New Sesterterpene Sulfates from the Sponge *Darwinella australensis*

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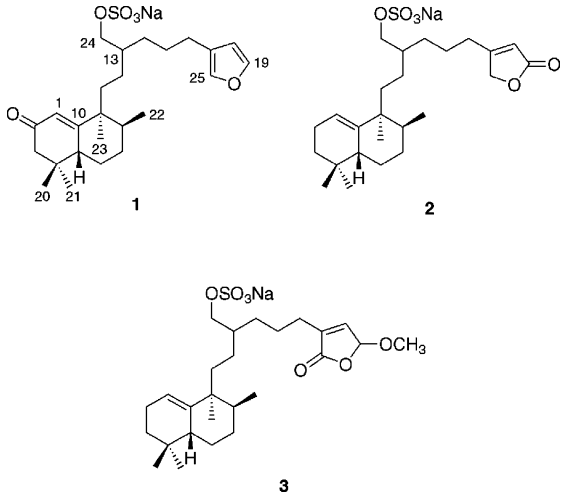
Three new sesterterpene sulfates, halisulfates 8−10 (**1**−**3**), were isolated from the Australian sponge *Darwinella australensis*. The structures of these compounds were determined by combined spectroscopic methods. The stereochemistry of the decalin moiety was assigned on the basis of NMR interpretations. Halisulfates 9 and 10 inhibited cell division of sea urchin eggs in moderate concentration.

Sponges have produced a wide variety of terpenoids and mixed biogenetic compounds possessing polyprenyl moieties.[1] Compared to other structural groups, however, sesterterpenoids are regarded as a relatively minor group and only a limited number of carbon skeletons have been reported.[1,2] Among the sponge-derived sesterterpenoids, halisulfates, isolated from a halichondriidae sponge and *Coscinoderma* sp., have distinguishing structural features by containing a sulfated side chain as well as a furan or hydroquinone moiety at the terminus of the molecule.[3−5] These compounds exhibited antimicrobial activity and inhibitory effects on phospholipase A_2, serine protease, and PMA-induced inflammation. Also related structurally are suvanine and sulfircin, guanidium salts of sesterterpene sulfates, from sponges of the genus *Ircinia*.[6−8]

During the course of our search for novel natural products from marine invertebrates, we collected the sponge *Darwinella australensis* (order Dendroceratida, family Aplysillidae) from the Australian coast of East Timor Sea. Guided by the combined results of bioassay and proton NMR measurement, secondary metabolites have been isolated employing various chromatographic techniques. We describe herein the structures of halisulfates 8−10 (**1**−**3**), novel sesterterpene sulfates possessing a furan moiety or its oxidized variants.

Halisulfate 8 (**1**) was isolated as a white amorphous solid, which analyzed for $C_{25}H_{37}O_6SNa$ by combined HRFABMS and ^{13}C NMR spectrometry. A preliminary analysis of the ^{13}C NMR data of this compound showed signals of a carbonyl carbon at δ 203.3 and six olefinic ones in the region δ 180−110. The chemical shift of the carbonyl carbon, coupled with an absorption band at 1660 cm^{-1} in the IR spectrum, revealed the presence of an α,β-unsaturated ketone. Also presented in the IR spectrum were strong absorption bands at 1260 and 1220 cm^{-1}, which were indicative of a sulfate functionality. Consideration of spectral data, in conjunction with seven degrees of unsaturation inherent in the molecular formula, suggested that halisulfate 8 possessed three rings.

Given this information, the gross structure of compound **1** was then determined by a combination of $^1H-^1H$ COSY, TOCSY, *g*HSQC, and *g*HMBC experiments. The presence



of an α,β-unsaturated carbonyl group was confirmed by long-range correlations of the carbonyl and olefinic carbons at δ 203.3 and 178.3, respectively, with a downfield proton at δ 5.82 (1H, s). The connection of the carbonyl carbon with an isopropyl group via a methylene was also evident from the *g*HMBC correlations between the carbons derived from these groups and the methylene protons at δ 2.38 (1H, d, J = 16.1 Hz) and 1.96 (1H, d, J = 16.1 Hz) (Table 1). Long-range correlations of the olefinic and gem-dimethyl carbons with a common methine proton at δ 2.31 (1H, dd, J = 12.7. 3.9 Hz) allowed a cyclohexenone ring to be constructed.

The 2D NMR correlations were extended to carbons and protons connected to the cyclohexenone ring. Detailed interpretation of the $^1H-^1H$ COSY and TOCSY correlations, beginning with the signal of a methine proton at δ 2.31 (H-5), revealed the presence of a linear spin system consisted of this and protons from two methylenes, a methine, and a methyl group that was confirmed by *g*HMBC experiment (Table 1). Also illustrated in the *g*HMBC data were long-range correlations of the isolated methyl protons at δ 1.14 (H-23) with the carbons at δ 178.3 (C, C-10), 47.8 (CH, C-8), and 46.2 (C, C-9), which established a cyclohexane ring substituted with two methyl groups.

Of the six olefinic carbons present in the molecule, chemical shifts of the remaining four at δ 143.9 (CH), 140.1 (CH), 126.3 (C), and 111.9 (CH) in the ^{13}C NMR data, as well as the mutual couplings among the protons attached

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Table 1. NMR Assignments for Halisulfates 8−10 (**1**−**3**) in CD_3OD[a]

	halisulfate 8 (**1**)			halisulfate 9 (**2**)		halisulfate 10 (**3**)	
position	^1H	^{13}C	HMBC	^1H	^{13}C	^1H	^{13}C
1	5.82, s	122.0 CH	C-2, C-3, C-5, C-9, C-10	5.34, dd (2.4, 2.4)	117.9 CH	5.34, dd (2.4, 2.4)	117.9 CH
2		203.3 C		2.06, m; 1.97, m	24.3 CH_2	2.06, m; 1.98, m	24.3 CH_2
3	2.38, d (16.1); 1.96, d (16.1)	48.0 CH_2	C-1, C-2, C-4, C-5 C-20, C-21	1.39, m; 1.09, m	32.4 CH_2	1.38, m; 1.10, m	32.4 CH_2
4		35.4 C			32.3 C		32.3 C
5	2.31, dd (12.7, 3.9)	46.6 CH	C-1, C-3, C-4, C-6, C-10, C-20, C-21	1.65, m	44.8 CH	1.65, m	44.8 CH
6	2.10, m; 1.35, m	31.1 CH_2		1.84, dddd (11.7, 3.9, 3.9, 3.4); 1.07, m	31.4 CH_2	1.84, dddd (11.7, 3.9, 3.9, 3.4); 1.06, m	31.4 CH_2
7	1.69, dddd (13.2, 13.2, 12.7 3.9); 1.60, m	31.5 CH_2	C-6, C-8	1.53, ddd (11.7, 11.7, 4.4)	32.5 CH_2	1.53, ddd (11.7, 11.2, 3.9)	32.5 CH_2
8	1.38, m	47.8 CH		1.25, m	46.2 CH	1.24, m	46.2 CH
9		46.2 C			43.7 C		43.7 C
10		178.3 C			148.0 C		147.6 C
11	2.05, ddd (12.2, 12.2, 4.4); 1.20, m	29.9 CH_2	C-9, C-10, C-12, C-23	1.77, m; 1.06, m	29.2 CH_2	1.75, m; 1.04, m	29.0 CH_2
12	1.18, m; 1.03, m	26.5 CH_2		1.11, m; 1.06, m	26.3 CH_2	1.10, m; 1.06, m	26.0 CH_2
13	1.60, m	39.7 CH		1.63, m	39.7 CH	1.59, m	39.7 CH
14	1.41, m; 1.33, m	32.3 CH_2	C-24	1.51, m; 1.39, m	32.2 CH_2	1.47, m; 1.34, m	31.7 CH_2
15	1.58, m	28.4 CH_2	C-17	1.66, m	25.4 CH_2	1.61, m	25.3 CH_2
16	2.41, t (7.3)	25.8 CH_2	C-14, C-15, C-17, C-18, C-25	2.48, t (7.3)	29.7 CH_2	2.28, t (7.3)	26.1 CH_2
17		126.3 C			174.6 C		134.0 C
18	6.30, br s	111.9 CH	C-17, C-19, C-25	5.89, t (1.5)	115.4 CH	7.01, br d (1.5)	144.8 CH
19	7.37, dd (2.0, 1.5)	143.9 CH	C-17, C-18, C-25		177.2 C	5.85, d (1.5)	104.4 CH
20	1.03, s	28.5 CH_3	C-3, C-4, C-5, C-21	0.86, s	28.4 CH_3	0.86, s	28.3 CH_3
21	1.03, s	27.6 CH_3	C-3, C-4, C-5, C-20	0.91, s	28.1 CH_3	0.91, s	28.1 CH_3
22	0.96, d (6.8)	16.4 CH_3	C-7, C-8, C-9	0.87, d (6.3)	16.8 CH_3	0.87, d (6.3)	16.8 CH_3
23	1.14, s	23.3 CH_3	C-8, C-9, C-10, C-11	1.02, s	23.8 CH_3	1.02, s	23.8 CH_3
24	3.98, dd (9.3, 4.9); 3.89, dd (9.3, 5.9)	70.7 CH_2	C-12, C-13, C-14	3.97, dd (9.8, 4.9); 3.84, dd (9.8, 3.4)	71.3 CH_2	3.94, dd (9.8, 4.8); 3.83, dd (9.8, 6.4)	71.5 CH_2
25	7.26, br s	140.1 CH	C-17, C-18, C-19	4.90, br s	75.0 CH_2		173.6 C
OMe						3.52, s	56.8 CH_3

[a] Measured at 500 and 125 MHz for ^1H and ^{13}C NMR, respectively. Assignments were aided by $^1H-^1H$ COSY, TOCSY, *g*HSQC, and *g*HMBC experiments.

at these carbons, revealed the presence of a 3-alkylfuran moiety that was consistent with the *g*HMBC data (Table 1). The connection between the furan and bicyclic system via a long chain was also determined by a combination of 2D NMR experiments.

Compound **1** possessed a sulfate functionality. Downfield shifts of the methylene protons at δ 3.98 (dd, J = 9.3, 4.9 Hz) and 3.89 (dd, J = 9.3, 5.9 Hz) indicated attachment of this methylene to a primary alcohol. The *g*HMBC correlations of these protons with carbons at δ 39.7 (CH, C-13), 32.3 (CH_2, C-14), and 26.5 (CH_2, C-12) located the sulfate ester group at C-13 of the side chain. Thus, the structure of halisulfate 8 (**1**) was defined as a furanosesterterpene sulfate containing a decalin moiety.

The bicyclic portion of halisulfate 8 (**1**) contained asymmetric carbon centers at C-5, C-8, and C-9. The stereochemistries at these centers were assigned on the basis of combined ROESY, 1D NOESY, and proton-decoupling experiments. The ROESY data showed mutual cross-peak correlations among H-5 (δ 2.31), H-7β (δ 1.69), and one of H-11 (δ 2.05), suggesting β-orientations for both H-5 and C-11 to the cyclohexane ring (Figure 1). Conversely, the other proton of H-11 (δ 1.20) displayed a cross-peak correlation with H-22 (δ 0.96), which in turn correlated with H-23 (δ 1.14), suggesting β-orientation for H-22 to the cyclohexane ring. A literature survey revealed that other halisulfates (notably halisulfates 3 and 7) having similar ring structures contained the C-22 methyl group α-oriented

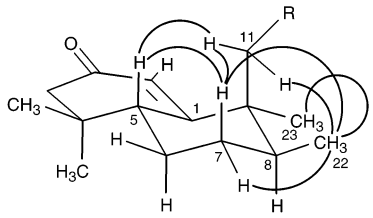

Figure 1. Selected NOE correlations for **1**.

to the cyclohexane ring.[3−5,9] To clarify this issue, proton decoupling of H-22 converted the splitting pattern of H-8 (δ 1.38) from a multiplet to a double-doublet (J = 12.7, 3.9 Hz) that indicated an α-orientation of H-8 and was consistent with the ROESY interpretation. The β-orientation of H-22 was further secured by its NOE correlations with H-7α (δ 1.60), H-7β (δ 1.69), H-8, H-11, and H-23 in the 1D NOESY data. Thus, the relative configurations of the asymmetric carbon centers were assigned as 5*R**, 8*S**, 9*S**.

The molecular formula of halisulfate 9 (**2**) was deduced as $C_{25}H_{39}O_6SNa$ on the basis of combined HRFABMS and ^{13}C NMR spectrometry. The NMR data of this compound were highly comparable with those obtained for **1**. The most noticeable difference in the ^{13}C NMR data was the replacement of the signal for the C-2 carbonyl carbon of **1** with that of a methylene carbon at δ 24.3 in **2**, and this was confirmed by combined 2D NMR experiments. Also sig-

nificantly changed in the ^{13}C NMR data were carbon signals of the furan moiety (C-17−C-19, C-25): δ 177.2 (C), 174.6 (C), 115.4 (CH), 75.0 (CH_2) (Table 1). Corresponding differences were observed in the ^1H NMR data, in which proton signals of the furan moiety were replaced with new ones at δ 5.89 (1H, t, $J = 1.5$ Hz) and 4.90 (2H, br s). These changes, coupled with the absorption band at 1740 cm^{-1} in the IR spectrum, indicated oxidative conversion of the furan moiety to an α,β-unsaturated γ-lactone. The *g*HMBC experiment showed long-range correlations of the carbons at δ 174.6, 115.4, and 75.0 with the allylic H-16 protons at δ 2.48, while the remaining carbon at δ 177.2 correlated with the ring protons at δ 5.89 and 4.90. Accordingly, the aromatic C-19 carbon at the furan moiety of **1** was oxidized to a carbonyl carbon in **2**, while the C-18 double bond shifted to C-17. Thus, the structure of halisulfate 9 (**2**) was determined to be a sesterterpene sulfate containing an unsaturated lactone moiety. The stereochemistry of the decalin portion was defined to be identical to that of compound **1** on the basis of ROESY and 1D NOESY correlations: H-5/H-11(δ 1.77), H-7/H-22, H-11(δ 1.06)/H-22, H-22/H-23.

The molecular formula of halisulfate 10 (**3**) was assigned as $C_{26}H_{41}O_7SNa$ on the basis of combined HRFABMS and ^{13}C NMR spectral analyses. Besides the appearance of an additional methoxy group (δ_H 3.52 (3H, s), δ_C 56.8 (CH_3)), the NMR data of this compound including those from 2D measurements were very similar to those of **2**. In particular, signals of protons and carbons at the decalin moiety and side chain were almost identical between these compounds. However, NMR signals corresponding to the γ-lactone ring shifted significantly (Table 1). Additional changes were also observed in the IR data, in which the carbonyl absorption band shifted from 1740 to 1770 cm^{-1} in **3**.

Of the four carbons forming the lactone moiety, those at δ 173.6 (C), 144.8 (CH), and 134.0 (C) showed long-range correlations with the neighboring H-16 protons at δ 2.28 in the *g*HMBC data, while the remaining carbon at δ 104.4 (CH) correlated with the olefinic and methoxy protons at δ 7.01 and 3.52, respectively. Accordingly, compound **3** had a carbonyl carbon at C-25 instead of C-19 in **2**. Thus, halisulfate 10 (**3**) possessed an α,β-unsaturated γ-methoxy-γ-lactone moiety. The stereochemistry of the decalin portion was defined to be identical to that of compound **1** on the basis of ROESY and 1D NOESY correlations: H-5/H-11(δ 1.75), H-5/H-22, H-7/H-22, H-11(δ 1.04)/H-22, H-22/H-23.

Halisulfates 1−7 exhibited diverse bioactivities.[3,5] However, in our evaluation, halisulfates 8−10 were neither cytotoxic (MTT method, $LC_{50} > 100$ μg/mL against the human leukemia K562 cell line) nor antimicrobial (paper-disk method, $D_{i.z.} < 3$ mm at the concentration of 25 μg against *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* sp.). Instead, halisulfates 9 (**2**) and 10 (**3**) inhibited cell division of the fertilized eggs of the sea urchin *Strongylocentrotus intermedius* at moderate concentration (IC_{50} 50 and 35 μg/mL for **2** and **3**, respectively).

Experimental Section

General Experimental Procedures. Melting points were measured on a Fisher-Jones apparatus and are uncorrected. Optical rotations were measured on a JASCO digital polarimeter using a 5 cm cell. UV spectra were obtained in methanol using a Milton-Roy spectrophotometer. IR spectra were recorded on a Mattson Galaxy spectrophotometer. NMR spectra were recorded in CD_3OD solutions containing Me_4Si as internal standard, on a Varian Unity 500 spectrometer. Proton and carbon NMR spectra were measured at 500 and 125 MHz, respectively. Mass spectral data were provided by the Korea Basic Science Institute, Taejeon, Korea. All solvents used were spectral grade or were distilled from glass prior to use.

Animal Material. The sponge *Darwinella australensis* (sample number 012-263) was collected by hand using scuba at 10 m depth at 14°04′01 S; 121°56′66 E (East Timor Sea) in November 1990, during the 12th scientific cruise of *r/v* "*Akademik Oparin*". The voucher specimens are deposited at Sponge Collection, Pacific Institute of Bioorganic Chemistry, Vladivostok, Russia.

Extraction and Isolation. The fresh collection was immediately frozen and kept until chemically investigated. The specimens were lyophilized (dry wt 55 g), macerated, and repeatedly extracted with EtOH (1 L × 2). The ethanolic extract after evaporation in vacuo was redissolved with EtOH and filtered through a silica gravity column. The ethanol-soluble fraction (4.34 g) was subjected to Sephadex LH-20 gel filtration chromatography using a mixture of CH_2Cl_2 and MeOH (v/v = 1:1) as an eluent. The yellow-colored fraction (1.47 g) was separated by silica column chromatography (230−400 mesh) using stepped gradient mixtures of $CHCl_3$ and MeOH as eluents. The fraction (586 mg) eluted with a mixture of $CHCl_3$ and MeOH (v/v = 4:1) was separated by C_{18} reversed-phase HPLC (YMC ODS-A column, 250 × 10 mm, 25% aqueous MeOH) to yield in order of elution **1**−**3**. Final purification was accomplished by C_{18} reversed-phase HPLC (MeOH−MeCN−H_2O, v/v = 2:1:2 for **1**, 1:2:2 for **2** and **3**, respectively) to afford 4.8, 5.8, and 13.5 mg of **1**−**3**, respectively.

Halisulfate 8 (1): amorphous solid, mp 55−57C; $[\alpha]^{25}_D$ −74.8° (*c* 0.36, MeOH); UV (MeOH) λ_{max} (log ϵ) 245 (4.22) nm; IR (KBr) ν_{max} 3600−3100 (br), 2920, 2850, 1660, 1540, 1460, 1260, 1220 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRFABMS *m/z* 511.2105 $[M + Na]^+$ (calcd for $C_{25}H_{37}O_6SNa_2$, 511.2106).

Halisulfate 9 (2): amorphous solid, mp 53−54C; $[\alpha]^{25}_D$ −57.9° (*c* 0.31, MeOH); UV (MeOH) λ_{max} (log ϵ) 206 (4.06) nm; IR (KBr) ν_{max} 3600−3100 (br), 2920, 2850, 1740, 1635, 1455, 1260, 1215 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRFABMS *m/z* 513.2261 $[M + Na]^+$ (calcd for $C_{25}H_{39}O_6SNa_2$, 513.2263).

Halisulfate 10 (3): amorphous solid, mp 69−71C; $[\alpha]^{25}_D$ −42.2° (*c* 0.21, MeOH); UV (MeOH) λ_{max} (log e) 204 (3.96) nm; IR (KBr) ν_{max} 3600−3100 (br), 2920, 2850, 1770, 1645, 1540, 1455, 1250, 1210 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRFABMS *m/z* 543.2366 $[M + Na]^+$ (calcd for $C_{26}H_{41}O_7SNa_2$, 543.2368).

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