Hipposulfates A and B, New Sesterterpene Sulfates from an Okinawan Sponge, *Hippospongia* cf. *metachromia*

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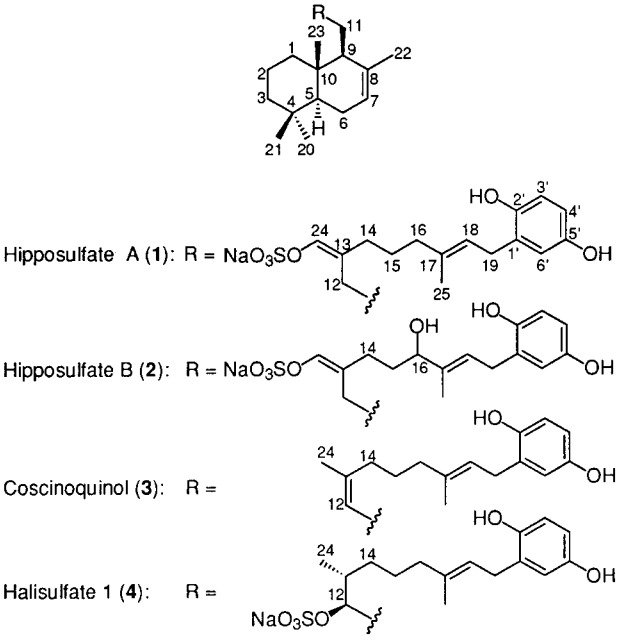
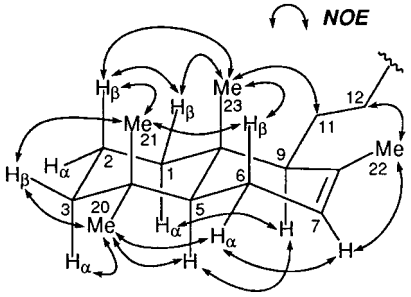
Two new sesterterpene sulfates, hipposulfates A (**1**) and B (**2**), have been isolated from an Okinawan sponge, *Hippospongia* cf. *metachromia* and their structures elucidated by interpretation of spectroscopic data. Both compounds contain an enolsulfate functionality. Hipposulfate A (**1**) showed moderate cytotoxicity.

In our search for bioactive compounds from marine organisms, we isolated two new merosesterterpene sulfates from a sponge, *Hippospongia* cf. *metachromia*, Spongiidae, in the late 1980s. One of the compounds showed cytotoxicity. However, we were unable to determine the structures at that time. We recently reexamined these compounds and have now established unambiguous structures for hipposulfates A (**1**) and B (**2**). Meroterpenes have been reported in abundance from marine organisms,[1] but merosesterterpenes are relatively rare. Related examples include coscinoquinol (**3**) from *Coscinoderma* sp.[2] and halisulfate 1 (**4**) from a species of the family Halichondriidae.[3] From a species of *Hippospongia*, the merosesquiterpenes dictyoceratins A and B[4] and metachromins A and B[5] were reported, but no merosesterterpenes have been described. In this paper we now report the isolation and structure elucidation of **1** and **2**.



A sample (700 g, wet weight) of *H.* cf. *metachromia*[6] was extracted with acetone, and the concentrated extract was partitioned between chloroform and water. A part (5.5 g) of the chloroform soluble material was separated by column chromatography on silica gel followed by successive chromatography on ODS Lobar and HPLC to afford hipposulfates A (**1**, 54 mg) and B (**2**, 6 mg) as colorless oils.

The molecular formula $C_{31}H_{45}NaO_6S$ of hipposulfate A (**1**) was determined by high-resolution FABMS ($[M + Na]^+$ *m/z* 591.2691 Δ −4.1 mmu). The IR bands at 3100−3700 (broad) and 1220 (strong) cm^{-1} suggested the presence of hydroxyl and sulfate groups, respectively. The ^1H and ^{13}C NMR exhibited six olefinic proton and 12 sp^2 carbon signals that could be assigned to a 2-substituted 1,4-hydroquinone [δ 6.43 (dd, J = 8, 3 Hz; H-4′), 6.54 (d, J = 3 Hz; H-6′), 6.58 (d, J = 8 Hz; H-3′)] and three trisubstituted olefins [δ_H 5.33 (2H, brs; H-7, H-18), 6.37 (brs; H-24)]. Two partial structures (**a**, **b**) were deduced from the COSY and HMBC correlations as shown in Figure 1. The HMBC data are given in Table 1. The COSY data observed are as follows: **a** [H_2-2/H_2-1, H_2-3; H-5/H_2-6, H_3-21; H-7/H-6α, H_3-22; H_2-11/H_2-12] and **b** [H_2-15/H_2-14, H_2-16; H-18/H_2-16, H_2-19, H_3-25; H_2-19/H-6′]. The *E* geometry of Δ^{17} was determined by NOE observation (H_3-25/H_2-19; H-16/H-18) and ^{13}C chemical shift for olefinic methyl (δ 16.1 for H_3-25). The relative stereochemistry of the partial structure **a** was deduced as follows. The ^1H NMR spectrum of **1** showed large vicinal coupling constants for H-1α/H-2β (J = 13 Hz), H-3α/H-2β (J = 13 Hz), H-5/H-6β (J = 12 Hz) and *W*-type long-range coupling between H-5 and H_3-21; H-1α and H_3-23, suggesting that each pair existed in *anti* diaxial orientation. The NOESY cross-peaks were consistent with these orientations (**1a**). The H-9 showed NOESY correlations to H-1α and H-5, also indicating the axial orientation of H-9. The relative stereochemistry of this portion of the molecule has thus been determined as shown in **1**. The partial structures **a** and **b** are found in the known coscinoquinol (**3**)[2] and halisulfate 1 (**4**).[3] The ^{13}C NMR data for



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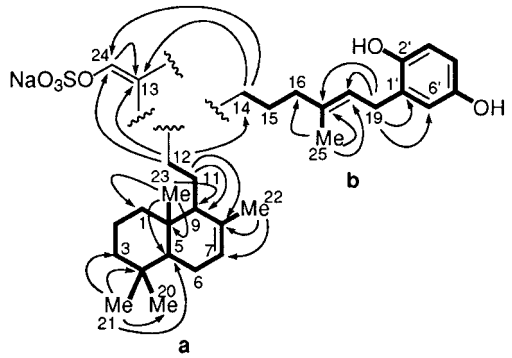

Figure 1. Partial structures (bold line) and HMBC correlations (arrow) for hipposulfate A (**1**).

a and **b** were superimposable with those reported for the corresponding moieties of **3** and **4**, as shown in Table 1. The remaining C_2HNaO_4S unit [C-13 (δ 126.4), C-24 (δ 134.1), H-24 (δ 6.37)] could be assigned as an enolsulfate. The HMBC data (H_2-12/C-13,C-14,C-24; H_2-14/C-12,C-13,C-24; H-24/C-12,C-13,C-14) connected the partial structures, concluding the structure of hipposulfate A as shown in **1**. The observation of NOE correlation between H-24/H_2-14 revealed the geometry of the enolsulfate moiety.

Hipposulfate B (**2**) has the molecular formula $C_{31}H_{45}NaO_7S$ based on its high-resolution FABMS data, which differed from **1** by 16 mass units. Analyses of the ^{1}H and ^{13}C NMR spectra (Table 2) indicated that **2** has a structure similar to that of **1**, except for the presence of an additional hydroxyl group at C-16. The HMBC connectivity from the carbinyl proton, H-16 (δ 3.97), to C-14, C-15, C-17, and C-18 and NOE correlation of H-24 (δ 6.38) to H_2-14 (δ 1.94, 2.00) supported this structure. The stereochemistry at C-16 remains to be solved.

Hipposulfate A (**1**) was cytotoxic to P388, A-549, HT-29, and MEL-28 cells, with an IC_{50} of 2 μg/mL.

Experimental Section

General Experimental Procedures. The IR spectra were measured using a Hitachi infrared spectrophotometer 260-10 and a JASCO FT/IR-300. The UV spectra were obtained in methanol using a JASCO UVIDEC 610 spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a JEOL EX-270, a JEOL α-500, and a GE QE-500 spectrometer. Merck silica gel 60 H was used for vacuum liquid chromatography (VLC). HPLC and medium-pressure liquid chromatography columns were Nacalai Cosmosil 5C18-AR and Lobar LiChroprep RP-18, respectively.

Extraction and Isolation. A sample (700 g wet weight) of *Hippospongia* cf. *metachromia* was collected by scuba at Hedo, Okinawa, in 1987, and extracted with acetone. The extract was concentrated and the residue was partitioned between chloroform and water. The chloroform layer was concentrated under reduced pressure to give an oil (9.5 g). A portion (5.5 g) of the oil was separated by silica gel VLC using

Table 1. NMR Data for Hipposulfate A (**1**) and Comparison with ^{13}C NMR Data of Coscinoquinol (**3**) and Halisulfate 1 (**4**) in CD_3OD

	1				**3**[2]	**4**[3]
no.	^{13}C NMR (mult.)[a]	^{1}H NMR	(mult., *J* in Hz)[b]	HMBC (H → C)	^{13}C NMR	^{13}C NMR
1α	δ 40.3 (t)	δ 0.98	(td, 13, 4)	2α,β, 3α,β, 23	δ 40.8	δ 39.8
β		1.89	(brd, 13)			
2α	19.8 (t)	1.41	(dquint, 13, 4)	1α, 3α	19.9	19.9
β		1.53	(m)			
3α	43.5 (t)	1.16	(td, 13, 4)	20, 21	43.5	43.3
β		1.38	(brd, 13)			
4	33.7 (s)			2α, 3α,β, 5, 20, 21	33.8	33.6
5	51.4 (d)	1.17	(dd, 12, 5)	3, 20, 21, 23	51.7	52.0
6α	24.8 (t)	1.93	(m)	5	24.8	24.9
β		1.84	(m)			
7	122.7 (d)	5.33	(brs)	22	123.3[c]	123.9
8	136.8 (s)			9, 11a, 22	136.8[d]	136.8
9	56.2 (d)	1.60	(m)	1α, 5, 11a,b, 12a,b, 23	56.9	49.3
10	37.9 (s)			1α, 2α,β, 5, 9, 11b, 23	38.0	37.4
11a	26.5 (t)	1.25	(m)	12a,b	26.6	27.7
b		1.55	(m)			
12a	30.9 (t)	2.05	(m)	9, 11b, 14, 24	128.7	85.7
b		2.31	(td, 13, 5)			
13	126.4 (s)			12a,b, 14, 24	134.8	38.2
14	32.2 (t)	1.96	(t, 7)	12a,b, 15, 16, 24	32.6	34.3
15	27.3 (t)	1.55	(quint, 7)	14, 16	27.0	27.4
16	40.3 (t)	2.04	(t, 7)	14, 18, 25	41.0	41.0
17	136.6 (s)			15, 16, 19, 25	136.5[d]	136.7
18	124.1 (d)	5.33	(brs)	16, 19, 25	124.1[c]	124.8
19	29.1 (t)	3.24	(brd, 7)	18, 6′	29.1	29.2
20	33.8 (q)	0.84	(s)	3α,β, 5, 21	33.8	33.7
21	22.3 (q)	0.87	(s)	20	22.4	22.5
22	22.6 (q)	1.75	(s)		22.9	23.0
23	14.0 (q)	0.74	(s)	5	14.5	14.2
24	134.1 (d)	6.37	(brs)	12a,b, 14	23.7	13.7
25	16.1 (q)	1.68	(s)	16, 18	16.2	16.3
1′	130.1 (s)			19, 3′	130.2	130.1
2′	148.9 (s)			3′, 4′, 6′	149.0	148.6
3′	116.5 (d)	6.58	(d, 8)	4′	116.5	117.5
4′	113.8 (d)	6.43	(dd, 8, 3)	3′, 6′	113.9	114.8
5′	151.0 (s)			3′, 4′, 6′	151.1	150.8
6′	117.0 (d)	6.54	(d, 3)	19, 4′	117.1	118.0

[a] *C*D_3OD (49.0 ppm) signal was used as internal standard for ^{13}C (67.5 MHz) NMR. [b] CD_2*H*OD (3.30 ppm) signal was used as internal standard for ^{1}H NMR (500 MHz). [c,d] Interchangeable signals.

Table 2. NMR Data for Hipposulfate B (**2**) in CD_3OD

no.	^{13}C NMR (mult.)[a]	^{1}H NMR (mult., *J* in Hz)[b]	HMBC (H → C)	NOESY (NOEDS)
1α	*δ* 40.3 (t)	*δ* 0.98 (td, 13, 3)	2α, 23	1*β*
β		1.90 (brd, 13)	1α, 2*β*, 23	
2α	19.8 (t)	1.41 (dquint, 13, 3)		2*β*, 3α
β		1.54 (m)		1*β*, 2α, 21, 23
3α	43.5 (t)	1.17 (td, 13, 3)	20, 21	2α, 3*β*, 20
β		1.38 (brd, 13)	3α, 20, 21	
4	33.9 (s)		2α, 3α, 5, 20, 21	
5	51.5 (d)	1.17 (dd, 12, 5)	3, 6α,*β*, 7, 20, 21, 23	9, 20
6α	24.9 (t)	1.94 (m)	5, 7	6*β*, 7, 20
β		1.85 (m)		6α, 21, 23
7	122.6 (d)	5.33 (brs)	22	6α, 22
8	136.9 (s)		11a,b, 22	
9	56.2 (d)	1.60 (m)	5, 7, 11a,b, 12a,b, 22, 23	5
10	37.9 (s)		1α,*β*, 5, 11b, 23	
11a	26.5 (t)	1.26 (qd, 12, 7)	12a,b	11b
b		1.54 (m)		11a
12a	30.9 (t)	2.07 (ddd, 12, 11, 7)	11, 14, 24	12b
b		2.31 (td, 12, 5)		12a
13	126.1 (s)		12a,b, 14, 24	
14a	29.1 (t)	1.94 (dd, 15, 7)	12a,b, 16, 24	(24)
b		2.00 (dd, 15, 9)		(24)
15	34.3 (t)	1.65 (m)	14, 16	
16	78.1 (d)	3.97 (t, 7)	14, 18, 25	18
17	138.5 (s)		16, 19, 25	
18	126.1 (d)	5.57 (t, 7)	16, 19, 25	16
19	29.1 (t)	3.28 (d, 7)	18, 6′	6′
20	33.7 (q)	0.84 (s)		3α,*β*, 5, 6α
21	22.3 (q)	0.88 (s)	3α, 5, 20	2*β*, 3*β*, 6*β*
22	22.7 (q)	1.75 (s)	7	7
23	14.0 (q)	0.74 (s)	1α, 5	1*β*, 2*β*, 6*β*
24	134.3 (d)	6.38 (brs)	12a,b, 14	(14a,b)
25	11.4 (q)	1.69 (s)	16, 18	
1′	129.9 (s)		18, 19, 3′	
2′	149.0 (s)		3′, 4′, 6′, 19	
3′	116.6 (d)	6.58 (d, 9)		
4′	114.1 (d)	6.43 (dd, 9, 3)	3′	
5′	151.2 (s)		3′, 4′	
6′	117.3 (d)	6.56 (d, 3)	19	19

[a] *C*D_3OD (49.0 ppm) signal was used as internal standard for ^{13}C (67.5 MHz) NMR. [b] CD_2*H*OD (3.30 ppm) signal was used as internal standard for ^{1}H NMR (500 MHz).

CH_2Cl_2−EtOAc (6:1), CH_2Cl_2−EtOAc (1:1), CH_2Cl_2−MeOH (10:1), and MeOH as eluent. The third fraction (1.59 g) was separated by medium-pressure liquid chromatography (MeOH−H_2O, 10:1) followed by reversed-phase HPLC on ODS (MeOH−H_2O, 4:1, CH_3CN−H_2O, 2:3, CH_3CN−H_2O, 1:1) to afford hipposulfates A (**1**, 54 mg) and B (**2**, 6 mg).

Hipposulfate A (1): colorless oil; $[\alpha]_D$ +8.1° (*c* 0.31, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (4.08), 293 nm (3.23); IR (KBr) 3100−3700, 2920, 1630, 1460, 1220, 1040, 810 cm^{-1}; ^{1}H and ^{13}C NMR data are shown in Table 1; FABMS *m*/*z* 591 ([M + Na]$^+$), 489 ([M + Na − SO_3Na + H]$^+$); HRFABMS *m*/*z* 591.2691 ([M + Na]$^+$, calcd for $C_{31}H_{45}Na_2O_6S$ 591.2732, Δ −4.1 mmu).

Hipposulfate B (2): colorless oil; $[\alpha]_D$ +36.8° (*c* 0.07, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (4.06), 293 nm (3.24); IR (KBr) 3100−3700, 2940, 1640, 1460, 1220, 1040, 810 cm^{-1}; ^{1}H and ^{13}C NMR data are shown in Table 2; HRFABMS *m*/*z* 607.2667 ([M + Na]$^+$, calcd for $C_{31}H_{45}Na_2O_7S$ 607.2682, Δ −1.5 mmu).

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References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **2000**, *17*, 7−55, and previous reports in this series.
(2) Alea, G. V.; Carroll, A. R.; Bowden, B. F. *Aust. J. Chem.* **1994**, *47*, 191−194.
(3) Kernan, M. R.; Faulkner, D. J. *J. Org. Chem.* **1988**, *53*, 4574−4578.
(4) Nakamura, H.; Deng, S.; Kobayashi, J.; Ohizumi, T.; Hirata, Y. *Tetrahedron* **1986**, *42*, 4197−4201.
(5) Ishibashi, M.; Ohizumi, Y.; Cheng, J.; Nakamura, H.; Hirata, Y.; Sasaki, T.; Kobayashi, J. *J. Org. Chem.* **1988**, *53*, 2855−2858.
(6) Taxonomic examination of the sponge was carried out by the late Dr. T. Hoshino. A voucher specimen (No. TII-132) has been deposited in our laboratory.

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