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Absolute Configuration of Halisulfate 3 from the Sponge *Ircinia* sp.

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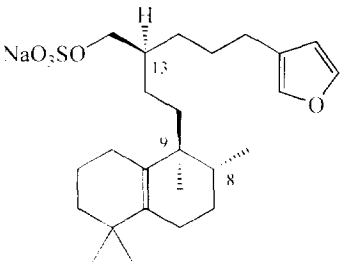
Abstract: The absolute configuration of halisulfate 3 (**1**), a metabolite of the sponge *Ircinia* sp. from the Philippines, was determined in two steps. The (13*S*) absolute configuration was derived by application of the chiral amide method using acid **5**. The (8*R*,9*S*) stereochemistry of the bicyclic ring system was obtained by comparison of the optical rotation of the degradation product **8** with that of an ester **9** of known absolute configuration. © 1997 Elsevier Science Ltd.

In 1988, we reported the isolation of the halisulfates from a Californian sponge of the family Halichondriidae.[1] At that time, halisulfates 3 (**1**) and 4 (**2**) were isolated as an inseparable 1:1 mixture.



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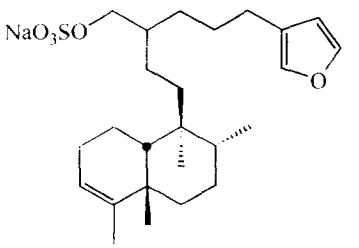
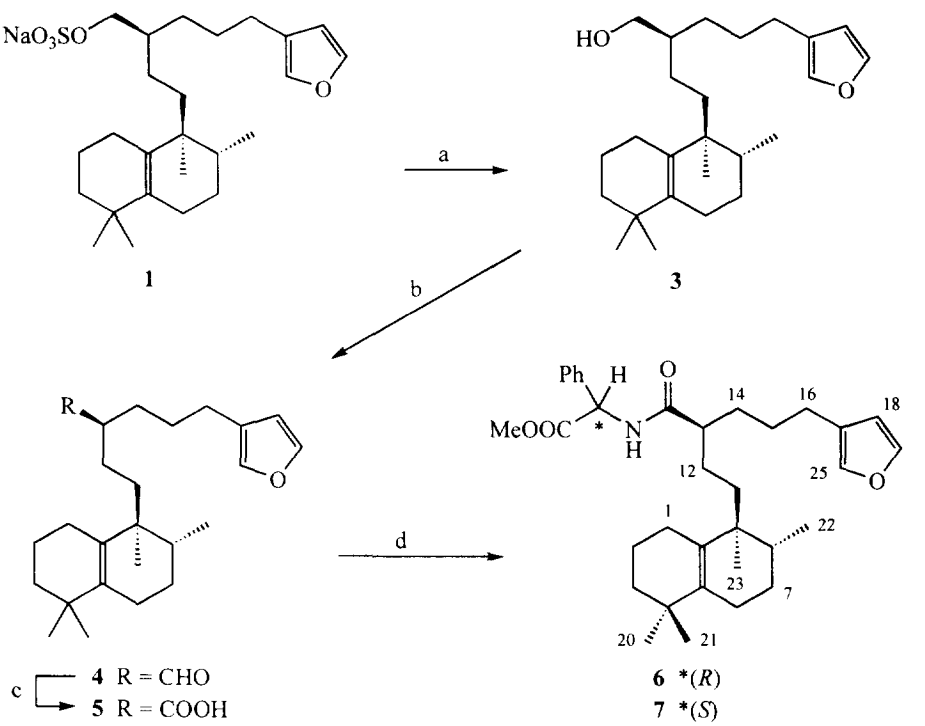


2

The relative stereochemistry about the bicyclic ring system of halisulfate 3 (**1**) was reported but the stereochemistry at C-13 remained unknown, as did the absolute configuration. At that time, there was no simple method available to determine the absolute or relative stereochemistry at a remote chiral center on a hydrocarbon chain. With the advent of high field NMR methods, originally suggested by Mosher,[2] to determine the absolute configuration of secondary alcohols[3] or of acids,[4] it became relatively simple to determine the absolute stereochemistry of natural products. In this paper, we present a general strategy for the determination of the absolute stereochemistry at a chiral center adjacent to a primary alcohol which was used

to determine the absolute configuration at C-13 of halisulfate 3 (**1**). Since we were not able to relate the stereochemistry at C-13 to that of the bicyclic ring system by cyclization or other methods, we had to determine the absolute stereochemistry at C-8 and C-9 by comparison of the optical rotation of a derivative of halisulfate 3 (**1**) with that of an analogue of known absolute configuration.



Scheme 1. a) 0.01M H_2SO_4, Et_2O, 2 h (30%). b) Periodinane (1.3 eq.), CH_2Cl_2, 30 min. c) $NaClO_2$, pH 7 buffer, 2-methyl-2-butene, 4 °C, 15 h (90%). d) (*R*)- or (*S*)-PGME.HCl, PyBOP (1.4 eq.), HOBT (1.4 eq.), *N*-methylmorpholine (cat.), 3 h. (90%).

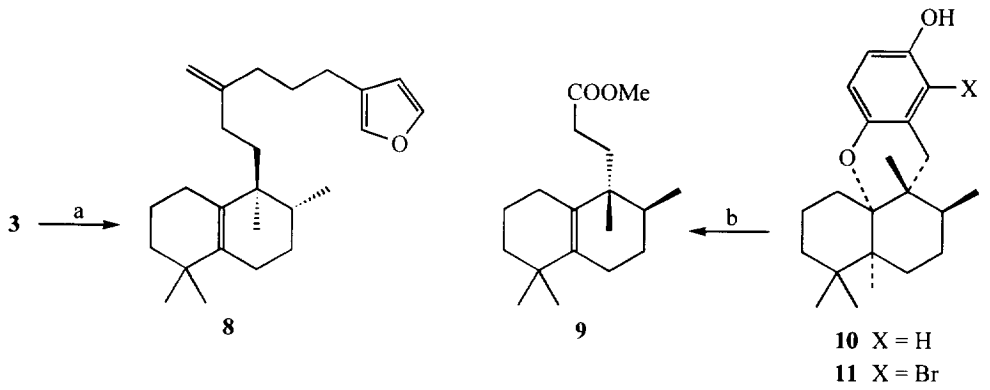
Using established chromatographic methods,[1] halisulfate 3 (**1**) was isolated as a major metabolite from a specimen of *Ircinia* sp. (NCI 1556) from Bisucay Island in the Philippines. The isolated material had identical spectral data to those of the authentic material. Acid catalyzed hydrolysis of halisulfate 3 (**1**) yielded the corresponding alcohol **3** (Scheme 1), that also showed identical spectral data to those of authentic material and had the same optical rotation, $[\alpha]_D = +55.2°$ (c 0.84, $CHCl_3$).[1] The alcohol **3** was oxidized with the Dess-Martin periodinane reagent[5] to obtain the aldehyde **4** that was subsequently converted into the acid **5** using buffered sodium perchlorate (Scheme 1).[6] The acid **5** was reacted with both (*R*)- and (*S*)-phenylglycine

Table 1. Selected ^{1}H NMR ($CDCl_3$, 500 MHz) data for the (*R*)- and (*S*)-PGME amides **6** and **7**.

H#	δ_R (**6**)	mult, *J*(Hz)	δ_S (**7**)	mult, *J*(Hz)	$\Delta\delta_{(S-R)}$
25	7.199	s	7.108	s	-0.091
19	7.332	s	7.311	s	-0.022
18	6.246	br s	6.155	br s	-0.091
16	2.390	t, 7.5	2.320	t, 7.5	-0.07
14	1.458	m	1.415	m	-0.043
23	0.667	s, 3 H	0.768	s, 3 H	+0.101
22	0.743	d, 3 H, 6.5	0.795	d, 3 H, 6.5	+0.052
21	0.891	s, 3 H	0.924	s, 3 H	+0.033
20	0.914	s, 3 H	0.943	s, 3 H	+0.029
12	1.58	m	1.59	m	+0.01
7	1.626	m	1.693	m	+0.067
1	1.885	m	1.937	m	+0.052

methyl ester (PGME) to form the corresponding amides **6** and **7**. The ^{1}H NMR spectra of amides **6** and **7** indicated that isomerization had not occurred during the reaction sequence. Application of Mosher's method (Table 1) revealed the (13*S*) configuration of halisulfate 3 (**1**).

After confirming the relative stereochemistry at C-8 and C-9 in alcohol **3** by NOEDS experiments, we found that we could not form any cyclized derivative that would relate these centers to C-13 and therefore had to independently determine the absolute stereochemistry about the bicyclic ring system. During their studies of the absolute configuration of ilimaquinone,[7] Capon and MacLeod had cautioned against predicting the absolute configuration of similar compounds using weak CD measurements, such as those recorded for halisulfate 3 and its derivatives. In order to determine the absolute configuration about the bicyclic ring system, we had first to remove the chiral center at C-13. After attempting to decarboxylate the acid **5** by various methods,[8,9] we found that dehydration of the alcohol **3** occurred relatively cleanly, albeit in modest yield. Acid catalyzed dehydration of alcohol **3** gave the olefin **8**, $[\alpha]_D$ = +44.8° (*c* 0.21, $CHCl_3$), with an optical rotation of opposite sign to that of ester **9**, $[\alpha]_D$ = -48.4° (*c* 0.4, $CHCl_3$), which had been obtained by degradation of aureol (**10**),[10] the absolute configuration of which had been determined by X-ray analysis of a brominated derivative **11**.[11] Thus the absolute configuration of halisulfate 3 (**1**) is (8*R*,9*S*,13*S*).



Scheme 2. a) H_2SO_4 (0.1 eq.), Et_2O, 4 h. (24%). b) see reference 11.

Experimental Section

General Methods: Melting points were determined on a MEL-TEMP apparatus and are uncorrected. Optical rotations were measured on a Rudolph Research Autopol III polarimeter. IR spectra were recorded on a Perkin Elmer 1600 FT-IR spectrometer. NMR spectra were obtained on Varian Unity 500 or Gemini 400 spectrometers; ^1H NMR spectra were referenced to TMS (δ = 0 ppm) and ^{13}C NMR spectra were referenced to $CDCl_3$ (77.0 ppm) or MeOH-d_4 (49.0 ppm). Low resolution electron impact mass spectra were measured on a Hewlett Packard 5988A instrument. HRCIMS data were obtained from the UC Riverside Regional Facility. All solvents were distilled prior to use.

Collection and Extraction: The brown sponge, *Ircinia* sp.(400 g), was collected by hand using SCUBA (-10 m) near Bisucay Island, the Philippines, and was immediately frozen. The frozen sponge was diced and soaked in MeOH (750 mL) for 48 hours. The MeOH extract was evaporated to obtain an aqueous suspension that was extracted with di-isopropyl ether (3 x 150 mL). The organic fraction was chromatographed on Sephadex LH-20 using 1:1 MeOH/CH_2Cl_2 as eluant to obtain five major fractions. The fourth fraction was rechromatographed on silica gel (230-400 mesh, 40-63 μm) using 30% EtOAc in hexane as eluant to obtain halisulfate 3 (**1**, 132.4 mg, 0.033% dry wt.).

Halisulfate 3 (1): white solid, mp. 162-166 °C; IR (film) 3600-3200, 1640, 1455, 1190, 1075 cm^{-1}; ^1H NMR (500 MHz, MeOH-d_4) δ 7.32 (s, 1 H), 7.21 (s, 1 H), 6.24 (s, 1 H), 3.86 (m, 2 H), 2.34 (t, 2 H, J = 7 Hz), 0.93 (s, 3 H), 0.91 (s, 3 H), 0.80 (d, 3 H, J = 7.5 Hz), 0.77 (s, 3 H); ^{13}C NMR (125 MHz, MeOH-d_4) δ 143.9, 140.0, 138.0, 134.2, 126.3, 111.9, 71.9, 41.7, 41.1, 39.8, 35.4, 34.9, 34.3, 31.6, 29.6, 28.4, 28.3, 28.1, 26.9, 26.3, 26.2, 25.9, 21.7, 21.0, 16.6; EIMS, *m/z* 371 $[M\text{-}SO_3Na]^+$, 261, 191 (100%).

Hydrolysis of halisulfate 3: Concentrated sulfuric acid (18 μL) and water (8 μL) were added to a stirred solution of halisulfate 3 (**1**, 120 mg, 0.25 mmol) in Et_2O (15 mL). After 2 hours, the reaction was quenched by addition of satd. $NaHCO_3$ (5 mL) and extracted with $CHCl_3$ (3 x 10 mL). The crude reaction mixture was chromatographed on silica gel using $CHCl_3$ as eluant to obtain alcohol **3** as a colorless oil that had identical IR, ^1H NMR, ^{13}C NMR and MS data to those measured previously.[1]

Preparation of PGME amides 6 and 7: The Dess-Martin periodinane reagent (15 mg, 0.035 mmol) was added to a stirred solution of the alcohol (**3**, 10 mg, 0.027 mmol) in CH_2Cl_2 at 25 °C. After 30 min., the reaction was quenched by addition of satd. $NaHCO_3$ (5 mL) and $Na_2S_2O_3$ (7-fold excess). The organic layer was separated, dried over Na_2SO_4 and concentrated to obtain the aldehyde **4**, that was used without further purification. A solution of the aldehyde **4** in 2-methyl-2-butene (1.2 mL) and *t*-butanol (5 mL) was cooled to 5°C and an ice-cold 1.1 M solution of $NaClO_2$ in pH 7 buffer was added with stirring. After 14 hours, the reaction was quenched by addition of a pH 2 phosphate buffer (5 mL) and extracted with $CHCl_3$ (3 x 5 mL). The $CHCl_3$ extract was dried over Na_2SO_4 and the solvent evaporated to obtain the acid **5** (9.4 mg, 90% overall yield), which was again used without further purification. This procedure was duplicated with identical results. To a stirred solution of the acid **5** (9.4 mg, 0.025 mmol) and either (*R*)- or (*S*)-phenylglycine methyl ester (PGME, Aldrich, 5.0 mg, 0.03 mmol) in dry DMF (1 mL) at 5 °C were added benzotriazolyloxytri(pyrrolidinyl)phosphonium hexafluorophosphate (PyBOP, 15.6 mg, 0.03 mmol), 1-hydroxybenzotriazole (HOBT, 4.6 mg, 0.03 mmol) and *N*-methylmorpholine (9.1 mg, 0.09 mmol). The reaction mixture was allowed to warm to room temperature and stirring was continued for 3 hours, after which time benzene (10 mL) and EtOAc (20 mL) were added. The organic phase was washed successively with 1N HCl (10 mL), satd. $NaHCO_3$ (10 mL) and brine (10 mL). The crude amide was chromatographed on silica gel using $CHCl_3$ as eluant to obtain two major fractions, the second of which was the amide [*R* (**6**) - 6.4 mg, 48%; *S* (**7**) - 6.5 mg, 49%].

(*R*)-PGME amide 6: colorless oil; IR (film) 3600-3200, 1745, 1655, 1455, 1215, 1175, 1075 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.35 (m, 5 H), 7.33 (s, 1 H), 7.20 (s, 1 H), 6.43 (d, 1 H, J = 7 Hz), 6.25 (s, 1 H), 5.59 (d, 1 H, J = 7 Hz), 3.78 (s, 3 H), 2.39 (t, 2 H, J = 7 Hz), 0.91 (s, 3 H), 0.89 (s, 3 H), 0.74 (d, 3 H, J = 7.5 Hz), 0.67 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.0, 171.5, 142.9, 139.0, 136.9, 136.7, 132.4, 128.9, 128.5, 127.4, 125.1, 111.1, 56.1, 52.8, 48.1, 40.4, 39.9, 34.6, 34.0, 33.5, 32.4, 29.8, 29.0, 27.9, 27.6, 27.3, 27.1, 25.5, 24.9, 21.1, 19.9, 15.9; EIMS, *m/z* 533.3 $(M)^+$, 353 $(M-C_{10}H_{10}NO_3)^+$.

(*S*)-PGME amide 7: colorless oil; IR (film) 3600-3200, 1745, 1655, 1455, 1215, 1175, 1075 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.33 (m, 5 H), 7.31 (s, 1 H), 7.11 (s, 1 H), 6.37 (d, 1 H, J = 7 Hz), 6.16 (s, 1 H), 5.61 (d, 1 H, J = 7 Hz), 3.73 (s, 3 H), 2.32 (t, 2 H, J = 7 Hz), 0.94 (s, 3 H), 0.92 (s, 3 H), 0.80 (d, 3 H, J = 7.5 Hz), 0.77 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.0, 171.4, 142.6, 138.8,

136.8, 136.7, 132.4, 128.9, 128.7, 127.4, 110.9, 56.1, 52.7, 48.1, 40.4, 39.9, 34.4, 34.0, 33.5, 32.5, 29.7, 29.1, 27.8, 27.7, 27.4, 27.2, 25.6, 25.0, 21.2, 19.9, 16.1; EIMS, *m/z* 533.3 $(M)^+$, 353 $(M-C_{10}H_{10}NO_3)^+$.

Dehydration of alcohol 3: Concentrated sulfuric acid (35 μL) was added to a solution of the alcohol **3** (30 mg, 0.081 mmol) in Et_2O (25 mL) at 25 °C. After 2 hours, the reaction was quenched by addition of satd. $NaHCO_3$ (15 mL) and extracted with $CHCl_3$ (3 x 10 mL). The combined organic phases were dried over Na_2SO_4 and concentrated. The crude reaction mixture was chromatographed on silica gel using 15% EtOAc in hexane as eluant to obtain the olefin **8** (4.8 mg, 17%) in the first major fraction.

Olefin 8: colorless oil; $[\alpha]_D$ = +44.8 (c 0.21, $CHCl_3$); IR (film) 1460, 1220, 900 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.33 (s, 1 H), 7.20 (s, 1 H), 6.25 (s, 1 H), 5.06 (br s, 1 H), 5.05 (br s, 1 H), 2.38 (t, 2 H, *J* = 7 Hz), 0.95 (s, 3 H), 0.93 (s, 3 H), 0.79 (d, 3 H, *J* = 7.5 Hz), 0.77 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 142.6, 138.7, 136.5, 132.5, 130.9, 128.8, 125.1, 111.0, 40.9, 40.0, 34.4, 33.5, 33.4, 32.3, 30.4, 29.7, 29.2, 27.7, 27.3, 27.2, 25.7, 25.2, 23.6, 20.0, 16.2; EIMS, *m/z* 354 $[M]^+$, 353 $[M-H]^+$, 191 ($C_{14}H_{23}$, 100%); HRCIMS, obsd. *m/z* 355.2969, $C_{25}H_{39}O$ $[M+H]^+$ requires 355.3001.

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