{17}O_4SNa$, as established by hrfabms. The ^{1}H-nmr spectrum of **2**, which revealed four olefinic protons at about δ 5.30, two methylene protons bearing a sulfate group at δ 3.71

(2H, m, H_2-1), a methyl group at δ 0.91 (3H, d, J=7.0 Hz, H_3-10), and eight methylene protons at δ 1.5–2.7, was almost superimposable on that of **3** (Table 1). The ^{1}H-^{1}H COSY nmr spectra of these compounds were consistent with the structure, 4,7-octadienyl sulfate. The geometry of the two double bonds in **2** was deduced to be 4*Z*,7*Z* from the ^{13}C-nmr chemical shifts (Table 1) of allylic methylenes, with sterically induced upfield shifts being observed at C-6 and C-9 of **2** (11), whereas **3** had 4 *Z*,7*E*-geometry.

The presence of a terminal vinyl group in **4** was inferred from three mutually coupled olefinic protons at δ 4.96 (1H, dd, J=10 and 1.8 Hz, H-10), 5.03 (1H, dd, J=17 and 1.8 Hz, H-10), and 5.78 (1H, dd, J=17 and 10 Hz, H-9). The ^{1}H-^{1}H COSY nmr spectrum of **4** revealed cross-peaks [δ 3.68 (2H, m, H_2-1)/2.27 (2H, m, H_2-2)/5.35 (1H, m, H-3)/5.37 (1H, m, H-4)/2.76 (2H, m, H_2-5)/5.37 (1H, m, H-6)/5.37 (1H, m, H-7)/2.79 (2H, m, H_2-8)/5.78/4.96 and 5.03], which secured connectivities from C-1 to C-10. Although three olefinic protons at δ 5.37 were overlapping, the structure was confirmed not only by HMBC nmr cross-peaks [δ 3.68 (H_2-1)/δ 126.1 (C-3), δ 2.27 (H_2-2)/δ 126.7 (C-4), δ 2.79 (H-8)/δ 136.7 (C-9), 128.8, and 129.2 (C-6 and C-7), and δ 5.03 (H-10)/δ 31.0 (C-8)], but also by homoallylic coupling between H_2-2 (δ 2.27) and H_2-5 (δ 2.76). Thus, **4** was 3,6,9-decatrienyl sulfate. A diagnostic carbon signal at δ 25.3 indicated 3*Z*,6*Z*-geometry (11). Consequently, the structures of **1–4** were established.

Compounds **1–4** showed 12-mm zones of inhibition against *Vibrio alginoliticus* and 10-mm zones of inhibition against *Mortierella ramaniana* at 0.2 mg/disk, respectively.

The distribution of these sulfates in other ascidians was investigated by hplc analysis. Similar amounts of **1–4** were found in the hepatopancreas of *H. roretzi* (Drasche) (Pyuridae), collected in 1984–1987 as well as 1994, and *H. aurantium*. However, other tissues of *H. roretzi* (gonad, tunic, body muscle, and hemolymph) did not contain these sulfates. Moreover, the ^{1}H-nmr spectrum of the extract from *Styela plicata* (Styelinae) indicated the presence of these compounds, even though their composition was not the same as in *H. roretzi*. The sulfates were not detected in the extract of the ascidians

TABLE 1. ^{1}H- and ^{13}C-Nmr Data for **2** and **3**.[a]

Position	Compound			
	2		**3**	
	^{1}H[b]	^{13}C[c]	^{1}H[b]	^{13}C[c]
1	3.71 (2H, m)	65.3 (t)	3.67 (2H, m)	65.0 (t)
2	1.54 (2H, m)	29.2 (t)	1.53 (2H, m)	29.1 (t)
3	2.05 (2H, m)	23.3 (t)	2.02 (2H, m)	23.2 (t)
4	5.27 (1H, m)[d]	127.2 (d)[e]	5.36 (1H, m)	127.2 (d)[f]
5	5.32 (1H, m)[d]	128.3 (d)[e]	5.36 (1H, m)	127.9 (d)[f]
6	2.73 (2H, m)	25.1 (t)	2.69 (2H, m)	29.8 (t)
7	5.32 (1H, m)[d]	129.2 (d)[e]	5.36 (1H, m)	129.5 (d)[f]
8	5.32 (1H, m)[d]	131.5 (d)[e]	5.43 (1H, m)	131.8 (d)[f]
9	2.02 (2H, m)	20.1 (t)	1.96 (2H, m)	23.2 (t)
10	0.91 (3H, t, 7.0)	14.2 (q)	0.91 (3H, t, 7.0)	13.7 (q)

[a]Data recorded in DMSO-d_6 at 500 MHz (^{1}H) and 125 MHz (^{13}C) at 27°.
[b]Number of proton(s), multiplicity and J in Hz are in parentheses.
[c]Multiplicities were determined by an HMQC experiment.
[d–f]These resonances may be interchangeable.

Herdmania momus (Pyuridae), *Botrylloides* sp. (Botryllinae), *Ascidia sydneiensis samea* (Oka) (Ascidiidae), *Ciona savignyi* (Cionidae), and *Pseudodistoma antinboja* (Polyclininae). It is likely that these simple sulfates may play some physiological role in the digestive systems of certain ascidians.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The optical rotation was determined with a Jasco DIP-371 digital polarimeter. The cd spectrum was measured on a Jasco J-720W spectropolarimeter in MeOH. Ir spectra were measured on a Jasco IR-700 spectrometer. ^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker ARX-500 nmr spectrometer at 27° in DMSO-d_6. Residual CHD_2SOCD_3 (2.49 ppm) and CD_3SOCD_3 (39.5 ppm) signals were used as internal standards. Multiplicities of ^{13}C-nmr signals were determined by HMQC experiments. Mass spectra were measured on a JEOL SX-102 mass spectrometer.

ANIMAL MATERIALS.—*Halocynthia roretzi* was collected in 1984–1987 and in 1994 in Mutsu Bay, Aomori, Japan. *Halocynthia aurantium* was collected in Nemuro Bay, Hokkaido, Japan; *Herdmania momus* and *S. plicata* off Miura Peninsula, Kanagawa, Japan; a compound tunicate of the genus *Botrylloides* from Sagami Bay, Kanagawa, Japan; *A. sydneiensis samea* and *C. savignyi* in Mutsu Bay, Japan; and *P. antinboja* off Izu Peninsula, Shizuoka, Japan. Voucher specimens are kept in this laboratory.

EXTRACTION AND ISOLATION.—The hepatopancreases (1.12 kg, wet wt) were dissected from *H. roretzi* (356 specimens) and extracted with MeOH. The concentrated aqueous residue was extracted with Et_2O and *n*-BuOH, successively. A part (11.18 g) of the *n*-BuOH layer (18.18 g) was chromatographed on Si gel (4×40 cm) with 5, 10, 20, and 40% MeOH/$CHCl_3$ (each 400 ml). The antibacterial/antifungal fractions (1.42 g) eluted with 40% MeOH/$CHCl_3$ were passed through a Sephadex LH-20 column (2.2×70 cm) with MeOH, and a fraction (110–160 ml, 1.06 g) was purified by reversed-phase hplc (Asahipak ODP-50, 5 μm, 10×250 mm, Asahi Chemical Industry, Co., Ltd., Kawasaki, Japan; 28% CH_3CN/0.1 N $NaClO_4$; 1.5 ml/min) to afford **1** (R_t 28.5 min; yield: 3.8×10^{-3}%, wet wt), **2** (25 min, 6.5×10^{-3}%), **3** (27 min, 4.7×10^{-4}%), and **4** (20 min, 1.9×10^{-3}%).

2,6-Dimethylheptyl sulfate [**1**].—$[\alpha]^{20}D$ 0° (*c*=0.40, MeOH); ir (KBr) ν max 3400 and 1210 cm^{-1}; negative fabms (TEA) *m/z* $[M-Na]^-$ 223; negative hrfabms *m/z* 223.0998 (calcd for $C_9H_{19}O_4S$, 223.1004); ^{1}H nmr (DMSO-d_6) δ 0.82 (3H, d, *J*=6.9 Hz, Me-2), 0.84 (6H, d, *J*=6.6 Hz, H_3-7 and Me-6), 1.00 (1H, m, H-3), 1.12 (2H, m, H_2-5), 1.20 (1H, m, H-4), 1.27 (1H, m, H-4), 1.28 (1H, m, H-3), 1.50 (1H, septet, *J*=6.6 Hz, H-6), 1.61 (1H, m, H-2), 3.45 (1H, dd, *J*=9.5 and 6.8 Hz, H-1), and 3.54 (1H, dd, *J*=9.5 and 5.9 Hz, H-1); ^{13}C nmr (DMSO-d_6) δ 16.9 (q, Me-2), 22.5 and 22.6 (each q, C-7 and Me-6), 24.0 (t, C-4), 27.4 (d, C-6), 32.6 (d, C-2), 33.2 (t, C-3), 38.8 (t, C-5), and 70.5 (t, C-1).

(4Z,7Z)-4,7-Decadienyl sulfate [**2**].—Ir (KBr) ν max 3400 and 1210 cm^{-1}; negative fabms (TEA) *m/z* $[M-Na]^-$ 233; negative hrfabms *m/z* 233.0866 (calcd for $C_{10}H_{17}O_4S$, 233.0848); ^{1}H and ^{13}C nmr, see Table 1.

(4Z,7E)-4,7-Decadienyl sulfate [**3**].—Ir (KBr) ν max 3400 and 1210 cm^{-1}; negative fabms (TEA) *m/z* $[M-Na]^-$ 233; negative hrfabms *m/z* 233.0822 (calcd for $C_{10}H_{17}O_4S$, 233.0848); ^{1}H and ^{13}C nmr, see Table 1.

(3Z,6Z)-3,6,9-Decatrienyl sulfate [**4**].—Ir (KBr) ν max 3400 and 1210 cm^{-1}; negative fabms (TEA) *m/z* $[M-Na]^-$ 231; negative hrfabms *m/z* 231.0686 (calcd for $C_{10}H_{15}O_4S$, 231.0691); ^{1}H nmr (DMSO-d_6) δ 2.27 (2H, m, H_2-2), 2.76 (2H, m, H_2-5), 2.79 (2H, m, H_2-8), 3.68 (2H, m, H_2-1), 4.96 (1H, dd, *J*=10 and 1.8 Hz, H-10), 5.03 (1H, dd, *J*=17 and 1.8 Hz, H-10), 5.35 (1H, m, H-3), 5.37 (3H, m, H_3-4, 6, and 7), and 5.78 (1H, dd, *J*=17 and 10 Hz, H-9); ^{13}C nmr δ 25.3 (t, C-5), 27.4 (t, C-2), 31.0 (t, C-8), 65.0 (t, C-1), 114.9 (t, C-10), 126.1 (d, C-3), 126.7 (d, C-4), 128.8 and 129.2 (each d, C-6 and C-7), and 136.7 (d, C-9).

ANTIBACTERIAL AND ANTIFUNGAL ASSAYS.—Antibacterial and antifungal activities were determined by the paper disk method. Paper disks (thickness, 8 mm, Toyo Roshi Kaisha, Ltd., Tokyo), impregnated with 0.2 mg of each sample, were incubated on agar plates containing *Vibrio alginoliticus* ATCC 17749 or *Mortierella ramaniana* for 24 h at 28°.

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