Halisulfate 7, a New Sesterterpene Sulfate from a Sponge, *Coscinoderma* sp.

Xiong Fu,[†] Maria L. G. Ferreira,[†] Francis J. Schmitz,*[,†] and Michelle Kelly[‡]

Department of Chemistry and Biochemistry, University of Oklahoma, Norman, Oklahoma 73019, and Department of Civil and Environmental Engineering, Faculty of Health, Science and Technology, UNITEC Institute of Technology, Private Bag 92025, Auckland, New Zealand

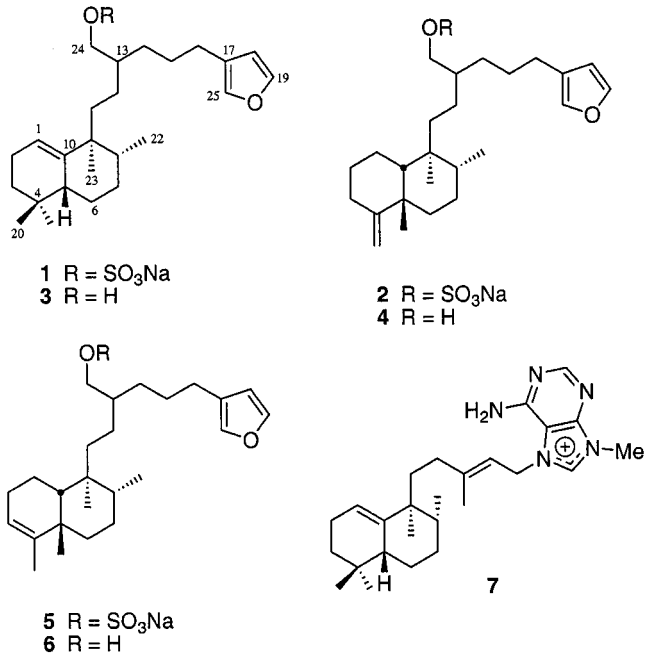
Received February 26, 1999

A new sesterterpene sulfate, halisulfate 7 (**1**), has been isolated from a *Coscinoderma* sp. of sponge collected at Yap, Federated States of Micronesia. The structure was determined from spectroscopic evidence.

Over 100 sulfate-containing compounds have been isolated from marine sources[1,2] such as sponges and echinoderms. Steroidal sulfates[2−4] account for most of these sulfate-containing compounds, although several sesterterpene sulfates have also been reported from sponges.[5−12] Examples of the sesterterpene sulfates include halisulfates 1−6[9] isolated from a sponge of the family Halichondriidae. The halisulfates exhibit antimicrobial activity and inhibitory effects on phospholipase A_2 and PMA-induced inflammation.[9] Most recently, l-methylherbipoline salts of halisulfate-1 and of suvanine have been reported as serine protease inhibitors from the sponge *Coscinoderma mathewsi*.[12] Our investigation of a *Coscinoderma* sp. of sponge collected from Yap led to isolation of a new sesterterpene sulfate, halisulfate 7 (**1**), which is closely related to the halisulfates.[9] In this paper, we report the isolation and structure elucidation of this new member of the sulfated sesterterpene family.

Specimens of *Coscinoderma* sp. were collected from Yap and extracted with MeOH followed by MeOH−CH_2Cl_2 (1:1). The CH_2Cl_2-soluble portion, obtained from solvent partitioning[3] of the combined concentrated extracts, was fractionated on a silica gel open column to yield a mixture of halisulfates 7 (**1**) and 5 (**2**)[13] which could not be separated on reversed-phase HPLC by using mixtures of MeOH and H_2O containing either 0.5 M NH_4OAc or 0.08 M HCO_2NH_4.

The isomeric mixture of halisulfate 7 (**1**) and halisulfate 5 (**2**) (95:5, respectively, by NMR analysis) was obtained as an amorphous powder. The molecular formula $C_{25}H_{39}O_5SNa$ for the isomers was established by HRFABMS. ^1H and ^{13}C NMR data (Table 1) assigned for the major compound **1** by DEPT (135°), COSY, RELAY, HMQC, and HMBC experiments were consistent with this formula. A search for this formula in the Marinlit database[14] revealed that halisulfate 7 (**1**) was an isomer of halisulfates 3−5,[9] and this facilitated the structure elucidation. Thus, the IR, ^1H NMR, and ^{13}C NMR data (Table 1) supported the existence of a 3-substituted furan and a sulfate ester of a primary alcohol. The presence and location of the geminal methyls was evident from HMBC correlations between each of two methyl singlet proton signals (δ 0.82 s and 0.83 s) and C-3 (δ 31.3 t), C-4 (δ 31.2 s), and C-5 (δ 43.6 d). In addition, H's-20 and H's-21 exhibited cross peaks to C-21 and C-20, respectively, in the HMBC spectrum. The olefinic proton (H-1) showed HMBC correlations to C-2, C-3, C-5, and C-9 which supported formulation of the cyclohexene ring. The quaternary methyl proton signal (H-23) showed HMBC correlations to C-8, C-9, C-10, and C- 11, while H's-22 showed correlations with C-7, C-8, and C-9. Thus all of the bicyclic skeleton was accounted for except C-6. With the NMR signals for H/C-7, -8, -9 identified, it was possible to identify H-'s 6 from the correlations to C-8 in the HMBC spectrum and then the signal for C-6 was identified from the HMQC spectrum. With these connectivities firmly established it became clear that the NMR data for the bicyclic portion of **1** matched closely ($\Delta\delta \approx 1$) that of *epi*-agelasine (**7**), an antifouling agent isolated from the sponge *Agelas mauritania*.[15] This provided support for assigning the bicyclic carbon skeleton and relative stereochemistry shown for **1**.



Proton coupling data (COSY) confirmed that the sulfated primary carbon was connected to a methine group, and HMBC data identified the neighboring carbons as C-13 and C-12, -14. HMBC correlations from H-16 to C's-14, -15, -17, -18, and -25 confirmed the side chain structure. The configuration at C-13 is undetermined.

Upon treatment of the mixture of halisulfate 7 (**1**) and 5 (**2**) with 1 N HCl[9] a mixture of alcohols **3** and **6** which could be separated by chromatography was obtained. The major alcohol **3**, corresponding to sulfate **1**, was fully characterized by spectral data (COSY, Relayed COSY, HMQC, HMBC) and complete NMR assignments are reported in Table 1. The minor alcohol product was identified as the known compound **6** by comparison of its FABMS, ^1H NMR,

* To whom correspondence should be addressed. Tel.: (405) 325-5581. Fax: (405) 325-6111. E-mail: fjschmitz@ou.edu.
[†] University of Oklahoma.
[‡] UNITEC Institute of Technology.

10.1021/np990074s CCC: $18.00
Published on Web 08/05/1999

Table 1. NMR Data for Halisulfate 7 (**1**) and Its Corresponding Alcohol (**3**)[a]

position	halisulfate 7 (**1**)		alcohol **3**	
	^{13}C[b]	^{1}H[c] (mult., *J* in Hz)	^{13}C[b]	^{1}H[c] (mult., *J* in Hz)
1	116.8 (d)	5.28 (bs)	116.7 (d)	5.30 (dd)
2	23.2 (t)	1.98 (m)	23.2 (t)	2.00 (m)
3	31.3 (t)	1.07 (m); 1.36 (m)	31.3 (t)	1.08 (m); 1.36 (m)
4	31.2 (s)		31.2 (s)	
5	43.6 (d)	1.49 (m)	43.6 (d)	1.50 (m)
6	30.1 (t)	1.76 (m); 1.05 (m)	30.2 (t)	1.78 (m); 1.04 (m)
7	31.3 (t)	1.50 (m); 1.35 (m)	31.1 (t)	1.50 (m); 1.35 (m)
8	44.6 (d)	1.24 (m)	44.6 (d)	1.25 (m)
9	42.5 (s)		42.5 (s)	
10	146.0 (s)		146.1 (s)	
11	26.7 (t)	1.57 (m)	28.02 (t)	1.00 (m); 1.60 (m)
12	23.9 (t)	1.05 (m)	24.5 (t)	1.05 (m)
13	37.9 (d)	1.64 (m)	41.2 (d)	1.38 (m)
14	29.7 (t)	1.77 (m)	30.6 (t)	1.25 (m); 1.45 (m)
15	26.8 (t)	1.54 (m)	27.2 (t)	1.56 (m)
16	24.9 (t)	2.37 (t, 7.5)	25.0 (t)	2.41 (t, 7.5)
17	125.0 (s)		125.0 (s)	
18	111.1 (d)	6.24 (bs)	110.9 (d)	6.26 (bs)
19	142.5 (d)	7.32 (bs)	142.6 (d)	7.34 (t, 2.6)
20	27.8 (q)	0.82 (s)	27.97 (q)	0.84 (s)
21	27.5 (q)	0.83 (s)	27.5 (q)	0.85 (s)
22	16.5 (q)	0.81 (d, 7.0)	16.4 (a)	0.83 (d, 6.9)
23	23.1 (q)	0.95 (s)	23.1 (q)	0.98 (s)
24	71.9 (t)	3.82 (t, 8.0) 3.94 (dd, 9.5, 5.0)	65.7 (t)	3.47 (dd, 5.3, 10.6) 3.53 (dd, 5.3, 10.6)
25	138.9(d)	7.21 (bs)	138.7 (d)	7.20 (bs)

[a] Spectra were recorded in $CDCl_3$. [b] ^{13}C NMR at 125 MHz, referenced to $CDCl_3$ (δ 77), multiplicities determined by DEPT experiment. [c] ^{1}H NMR at 500 MHz, referenced to residual solvent $CDC1_3$ (δ 7.26).

and ^{13}C NMR data with literature values.[9] Since no signals for the corresponding sulfate **5** (i.e., halisulfate 4[9]) were observed in the original mixture of sulfates, alcohol **6** must arise by rearrangement from **2** and/or **1** during the acid catalyzed hydrolysis.

Experimental Section

General Experimental Procedures. All solvents were redistilled. Merck Si gel 60 (230−240 mesh) was used for vacuum flash chromatography. IR spectra were taken on a Bio-Rad 3240-SPC FT instrument. NMR experiments were conducted with a Varian VXR-500 instrument equipped with a 3 mm $^{1}H/^{13}C$ switchable gradient microprobe (MDG-500-3) and a pulsed field gradient driver; signals are reported in parts per million (δ), referenced to the solvent used. FABMS were measured on a VG ZAB-E mass spectrometer, and optical rotations on a Rudolph Autopol III Automatic Polarimeter.

Animal Material. The sponge was collected from 10 m depth at Gilman Point, Yap Caverns, Yap, Federated States of Micronesia, on July 8, 1995. The sponge was a thickly encrusting mass, 10 cm long and 3 cm thick, with an irregular mounded surface. The surface is conulose, texture rubbery and difficult to tear, oscules are grouped in shallow cavities. The external colour in life is dark grey, the interior beige. The skeleton consists of broad fasculate primary spongin fibres, embedded with sand grains, with a tertiary skeleton of thick, wooley spaghetti-like fibres interconnected occasionally by short thin junctions. The surface and interior is loosely charged with sandgrains visible to the unaided eye. The sample is a undescribed species of *Coscinoderma* (order Dictyoceratida, family Spongiidae). Voucher specimens have been deposited at the Natural History Museum, London, United Kingdom (BMNH 1998.1.27.1), and at the University of Oklahoma (39YA95).

Extraction and Isolation. Freshly thawed specimens of the sponge (173 g wet wt; 44 g dry wt after extraction) were cut into small pieces and extracted with MeOH−CH_2Cl_2 (1:1) twice. All extracts were combined after removal of solvents *in vacuo* and subjected to solvent partitioning as described previously.[3] This gave, after evaporation of solvents under reduced pressure, fractions soluble in hexane (0.46 g), CH_2Cl_2 (3.27 g), and *n*-BuOH (1.34 g). Both the CH_2Cl_2 and *n*-BuOH extracts contained halisulfate type compounds as judged by ^{1}H NMR. Fractionation of the CH_2Cl_2 solubles by flash chromatography over silica gel using increasing amounts of MeOH in CH_2Cl_2 as eluent (10% MeOH/CH_2Cl_2 to MeOH) gave an inseparable mixture (856 mg) of halisulfate 7 (**1**) (∼95%) and halisulfate 5 (**2**) (∼5%).

Mixture of Halisulfate 7 (1) and Halisulfate 5 (2). Amorphous powder; IR (NaCl) ν_{max} 3600−3100, 1720, 1652, 1502, 1460, 1365, 1281 cm^{-1}; ^{1}H and ^{13}C NMR for the major compound **1**, see Table 1; HRFABMS *m/z* $[M + Na]^+$ 497.2345 (calcd for $C_{25}H_{39}O_5SNa_2$, 497.2314).

Hydrolysis of Halisulfates. A 95:5 mixture (200 mg) of halisulfate 7 (**1**) and 5 (**2**) was treated as described by Kernan and Faulkner.[9] After workup, the resultant residue was subjected to vacuum liquid chromatography over silica gel using 5% to 10% EtOAc−hexane as eluent to afford the alcohols **3** (102.6 mg) and **6** (6.2 mg).

Alcohol 3. Colorless oil, $[\alpha]_D$ −305.4° (*c* 0.83, $CHCl_3$); IR (NaCl) ν_{max} 3370 (br), 1651, 1562, 1502, 1460, 1380, 1163, 1025 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; FABMS *m/z* 373 (9%) $[M + H]^+$, 355 (3%) $[M - OH]^+$, 191 (100%) [M − side chain]$^+$.

Alcohol 6. Colorless oil, $[\alpha]_D$ +5.9° (*c* 0.29, $CHCl_3$); FABMS *m/z* 373 (17%), 355 (4%), 191 (100%) [M − side chain]$^+$.

Acknowledgment. This work was supported by National Cancer Institute Grant No. CA 52955. We thank the government of Yap, Federated States of Micronesia, for permission to collect specimens and the Coral Reef Research Foundation for assistance in the collection.

References and Notes

(1) Faulkner, D. J. *Nat. Prod. Reports* **1998**, *15*, 113−158 and previous reviews in this series.
(2) D'Auria, M. V.; Minale, L.; Riccio, R. *Chem. Rev.* **1993**, *93*, 1839−1895.
(3) Fu, X.; Schmitz, F. J.; Lee, R. H.; Papkoff, J. S.; Slate, D. L. *J. Nat. Prod.* **1994**, *57*, 1591−1594.
(4) For recent examples, see: (a) Sperry, S.; Crews, P. *J. Nat. Prod.* **1997**, *60*, 29−32. (b) Iorizzi, M.; De Marino, S.; Minale, L.; Zollo, F.; Le Bert, V.; Roussakis, C. *Tetrahedron* **1996**, *52*, 10997−10110. (c) Patil, A. D.; Freyer, A. J.; Breen, A.; Carte, B.; Johnson, R. K. *J. Nat. Prod.* ***1996***, *59*, 606−608. (d) Roccatagliata, A. J.; Maier, M. S.; Seldes, A. M.; Pujol, C. A.; Damonte, E. B. *J. Nat. Prod.* ***1996***, *59*, 887−889.
(5) De Rosa, S.; Milone, A.; De Giulio, A.; Crispino, A.; Iodice, C. *Nat. Prod. Lett.* **1996**, *8*, 245−251.
(6) De Rosa, S.; De Giulio, A.; Crispino, A.; Iodice, C.; Tommonaro, G. *Nat. Prod. Lett.* **1997**, *10*, 7−12.
(7) Coll, J. C.; Kearns, P. S.; Rideout, J. A.; Hooper, J. *J. Nat. Prod.* **1997**, *60*, 1178−1179.
(8) Manes, L. V.; Crews, P.; Kernan, M. R.; Faulkner, D. J.; Fronczek, F. R.; Gandour, R. D. *J. Org. Chem.* **1988**, *53*, 570−573.
(9) Kernan, M. R.; Faulkner, D. J. *J. Org. Chem.* **1988**, *53*, 4574−4578.
(10) Müller, E.; Faulkner, D. J. *Tetrahedron* **1997**, *53*, 5373−5378.
(11) Wright, A. E.; McCarthy, P. J. *J. Org. Chem.* **1989**, *54*, 3472−3474.
(12) Kimura, J.; Ishizuka, E.; Nakao, Y.; Yoshida, W. Y.; Scheuer, P. J.; Kelly-Borges, M. *J. Nat. Prod.* **1998**, *61*, 248−250.
(13) The presence of halisulfate 5 (**2**) was indicated by two broad singlets in the ^{1}H NMR spectrum at around 4.70 ppm typical for the *exo*-methylene protons,[9] although the rest of ^{1}H NMR signals were immersed in the signals of the major compound **1**. The ^{13}C NMR signals for halisulfate 5 (**2**) were not observed due to the small percentage of **2**.
(14) *Marinlit*, marine natural products database by J. W. Blunt and M. H. G. Munro, Department of Chemistry, University of Canterbury, Christchurch, New Zealand.
(15) Hattori, T.; Adachi, K.; Shizuri, Y. *J. Nat. Prod.* **1997**, *60*, 411−413.

NP990074S