New Triterpenoid Sulfates from the Red Alga *Tricleocarpa fragilis*[1]

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Ten new sulfated terpenoids, including six cycloartenol sulfates (**1**−**6**), two 29-*nor*-cycloartenol sulfates (**7**,**8**), and two 29-*nor*-lanosterol sulfates (**9**,**10**), were isolated from brine shrimp-toxic fractions of the methanolic extract of the red alga *Tricleocarpa fragilis* collected in Hawaiian waters. Structures **1**−**10** were elucidated by spectral methods, and the absolute stereochemistry for compound **1** at C23 was determined by Mosher analysis. Compounds **7** and **10** showed brine shrimp toxicity at 50 μg/mL, while **1** and **3** showed substantial activity at 17 μg/mL. Compounds **2**, **4**, **5**, and **9** were inactive. In cytotoxicity assays, compounds **1**−**10** were inactive at concentrations tested.

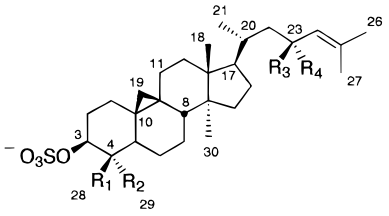
Tricleocarpa fragilis (L.) Huisman & Townsend (Galaxauraceae) is a weakly calcified red alga with distribution throughout the world's warmer oceans[2] and is a common species in the waters off Oahu, Hawaii. Despite its wide distribution, there are no chemical reports in the literature, although antiinflammatory activity has been reported for both $CHCl_3$ and MeOH extracts of the plant.[3] In an ongoing effort to isolate and identify biologically active substances from marine organisms, we investigated the MeOH extract of a Hawaiian collection of this alga after the $CHCl_3$ partition residue demonstrated activity in the brine shrimp toxicity assay.

Results and Discussion

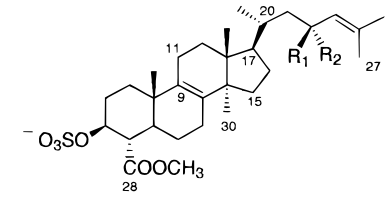
Whole plants of *Tricleocarpa fragilis* totaling 2.6 kg (wet wt) were collected from a depth of 3 m off the west coast of Oahu. The $CHCl_3$ partition residue showed moderate brine shrimp toxicity and was subjected to normal-phase vacuum liquid chromatography (VLC). The fractions, which eluted with $CHCl_3$−MeOH (85:15 and 75:25), were further fractionated by size exclusion chromatography and/or repeated reversed-phase HPLC leading to the isolation of compounds **1**−**10**.

The IR spectra of all 10 compounds showed a strong broad absorption near 1220 cm^{-1} along with an absorption near 1060 cm^{-1}, suggesting the presence of a sulfate group.[4] Together with HRFABMS analyses, these data indicated that **1**−**10** are monosulfated organic anions. This claim is further supported by substantial [M + 2] peaks that were observed in the LRMS.

Based on the HPLC profile of each isolate, **1**−**10** were obtained as either monovalent or divalent cation salts (presumably Na^+ and Ca^{2+} or Mg^{2+}), or, in some cases, both forms were obtained. When both monovalent and divalent cation salts were isolated, they gave identical NMR and MS data, but the chromatographic behavior of the monovalent cation salts was markedly different from that of the divalent cation salts. The former showed shorter retention times and gave relatively sharp Gaussian peaks when subjected to C_{18} HPLC (MeOH−H_2O mixtures), whereas the latter eluted with longer retention times in broad fronting peaks. In general, conversion of the organic sulfates **1**−**10** to their Na^+ or K^+ salt could be achieved by briefly incubating the sample with aqueous Na_2SO_4 or KH_2PO_4 prior to C_{18} HPLC. Both Na^+ or K^+ salts of a given compound showed identical HPLC profiles. Likewise, the Mg^{2+} and Ca^{2+} salts gave identical HPLC chromatograms and could be obtained by treatment of the organic sulfates with aqueous $MgSO_4$ or $CaCl_2$. Use of Na_2SO_4 as a HPLC mobile-phase modifier, as employed previously for related disulfates,[5] destroyed resolution.



	R_1	R_2	R_3	R_4
1:	CH_3	$COOCH_3$	H	OH
2:	CH_3	$COOCH_3$	=O	
3:	CH_3	CH_2OH	H	OH
4:	CH_3	CH_2OH	=O	
5:	CH_3	CH_3	H	OH
6:	CH_3	CH_3	=O	
7:	H	$COOCH_3$	H	OH
8:	H	$COOCH_3$	=O	



	R_1	R_2
9:	H	OH
10:	=O	

Compounds **2**, **4**, **5**, and **6** were isolated only as divalent cation salts based on their characteristic HPLC profiles, while **7** was isolated only in association with a monovalent cation. Both forms of compounds **1**, **3**, and **9** were obtained. Compounds **8** and **10** were difficult to separate. Both showed HPLC profiles consistent with association with divalent cations but could only be separated by conversion to their Na^+ salts as described above.

HRFABMS data for compound **1** suggested an organic sulfate anion with a molecular formula of $C_{31}H_{49}O_7S^-$. 1D NMR experiments provided evidence of a cycloartane skeleton. The ^{13}C NMR spectrum displayed 31 signals,

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including one OMe signal, indicating a 30-carbon skeleton. The ^{1}H NMR spectrum showed three Me singlets and one Me doublet. The fifth Me group expected for a cycloartane nucleus is accounted for by a Me ester at C28 (IR ν_{max} 1718 cm^{-1}; carbonyl carbon and OMe signals at δ 178.0 and 52.4, respectively). Furthermore, two coupled doublets near δ 0.4 and 0.6 (H19) are characteristic of the tetrasubstituted cyclopropyl group of cycloartanes.[6] Last, the methine at δ 4.74 (H3) showed a splitting pattern (doublet of doublet) consistent with 4,4-disubstitution in ring A.

Evidence of further structural features was also obtained from 1D NMR experiments. Two olefinic Me groups resonated at δ 1.67 (H27) and 1.70 (H26), suggesting an unsaturated side chain at C24. A second downfield methine carbon (δ 66.7) pointed to an additional site of oxygenation (C23).

Unambiguous proton and carbon chemical shift assignments (Tables 1 and 2) and, with the exception of the sulfate group, functional group positions were obtained or confirmed through analysis of COSY, DEPT, HSQC, and HMBC experiments. Key HMBC correlations are shown in Figure 1. The position of the sulfate group was assigned to C3 on the basis of the downfield chemical shifts of H3 (δ 4.74) and C3 (δ 83.3), which are farther downfield than expected for a 3-OH substitution and are consistent with the presence of a sulfate group.[5,7] This was later confirmed by the preparation of Mosher derivatives at 23-OH (discussed below), which is the only other possible position of the sulfate.

The relative stereochemistry of the ring system of **1**, including assignment of α- and β-orientation of geminal protons, was determined by interpretation of NOESY data. Key correlations were seen between H3 and H5, indicating a 3-β-sulfate; this is confirmed by the axial coupling of H3 with H2α (J = 11.6 Hz). The β-position of the 4-Me group (H29) was settled by NOESY correlations with the cyclopropyl hydrogens (H19). Last, H17 was assigned α orientation, based on cross-peaks with the 14-Me group (H30).

With respect to ring system conformation, notable correlations were seen between the H30 Me protons and H11α, which serves as evidence of a boatlike conformation of ring C. This was supported by the energy-minimized molecular model of compound **1** (HyperChem, MM+ force field, gas-phase), which predicts ring C to take on a somewhat flattened, twisted-boat conformation, with a distance of 2.2 Å between the protons of interest.

Compound **2** showed a HRFABMS negative molecular ion at *m*/*z* 563.3014, indicating a molecular formula of $C_{31}H_{47}O_7S^-$ for the organic anion, which suggests one additional degree of unsaturation as compared with **1**. This assignment was easily accounted for by the replacement of the allylic OH group at C23 in **1** with an α,β-unsaturated ketone in **2** (IR ν_{max} 1682 cm^{-1}, UV λ_{max} 238 nm). This was fully supported by the ^{1}H NMR data (Table 1,) which showed the absence of H23 in the spectrum of **2** and the downfield shifts of H24, H26, and H27 from δ 5.16 td (J = 1.4, 8.5 Hz), 1.70 d (J = 1.0 Hz), and 1.67 d (J = 1.3 Hz), respectively, in **1** to δ 6.16 t (J = 1.3 Hz), 1.90 d (J = 1.2 Hz), and 2.11 d (J = 1.2 Hz) in **2**. The ^{13}C spectrum of **2** also supported the proposed structure, indicating replacement of the methine carbon at δ 66.7 (C23) in **1** with a carbonyl carbon signal at δ 203.9 in **2**. The structure was confirmed, and proton and carbon assignments (Tables 1 and 2) were determined by DEPT, COSY, HETCOR, HMBC, and NOESY NMR experiments.

Compound **3** was assigned the molecular formula $C_{30}H_{49}O_6S^-$ on the basis of a HRFABMS disodium pseudomolecular ion at *m*/*z* 583.3050 (583.3045 expected for $C_{30}H_{49}O_6SNa_2$, Δ −0.5 mmu). This compound afforded a ^{1}H NMR spectrum similar to compound **1**, with important exceptions, among them the absence of the OMe signal, the upfield shifts of H3 (δ 4.74 in **1** to 4.39 in **3**), H5 (δ 2.09 in **1** to 1.80 in **3**), and H29 (δ 1.17 in **1** to 0.74 in **3**), and the presence of two geminal carbinol doublets at δ 3.33 and 3.57 (H28) in **3**. The obvious deduction that **3** is a C28-reduced derivative of **1** was borne out by analysis of DEPT, COSY, NOESY, HSQC, and HMBC NMR spectra. Absence of a carbonyl absorption in the IR spectrum supports this assignment. Tables 1 and 2 list the proton and carbon NMR assignments. Last, the assignment of α-configuration to the −CH_2OH group at C4 was established by weak NOESY cross-peaks between H19α/H19β and H29 and a correlation observed between the H28 doublet at δ 3.33 and the equatorial proton H6α at δ 1.64.

The HRFABMS molecular ion peak for compound **4** at *m*/*z* 535.3115 suggested a molecular formula of $C_{30}H_{47}O_6S^-$, corresponding to one additional degree of unsaturation as compared with **3**. In fact, the ^{1}H NMR spectrum of **4** was very similar to that of **3**, but showed the same side chain resonances as compound **2**, including δ 6.17 t (H24), 1.90 (H26), and 2.11 (H27), suggesting that **4** is the α,β-unsaturated ketone analogue (IR ν_{max} 1686 cm^{-1}, UV λ_{max} 238 nm) of compound **3**. Analysis of DEPT, COSY, NOESY, HETCOR, HMQC, and HMBC spectra of **4** confirmed the structure and led to the full proton and carbon assignments (Tables 1 and 2).

Compound **5** showed a ^{1}H NMR spectrum (CD_3OD) similar to that of compound **1**, with the notable absence of the OMe resonance (δ 3.67 in **1**), presence of an additional Me singlet, and upfield shift of H3 (δ 4.74 in **1** to 4.01 in **5**). The IR spectrum lacked carbonyl absorption. At the same time, HRFABMS afforded a peak at *m*/*z* 521.3311 consistent with a molecular formula of $C_{30}H_{49}O_5S^-$, differing from **1** by CO_2. These data strongly suggested that in **5** a C28-Me replaces a COOMe in **1**. This was confirmed by further NMR analysis of **5** (^{13}C, DEPT, COSY, HMQC, HMBC, NOESY), which was carried out in DMSO-d_6 due to poor solubility in CD_3OD. Tables 1 and 2 list the full NMR assignments of compound **5**. Assignment of α- and β-orientation of ring-system protons was based on NOESY correlations, or, for C16 protons, on comparison of chemical shifts with spectra of compounds **1**−**4**. NOESY correlations for protons on C6 and C7 were ambiguous due to overlapping signals of nearby protons.

Compound **6** showed similar solubility and NMR spectra as **5**. One additional degree of unsaturation as suggested by HRFABMS ($C_{30}H_{47}O_5S^-$, Δ −0.3), a carbonyl absorption in the IR spectrum (1684 cm^{-1}), and ^{1}H and ^{13}C NMR signals consistent with a 1,5-dimethyl-3-oxo-4-hexenyl side chain ([δ 6.13 br s (H24), 1.84 br s (H26), 2.04 br s (H27)]) indicated that **6** is the α,β-unsaturated ketone analogue of **5**. HMQC, COSY, and DEPT NMR experiments confirmed this conclusion and allowed assignment of proton and nonquaternary carbon chemical shifts (Tables 1 and 2). Quaternary carbon assignments and stereochemical assignments of protons on C1, C2, C11, and C19 were assigned based on comparison with spectra of **5**, while C16 proton orientations were deduced by comparison with data from compounds **1**−**4**.

Compound **7** gave a negative HRFABMS molecular ion peak at 551.3026, indicating an organic anion with a molecular formula of $C_{30}H_{47}O_7S^-$ (Δ +1.7). The ^{1}H NMR spectrum of **7** differs from that of **1** by the (a) absence of the 4-Me singlet (δ 1.17 in **1**, H29), (b) presence of a triplet

Table 1. ^{1}H NMR Chemical Shift Assignments of Compounds **1**−**10**[a]

	1		**2**		**3**[b]		**4**		**5**[c]		**6**[b,c,d]		**7**[d]		**8**[d]		**9**		**10**[d]	
position	^{1}H	mult (*J*)	^{1}H	mult (*J*)	^{1}H	mult (*J*)	^{1}H	mult (*J*)	^{1}H	mult (*J*)	^{1}H	mult (*J*)	^{1}H	mult (*J*)	^{1}H	mult (*J*)	^{1}H	mult (*J*)	^{1}H	mult (*J*)
1	1.67α	m	1.64α	m	1.58α	m	1.60α	m	1.42α	m	1.40α	m	1.61	m	1.61	m	1.83α	m	1.82α	m
	1.34*β*	m	1.36*β*	m	1.32*β*	m	1.32*β*	m	1.18*β*	m	1.18*β*	m	1.37	m	1.37	m	1.26*β*	m	1.26*β*	m
2	2.25α	m	2.26α	m	2.13α	m	2.13α	m	2.06α	m	2.03α	m	2.45α	m	2.45α	dq (11.9,3.9)	2.31α	m	2.31α	m
	1.71*β*	m	1.72*β*	qd (12.0,3.3)	1.79*β*	qd (12.6,4.6)	1.80*β*	qd (12.5,4.3)	1.43*β*	m	1.43*β*	m	1.52*β*	qd (11.8,3.2)	1.52*β*	qd (12.9,3.4)	1.62*β*	m	1.63*β*	m
3	4.74	dd (4.5,11.6)	4.77	dd (4.1,11.4)	4.39	dd (4.7,11.7)	4.42	dd (4.6,11.8)	3.67	dd (4.1,10.6)	3.67	dd (4.2,10.6)	4.55	td (10.8,4.7)	4.55	td (10.8,4.7)	4.44	td (11.0,5.2)	4.43	td (11.1,5.2)
4													2.26	t (10.8)	2.26	t (10.7)	2.45	t (11.2)	2.45	t (11.0)
5	2.09	dd (4.2,12.3)	2.09	m	1.80	dd (4.0,12.2)	1.80	dd (4.3,12.5)	1.26	m	1.27	m	1.84	td (11.6,4.3)	1.84	td (11.6,4.3)	1.59	m	1.59	m
6	1.06α	m	1.07α	m	1.64α	m	1.62α	m	1.50	m	1.50	m	1.27	m	1.30	m	1.27α	m	1.27α	m
	0.96*β*	m	0.97*β*	m	0.83*β*	m	0.84*β*	m	0.76	m	0.74	m	0.79	m	0.79	m	1.53*β*	m	1.53*β*	m
7	1.14α	m	1.15α	m	1.18α	m	1.18α	m	1.03	m	1.04	m	1.12	m	1.14	m	2.06	m	2.04	m
	1.31*β*	m	1.30*β*	m	1.35*β*	m	1.36*β*	m	1.27	m	1.27	m	1.31	m	1.33	m				
8	1.61	m	1.62	m	1.57	m	1.57	m	1.48	m	1.49	m	1.71	m	1.71	m				
9																				
10																				
11	2.04α	m	2.04α	m	2.04α	m	2.04α	m	1.91α	m	1.94α	m	2.01α	m	2.00α	m	2.11	m	2.12	m
	1.20*β*	m	1.21*β*	m	1.20*β*	m	1.20*β*	m	1.13*β*	m	1.10*β*	m	1.28*β*	m	1.29*β*	m				
12	1.68	m	1.67	m	1.68	m	1.66	m	1.58	m	1.54	m	1.67	m	1.66	m	1.79	m	1.77	m
13																				
14																				
15	1.32	m	1.32	m	1.32	m	1.34	m	1.24	m	1.25	m	1.30	m	1.32	m	1.63α	m	1.66α	m
																	1.20*β*	m	1.22*β*	m
16	1.92α	m	1.92α	m	1.93α	m	1.91α	m	1.82α	m	1.82α	m	1.92α	m	1.92α	m	1.94α	m	1.94α	m
	1.36*β*	m	1.31*β*	m	1.35*β*	m	1.32*β*	m	1.25*β*	m	1.27*β*	m	1.34*β*	m	1.32*β*	m	1.40*β*	m	1.35*β*	m
17	1.60	m	1.68	m	1.59	m	1.66	m	1.51	m	1.57	m	1.61	m	1.66	m	1.49	m	1.57	m
18	1.02	s	1.03	s	1.03	s	1.05	s	0.93	s	0.95	s	1.02	s	1.04	s	0.77	s	0.79	s
19	0.44α	d (4.2)	0.45α	d (4.2)	0.44α	d (4.1)	0.41α	d (4.0)	0.30α	d (3.9)	0.30α	d (3.7)	0.22α	d (4.1)	0.23α	d (4.0)	1.01	s	1.01	s
	0.63*β*	d (4.2)	0.64*β*	d (4.2)	0.59*β*	d (4.1)	0.61*β*	d (4.0)	0.48*β*	d (3.6)	0.49*β*	d (3.7)	0.50*β*	d (4.1)	0.51*β*	d (4.3)				
20	1.68	m	1.96	m	1.68	m	1.97	m	1.58	m	1.86	m	1.67	m	1.98	m	1.69	m	1.99	m
21	0.95	d (6.5)	0.87	d (6.4)	0.95	d (7.0)	0.86	d (6.3)	0.86	d (5.9)	0.79	d (6.3)	0.95	d (6.4)	0.86	d (6.4)	0.98	d (6.4)	0.89	d (6.4)
22	0.98	dd (3.2,9.7)	2.09	m	0.99	dd (3.7,10.1)	2.10	dd (10.1,14.8)	0.85	m	2.06	m	0.98	dd (3.7,10.0)	2.10	dd (3.2,14.8)	0.96	m	2.10	dd (9.9,14.9)
	1.65	m	2.52	dd (3.0,15.0)	1.65	m	2.53	dd (3.0,14.8)	1.49	m	2.44	dd (2.2,15.0)	1.65	m	2.53	dd (10.1,14.7)	1.63	m	2.52	dd (3.0,14.9)
23	4.41	td (9.1,3.3)			4.41	td (9.6,3.4)			4.21	m			4.41	td (9.1,3.2)			4.40	td (9.0,2.9)		
24	5.16	dt (8.5,1.4)	6.16	t (1.3)	5.16	dt (8.6,1.2)	6.17	t (1.2)	5.09	dt (8.2,1.3)	6.13	br s	5.16	dt (8.5,1.3)	6.17	t (1.2)	5.16	dt (8.5,1.4)	6.17	t (1.2)
25																				
26	1.70	d (1.0)	1.90	d (1.2)	1.70	d (0.7)	1.90	br s	1.61	d (1.0)	1.84		1.69	d (1.0)	1.90	d (1.0)	1.69	d (1.2)	1.90	d (1.1)
27	1.67	d (1.3)	2.11	d (1.2)	1.67	d (1.0)	2.11	br s	1.57	d (1.0)	2.04	br s	1.66	d (1.3)	2.11	d (1.0)	1.66	d (1.2)	2.11	d (1.1)
28					3.33	d (12.1)	3.37	d (12.3)	0.87	s	0.87	s								
					3.57	d (12.1)	3.57	d (12.3)												
29	1.17	s	1.17	s	0.74	s	0.74	s	0.71	s	0.71	s								
30	0.94	s	0.94	s	0.94	s	0.94	s	0.84	s	0.86	s	0.93	s	0.94	s	0.92	s	0.93	s
23-OH									4.29	d (4.9)										
28-OMe	3.67	s	3.68	s									3.67	s	3.67	s	3.68	s	3.69	s

[a] Spectra obtained at 500 MHz and in CD_3OD unless otherwise specified; offset set to δ 3.30 for C*H*D_2OD impurity; *J* values given in Hz; assignments based on ^{1}H, ^{13}C, DEPT, COSY, HMQC/HSQC/HETCOR, HMBC, and NOESY NMR experiments unless otherwise specified. [b] Spectra obtained at 400 MHz. [c] Spectra obtained in DMSO-d_6; DMSO-d_5 impurity referenced to δ 2.49. [d] Assignments based on ^{1}H, ^{13}C, DEPT, COSY, and HMQC NMR experiments.

Table 2. ^{13}C NMR Chemical Shift Assignments for Compounds **1**−**10**[a]

	1		**2**		**3**[b]		**4**		**5**[c]		**6**[b,c,d]		**7**[d]		**8**[d]		**9**		**10**[d]	
position	^{13}C	mult	^{13}C	mult	^{13}C	mult	^{13}C	mult	^{13}C	mult	^{13}C	mult	^{13}C	mult	^{13}C	mult	^{13}C	mult	^{13}C	mult
1	32.5	t	33.4	t	33.0	t	33.0	t	31.3	t	31.3	t	31.1	t	31.1	t	35.6	t	35.6	t
2	28.1	t	28.0	t	28.1	t	28.1	t	27.4	t	27.3	t	32.4	t	32.4	t	28.7	t	28.7	t
3	83.3	d	83.6	d	80.8	d	80.8	d	82.2	d	82.2	d	80.4	d	80.4	d	80.5	d	80.4	d
4	55.2	s	55.2	s	45.8	s	45.8	s	39.6	s	39.6	s	57.5	d	57.5	d	53.0	d	53.0	d
5	45.9	d	45.9	d	41.5	d	41.5	d	47.3	d	47.2	d	42.0	d	42.0	d	45.2	d	45.1	d
6	24.0	t	24.0	t	21.7	t	21.7	t	20.6	t	20.5	t	26.2	t	26.2	t	23.5	t	23.5	t
7	26.6	t	26.6	t	26.8	t	26.8	t	25.5	t	25.5	t	25.6	t	25.6	t	26.1	t	26.1	t
8	49.2	d	49.2	d	49.2	d	49.2	d	47.3	d	47.2	d	48.0	d	48.0	d	136.3	s	136.2	s
9	21.3	s	21.3	s	21.2	s	21.1	s	19.5	s	19.4	s	25.1[e]	s	25.0[e]	s	134.5	s	134.6	s
10	26.3	s	26.3	s	26.8	s	26.8	s	25.8	s	25.8	s	29.8[e]	s	29.8[e]	s	36.8	s	36.8	s
11	27.5	t	27.4	t	27.6	t	27.5	t	25.9	t	25.8	t	28.0	t	27.9	t	22.9	t	22.8	t
12	34.1	t	33.9	t	34.3	t	34.0	t	32.5	t	32.3	t	34.1	t	33.9	t	32.3	t	32.1	t
13	46.6	s	46.6	s	46.6[e]	s	46.6	s	44.9	s	44.9	s	46.6[f]	s	46.6[f]	s	45.9	s	45.8	s
14	50.0	s	50.1	s	50.1[e]	s	50.2	s	48.4	s	48.5	s	50.1[f]	s	50.2[f]	s	51.1	s	51.2	s
15	36.6	t	36.5	t	36.6	t	36.6	t	35.0	t	35.0	t	36.3	t	36.2	t	31.8	t	31.8	t
16	29.2	t	29.3	t	29.2	t	29.3	t	27.7	t	27.8	t	29.1	t	29.2	t	29.2	t	29.3	t
17	54.2	d	53.8	d	54.2	d	53.9	d	52.3	d	51.8	d	54.1	d	53.8	d	52.3	d	52.0	d
18	18.6	q	18.6	q	18.6	q	18.6	q	17.9	q	17.8	q	18.2	q	18.2	q	16.3	q	16.3	q
19	30.6	t	30.6	t	30.8	t	30.8	t	29.1	t	29.0	t	27.2	t	27.2	t	18.0	q	18.0	q
20	33.8	d	34.8	d	33.8	d	34.9	d	31.9	d	32.8	d	33.8	d	34.9	d	34.2	d	35.2	d
21	18.9	q	19.8	q	18.9	q	19.8	q	18.2	q	19.1	q	19.0	q	19.8	q	19.3	q	20.1	q
22	45.6	t	52.6	t	45.6	t	52.7	t	44.4	t	51.1	t	45.6	t	52.7	t	45.6	t	52.7	t
23	66.7	d	203.9	s	66.7	d	204.2	s	64.0	d	200.4	s	66.7	d	204.2	s	66.7	d	204.1	s
24	130.5	d	125.3	d	130.5	d	125.4	d	131.0	d	124.2	d	130.5[g]	d	125.3	d	130.5	d	125.3	d
25	133.4	s	156.8	s	133.4	s	156.9	s	129.5	s	153.6	s	133.4[g]	s	156.9	s	133.4	s	156.9	s
26	25.9	q	27.7	q	25.9	q	27.6	q	25.4	q	27.0	q	25.9	q	27.6	q	25.9	q	27.6	q
27	18.1	q	20.9	q	18.1	q	20.8	q	17.8	q	20.2	q	18.1	q	20.8	q	18.1	q	20.8	q
28	178.0	s	178.1	s	64.3	t	64.2	t	25.6	q	25.6	q	175.7	s	175.6	s	176.0	s	176.1	s
29	10.6	q	10.6	q	11.5	q	11.5	q	15.0	q	15.0	q	—		—		—		—	
30	19.7	q	19.8	q	19.8	q	19.8	q	19.0	q	19.1	q	19.5	q	19.5	q	24.8	q	24.8	q
28-OMe	52.4	q	52.6	q	—		—		—		—		52.1	q	52.1	q	52.2	q	52.2	q

[a] Spectra obtained at 125 MHz in CD_3OD unless otherwise specified with offset set to δ 49.0 for CD_3OD; assignments based on ^1H, ^{13}C, DEPT, COSY, HMQC/HSQC/HETCOR, HMBC, and NOESY NMR experiments unless otherwise stated. [b] Spectra obtained at 100 MHz. [c] Spectra obtained in DMSO-d_6; solvent peak referenced to δ 39.5. [d] Assignments based on ^1H, ^{13}C, DEPT, COSY, and HMQC NMR experiments. [e,f,g] Assignments may be reversed; assignments based on comparison of chemical shifts with other compounds in the table.

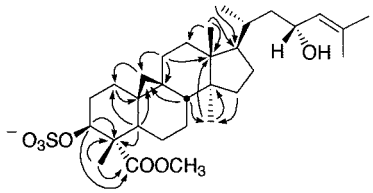

Figure 1. Key HMBC correlations for the pentacyclic ring system of compound **1**.

at δ 2.26 (H4), (c) upfield shift of H19 protons (δ 0.44 and 0.63 in **1** to δ 0.22 and 0.50 in **7**), and (d) change in the multiplicity of the H3 resonance (doublet of doublets in **1** to a triplet of doublets in **7**). The upfield shift of the H19 protons is indicative of a 29-*nor*-cycloartanol skeleton,[6] and, taken together, the FABMS and ^1H NMR data strongly suggested that **7** is the 29-*nor* analogue of **1**. IR data confirm the presence of a Me ester (1726 cm^{-1}). Analysis of ^{13}C, DEPT, COSY, and HMQC NMR experiments confirmed the assigned structure and allowed full assignment of proton and nonquaternary carbon atoms (Tables 1 and 2). The stereochemical assignment of H4β is apparent from its axial coupling with both H3 and H5 (J = 10.7 Hz).

The ^1H NMR spectrum of compound **8** displayed the same side chain resonances as those of **2** ([δ 6.17 t (H24), 1.90 (H26), and 2.11 (H27)]), but was nearly identical to **7** in all other respects, suggesting that **8** is its α,β-unsaturated ketone analogue. This is fully supported by IR data, which indicated the presence of two carbonyls (1724 and 1682 cm^{-1}); UV data (λ_{max} 239 nm); and HRFABMS measurements, which showed a molecular ion consistent with the molecular formula $C_{30}H_{45}O_7S^-$ (Δ −2.5 mmu). Further analysis of ^1H, ^{13}C, DEPT, COSY, and HMQC NMR spectra confirmed the structure and allowed assignment of proton resonances and nonquaternary carbon resonances (Tables 1 and 2). For both compounds **7** and **8**, the orientation of protons on C11 and C16 was assigned by comparison of chemical shifts with spectra of **1**−**4**, and C19 protons were compared with spectra of **5** to determine their α- and β-orientation.

The molecular formula of compound **9** was established as $C_{30}H_{47}O_7S^-$ by the HRFABMS negative molecular ion at m/z 551.3061 (551.3043 required, Δ −1.8 mmu). The ^1H and ^{13}C NMR spectra of this compound gave nearly identical side-chain resonances as compared with compounds **1** and **3** (Table 1), suggesting the presence of the same 3-hydroxy-1,5-dimethyl-4-hexenyl side chain. However, it was clear that **9** possessed a slightly different skeleton as compared with compounds **1**−**8**. Important differences included the absence of the upfield cyclopropyl protons (H19 of **1**−**8**), the doublet-of-triplets splitting pattern of H3 (δ 4.44), and the presence of a one-proton triplet at δ 2.45 (H4). The latter two observations suggested a 4-monosubstituted ring A for **9**. Additional structural features indicated by the ^1H, ^{13}C, and DEPT NMR spectra were one Me ester (carbonyl carbon at δ 176.0, IR ν_{max} 1723 cm^{-1}), one additional double bond (tetrasubstituted, δ 136.3 and 134.5), one Me doublet (δ 0.98), and three Me singlets (δ 0.77, 0.92, and 1.01). These data are consistent with a 29-*nor*-lanostadienol skeleton.[8]

Interpretation of COSY, HMQC, and HMBC spectra for **9** led to the assignment of functional group positions and proton and carbon resonances (Tables 1 and 2). A series of COSY cross-peaks connected C1 through C7 and allowed the assignment of the continuous spin system from C15 through C17−C20 through C27. COSY correlations also pointed to the presence of a two-carbon spin system (C11−C12). The position of the Me ester was indicated by an

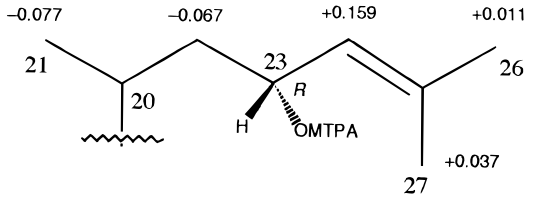

Figure 2. Selected $\delta\Delta$ values for the side chains of (*R*)- and (*S*)-MTPA esters of compound **1.** [$\delta\Delta$ = chemical shift of (*R*)-MTPA ester minus chemical shift of (*S*)-MTPA ester].

HMBC correlation between C28 and H4, while correlations between the olefinic carbons C8 and C9 and the Me protons H30 and H19, respectively, precluded assignment of the olefin to any other position. Other important HMBC correlations included C1/C10−H19, C5−H4/H19, C12−H18, C13/C14−H18/H30, C15−H30, C17−H18/H21, C28−(28-OMe).

The relative stereochemistry of the tetracyclic ring system of **9** was determined by NOESY data. The stereochemistry at C4 was evident from the cross-peak between H4 and the H19 Me protons, indicating that H4 has β-configuration. The axial coupling constants of H3 (J = 11.0 Hz × 2) and NOESY correlations between H3 and H5 are evidence of a β-sulfate group. The α-orientation of H17 was assignable on the basis of cross-peaks with the H30 Me protons.

Compound **10** demonstrated a ^{1}H NMR spectrum that differed from that of **9** only in the side chain resonances and suggested the presence of a 1,5-dimethyl-3-oxo-4-hexenyl side chain, as in **2**, **4**, **6**, and **8**. The assignment of **10** as the 23-oxo analogue of **9** was supported by IR data showing two carbonyl bands (1721 and 1686 cm^{-1}), a UV maximum at 239 nm, and HRFABMS data, which indicated a molecular formula ($C_{30}H_{45}O_7S^-$, Δ +1.6 mmu) with one additional degree of unsaturation compared with **9**. This structural assignment was fully consistent with subsequent analysis of ^{13}C, DEPT, COSY, and HMQC NMR spectra, which allowed the assignment of proton and nonquaternary carbon signals (Tables 1 and 2). The orientation assigned to geminal protons on C1, C2, C6, C15, and C16 was based on comparison of chemical shifts with the proton assignments of compound **9**.

The absolute stereochemistry shown for **1**−**8** assumes a typical cycloartane ring plus C20 configuration.[9] In support of this assumption, the dextrorotatory optical rotation of **1**−**8** (e.g., $[\alpha]^{27}_D$ +53° in **1**) is consistent with related cycloartenol sulfates described previously.[5,7] Likewise, the absolute stereochemistry shown for **9** and **10** assumes standard lanostane absolute stereochemistry for the ring system and C20.[10]

To determine the stereochemistry at C23 for compounds **1**, **3**, **5**, **7**, and **9**, Mosher derivatives[11] of **1** were prepared. Figure 2 shows $\delta\Delta$ values for key side-chain resonances of the (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) esters of **1**. Negative $\delta\Delta$ values to the left of C23 and positive values to the right suggest that, in the preferred conformation, the phenyl group of the (*R*)-MTPA ester of **1** is oriented toward the left-hand side of the side chain, requiring (*R*)-configuration at C23.

The identical ^{1}H NMR chemical shift values (CD_3OD) of the side-chain protons H21, H23, H24, H26, and H27 for **5** and **1**, and the nearly identical ^{13}C and ^{1}H NMR chemical shifts for C22 through C26 for compounds **1**, **3**, **7**, and **9** suggest the same stereochemistry at C23 for all 23-OH compounds isolated. It should be noted that methyl esters **1**, **2**, **7**, **8**, **9**, and **10** may be artifacts of naturally occurring corresponding carboxylic acids, inasmuch as MeOH was used in the extraction and purification process.

Compounds **1**−**7** and **9**−**10** were tested for brine shrimp toxicity. If both monovalent and divalent cation salts were isolated, the divalent cation salts were assayed. Compounds **2**, **4**, **5**, and **9** failed to show significant immobilization of brine shrimp at 50 μg/mL, while, at the same concentration, **7** and **10** showed 39.1 ± 11.0 and 35.5 ± 12.8% immobilization (90% confidence intervals given), respectively. Compounds **1** and **3** were more active, showing 55.7 ± 8.7 and 47.1 ± 15.1% immobilization respectively, at 17 μg/mL.

Toxicity toward P-388 (ATCC: CCL 46), A-549 (ATCC: CCL 8), MEL-28 (ATCC: HTB 72), and HT-29 (ATCC: HTB 38) cell lines was also evaluated. Fifty-percent inhibitory concentration (IC_{50}) values for **1**−**3** and **6** were >10 μg/mL; for **4**, **5**, and **9** >2 μg/mL; and for **7**, **8**, and **10** >1 μg/mL against all cell lines tested. Additionally, Na^+, Ca^{2+}, and Mg^{2+} salts of **2** showed IC_{50} values of >10 μg/mL against all cell lines tested.

Sulfated triterpenoids are common in the marine environment, particularly from echinoderms, and, to a lesser extent, sponges; in echinoderms, such compounds are usually found as glycosides, and, in both groups, polyhydroxylated sterol skeleta predominate.[12] Conversely, sulfated terpenoids are rare in algae. Exceptions include several cycloartenol disulfates, from the green alga *Tydemania expeditionitis* Weber van Bosse, which demonstrated inhibition of the oncogenic pp60^{v-src} protein tyrosine kinase at micromolar concentrations,[5] and a cycloartenol sulfate from the green alga *Tuemoya* sp., which inhibited viral proteases, also at micromolar concentrations.[7] To our knowledge, this is the first report of sulfated triterpenoids from a red alga.

Experimental Section

General Experimental Procedures. NMR experiments were performed on either a General Electric GN Omega 500 spectrometer operating at 500 and 125 MHz or a Varian Unity INOVA 400 operating at 400 and 100 MHz. HMQC/HSQC/HETCOR and HMBC were optimized for J_{CH} = 142 Hz and $^nJ_{CH}$ = 7 Hz, respectively. COSY experiments were double quantum filtered. Optical rotations were measured on a JASCO DIP-370 polarimeter. HRFABMS were measured on a VG ZAB2SE mass spectrometer. IR spectra were recorded on a Perkin-Elmer 1600 FTIR.

Plant Material. A sample of *T. fragilis* [2.6 kg, wet wt; synonyms: *T. oblongota*, Ellis et Solander; *Galaxaura oblongota* (Ellis et Solander) Lamouroux; *G. eburnea* Kjellman; *Corallina oblongata* Ellis et Solander; *C. tubulosa* Pallas; *Eschara fragilis* L.][2,13] was collected by free diving (−3 m) in waters adjacent to Nanakuli Beach Park (Oahu, Hawaii) on August 2, 1997. A voucher specimen, on file in the Department of Chemistry, University of Hawaii (no. 080297-NAN-1), was identified by Professor I. A. Abbott.

Extraction and Isolation. The fresh plant material was extracted exhaustively with MeOH, and the aqueous methanolic extract was concentrated and partitioned against hexanes. After removal of the MeOH from the polar phase under reduced pressure, the extract was partitioned against $CHCl_3$. The $CHCl_3$ residue (4 g) showed moderate toxicity toward brine shrimp (IC_{50} = 2.5−22 μg/mL), and a portion (2.1 g) was chromatographed by VLC (200−425 mesh Si gel, 127 g), eluting with hexanes−$CHCl_3$ (50:50), $CHCl_3$, $CHCl_3$−MeOH mixtures, and MeOH, sequentially. Fraction **f11** (540 mg), which eluted with $CHCl_3$−MeOH (75:25), showed the highest toxicity to brine shrimp (IC_{50} = 2.5−7.4 μg/mL). Part of fraction **f11** (460 mg) was separated by Sephadex chromatography [LH-20, 1.5 cm diameter column × 120 cm; mobile phase was $CHCl_3$−MeOH (50:50)]. Both the Sephadex fraction that eluted between 48 and 72 mL (84 mg) and the VLC fraction **f10** (558

mg), which eluted with $CHCl_3$−MeOH (85:15), were subjected to repeated reversed-phase HPLC separations [C_{18} Cosmosil, mobile phase MeOH−H_2O (60:40, 65:35, 67:33, 70:30, and 75:25)] leading to the isolation of divalent cation salts of **1** (58.3 mg, 0.0022% yield based on wet wt), **2** (34.5 mg, 0.0013% yield), **3** (10.7 mg, 0.00041% yield), **4** (2.9 mg, 0.00011% yield), **5** (2.6 mg, 0.00010% yield), **6** (5.4 mg, 0.00022), and **9** (3.3 mg, 0.00013% yield), as well as the monovalent cation salts of **3** (1.5 mg, 0.000058% yield), **7** (0.8 mg, 0.00003% yield), and **9** (1.3 mg, 0.000050% yield). A mixture of the divalent salts of compounds **8** and **10**, which we were unable to separate, was dissolved in a minimal amount of MeOH and incubated for several minutes after the addition of saturated aqueous Na_2SO_4 (ca. 20% total volume); the sample was filtered and subjected to HPLC [mobile phase MeOH−H_2O (65:35)] to yield the Na^+ salts of **8** (0.3 mg, 0.000012% yield) and **10** (1.0 mg, 0.000038% yield).

Methyl 3*β*,23(*R*)-dihydroxycycloart-24-en-28-oate 3-sulfate (1): amorphous white solid; $C_{31}H_{49}O_7S^-$; $[\alpha]^{27}_D$ +53° (MeOH, *c* 0.10); UV λ_{max} (in MeOH) 208 nm; IR ν_{max} (film on NaCl) 3453 br, 2937, 2869, 1717, 1643, 1438, 1376, 1236 str/br, 1064, 979, 962, 832 cm^{-1}; positive-mode FABMS *m/z* (rel int) 611 ($[M + 2Na]^+$, 100), 589 ($[M + HNa]^+$, 23), 491 (41), 473 (53); negative-mode FABMS *m/z* (rel int) 565 ($[M]^-$, 100); HRFABMS *m/z* 611.2928 ($C_{31}H_{49}O_7SNa_2$ requires 611.2994, Δ +6.6 mmu), 589.3200 ($C_{31}H_{50}O_7SNa$ requires 589.3175, Δ −2.5 mmu); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Preparation of (*R*)-<bold>α-Methoxy-<bold>α-trifluoromethylphenylacetic Acid Ester of 1. The divalent cation salt of compound **1** (4.0 mg) and 4-(dimethylamino)pyridine (2.3 mg) were dissolved in dry pyridine (200 μL) under argon, and (*S*)-MTPA chloride (12 μL) was added. After stirring at room temperature for 27 h, the reaction was terminated by adding MeOH (ca. 0.5 mL) and stirring for an additional 5 min. The solution was dried under a stream of N_2 and eluted from a silica Sep Pak (Waters 20810) using CH_2Cl_2 and CH_2Cl_2−MeOH mixtures. The unstable (*R*)-MTPA ester of **1** eluted with CH_2Cl_2−MeOH (95:5): negative-mode FABMS *m/z* (rel int) 548 (100), 781 ($[M]^-$, 53); HRFABMS *m/z* 781.3602 ($C_{41}H_{56}O_9F_3S$ requires 781.3597, Δ −0.5 mmu); ^1H NMR (CD_3OD, 400 MHz) *δ* 0.435 d (1H, *J* = 4.4, H19), 0.620 d (1H, *J* = 3.2 Hz, H19), 0.801 s (3H), 0.893 s (3H), 0.896 d (3H, *J* = 6.0, H21), 1.165 s (3H), 1.754 d (3H, *J* = 1.2 Hz, H27), 1.803 d (3H, *J* = 0.8 Hz, H26), 1.839 m (H22, from COSY), 3.526 br s (3H, MTPA OMe), 3.662 s (3H, 28-OMe), 4.731 dd (1H, *J* = 11.0, 4.2, H3), 5.212 br d (1H, *J* = 9.6, H24), 5.862 td (1H, *J* = 10.0, 2.0, H23), 7.36−7.51 (5H, MTPA aromatic protons) ppm.

Preparation of (*S*)-α-Methoxy-α-trifluoromethylphenylacetic Acid Ester of 1. The above procedure was repeated with 2.0 mg of the divalent cation salt of **1**, 1.3 mg 4-(dimethylamino)pyridine, and 10 μL of the (*R*)-MTPA chloride to give the unstable (*S*)-MTPA ester of **1**: negative-mode FABMS *m/z* (rel int) 548 (100), 781 ($[M]^-$, 67); HRFABMS *m/z* 781.3632 ($C_{41}H_{56}O_9F_3S$ requires 781.3597, Δ −3.5 mmu); ^1H NMR (CD_3OD, 400 MHz) *δ* 0.446 d (1H, *J* = 4.4, H19), 0.632 d (1H, *J* = 4.4 Hz, H19), 0.929 s (3H), 0.947 s (3H), 0.973 d (3H, *J* = 6.4, H21), 1.167 s (3H), 1.717 d (3H, *J* = 0.8 Hz, H27), 1.792 d (3H, *J* = 0.8 Hz, H26), 1.906 m (H22, from COSY), 3.544 d (3H, *J* = 1.2 Hz, MTPA OMe), 3.668 s (3H, 28-OMe), 4.739 dd (1H, *J* = 11.2, 4.5, H3), 5.053 br d (1H, *J* = 8.8, H24), 5.821 td (1H, *J* = 10.0, 2.6, H23), 7.37−7.48 (5H, MTPA aromatic protons) ppm.

Methyl 3*β*-hydroxy-23-oxocycloart-24-en-28-oate 3-sulfate (2): amorphous white solid; $C_{31}H_{47}O_7S^-$; $[\alpha]^{28}_D$ +24° (MeOH, *c* 0.44); UV λ_{max} (in MeOH) 238 nm; IR ν_{max} (film on NaCl) 3450 br, 2935, 2865, 1714, 1682, 1621, 1455, 1378, 1220 str/br, 1062, 977, 829 cm^{-1}; negative-mode FABMS *m/z* (rel int) 563 ($[M]^-$, 100); HRFABMS *m/z* 563.3014 ($C_{31}H_{47}O_7S$ requires 563.3043, Δ + 2.9 mmu); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Cycloart-24-en-3*β*,23(*R*),28-triol 3-sulfate (3): amorphous white solid; $C_{30}H_{49}O_6S^-$; $[\alpha]^{27}_D$ +35° (MeOH, *c* 0.14); UV λ_{max} (in MeOH) 208 nm; IR ν_{max} (film on NaCl) 3417 br, 2925, 2870, 1643 br, 1470, 1462, 1454, 1445, 1378, 1205 str/br, 1050, 952, 834 cm^{-1}; positive-mode FABMS *m/z* (rel int) 583 ($[M + 2Na]^+$, 100), 463 (45), 445 (66), 423 ($[M - (H_2O + HSO_4)]^+$, 4); negative-mode FABMS *m/z* (rel int) 537 ($[M]^-$, 100); HRFABMS *m/z* 583.3050 ($C_{30}H_{49}O_6SNa_2$ requires 583.3045, Δ −0.5 mmu), 423.3621 ($C_{30}H_{47}O$ requires 423.3627, Δ −0.6 mmu); ^1H and ^{13}C NMR data, see Tables 1 and 2.

3*β*,28-Dihydroxycycloart-24-en-23-one 3-sulfate (4): amorphous white solid; $C_{30}H_{47}O_6S^-$; $[\alpha]^{23}_D$ +28° (MeOH, *c* 0.12); UV λ_{max} (in MeOH) 238 nm; IR ν_{max} (film on NaCl) 3416 br, 2929, 2870, 1686, 1592 br, 1468, 1462, 1451, 1441, 1410, 1379, 1354, 1218 str/br, 1065, 1038, 975, 953, 877 cm^{-1}; negative-mode FABMS *m/z* (rel int) 535 ($[M]^-$, 100); HRFABMS *m/z* 535.3115 ($C_{30}H_{47}O_6S$ requires 535.3093, Δ − 2.2 mmu); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Cycloart-24-en-3*β*,23(*R*)-diol 3-sulfate (5): amorphous white solid; $C_{30}H_{49}O_5S^-$; $[\alpha]^{27}_D$ +35° (MeOH, *c* 0.24); UV λ_{max} (in MeOH) 201 nm; IR ν_{max} (film on NaCl) 3400 br, 2930, 2869, 1632 br, 1467, 1462, 1453, 1444, 1374, 1202 str/br, 1068, 1053, 945, 841 cm^{-1}; negative-mode FABMS *m/z* (rel int) 521 ($[M]^-$, 100); HRFABMS *m/z* 521.3311 ($C_{30}H_{49}O_5S$ requires 521.3301, Δ −1.0 mmu); ^1H NMR (CD_3OD, 400 MHz) *δ* 0.38 d (1H, *J* = 4.2, H19), 0.59 d (1H, *J* = 3.9 Hz, H19), 0.86 s (3H), 0.93 s (3H), 0.95 d (3H, *J* = 6.4, H21), 1.02 s (3H), 1.03 s (3H), 1.67 d (3H, *J* = 1.1 Hz, H27), 1.70 d (3H, *J* = 1.0 Hz, H26), 4.01 dd (1H, *J* = 4.5, 11.8, H3), 4.41 td (1H, *J* = 9.1, 3.2, H23), 5.16 dt (1H, *J* = 8.5, 1.3, H24) ppm; ^1H and ^{13}C NMR data (DMSO-d_6), see Tables 1 and 2.

3*β*-Hydroxycycloart-24-en-23-one 3-sulfate (6): amorphous white solid; $C_{30}H_{47}O_5S^-$; $[\alpha]^{28}_D$ +20° (MeOH, *c* 0.54); UV λ_{max} (in MeOH) 239 nm; IR ν_{max} (film on NaCl) 3454 br, 2945, 2867, 1684, 1624, 1466, 1446, 1377, 1357, 1248 str, 1217 str, 1070, 1039, 986, 875, 837 cm^{-1}; negative-mode FABMS *m/z* (rel int) ($[M]^-$, 100); HRFABMS *m/z* 519.3147 ($C_{30}H_{47}O_5S$ requires 519.3144, Δ −0.3 mmu); ^1H and ^{13}C NMR data (DMSO-d_6), see Tables 1 and 2.

Methyl 3*β*,23(*R*)-dihydroxy-29-*nor*-cycloart-24-en-28-oate 3-sulfate (7): amorphous white solid; $C_{30}H_{47}O_7S^-$; $[\alpha]^{28}_D$ +38° (MeOH, *c* 0.08); UV λ_{max} (in MeOH) 200 nm; IR ν_{max} (film on NaCl) 3436 br, 2930, 2870, 1726, 1587, 1463, 1440, 1411, 1378, 1328, 1231 str/br, 1066, 980, 829 cm^{-1}; negative-mode FABMS *m/z* (rel int) 551 ($[M]^-$, 100); HRFABMS *m/z* 551.3026 ($C_{30}H_{47}O_7S$ requires 551.3043, Δ +1.7 mmu); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Methyl 3*β*-Hydroxy-23-oxo-29-*nor*-cycloart-24-en-28-oate 3-sulfate (8): amorphous white solid; $C_{30}H_{45}O_7S^-$; $[\alpha]^{28}_D$ +40° (MeOH, *c* 0.03); UV λ_{max} (in MeOH) 239 nm; IR ν_{max} (film on NaCl) 3414 br, 2932, 2871, 1724, 1682, 1621, 1456, 1445, 1378, 1292, 1234 str/br, 1064, 994, 979, 811 cm^{-1}; negative-mode FABMS *m/z* (rel int) 549 ($[M]^-$, 100); HRFABMS *m/z* 549.2911 ($C_{30}H_{45}O_7S$ requires 549.2886, Δ −2.5 mmu); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Methyl 3*β*,23(*R*)-dihydroxy-29-*nor*-lanosta-8,24-dien-28-oate 3-sulfate (9): amorphous white solid; $C_{30}H_{47}O_7S^-$; $[\alpha]^{23}_D$ +53.5° (MeOH, *c* 0.11); UV λ_{max} (in MeOH) 210 nm; IR ν_{max} (film on NaCl) 3417 br, 2939, 2868, 1723, 1574 br, 1454, 1444, 1378, 1371, 1293, 1231 str/br, 1055, 986, 804 cm^{-1}; positive-mode FABMS *m/z* (rel int) 597 ($[M + 2Na]^+$, 100), 575 ($[M + HNa]^+$, 58); negative-mode FABMS *m/z* (rel int) 551 ($[M]^-$, 100); HRFABMS *m/z* 551.3061 ($C_{30}H_{47}O_7S$ requires 551.3043, Δ −1.8 mmu); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Methyl 3*β*-hydroxy-23-oxo-29-*nor*-lanosta-8,24-dien-28-oate 3-sulfate (10): amorphous white solid; $C_{30}H_{45}O_7S^-$; $[\alpha]^{28}_D$ +36° (MeOH, *c* 0.10); UV λ_{max} (in MeOH) 239 nm; IR ν_{max} (film on NaCl) 3400 br, 2939, 2871, 1721, 1686, 1617, 1561, 1443, 1409, 1376, 1293, 1231 str/br, 1210, 1080, 1057, 1035, 986, 804 cm^{-1}; negative-mode FABMS *m/z* (rel int) 549 ($[M]^-$, 100); HRFABMS *m/z* 549.2870 ($C_{30}H_{45}O_7S$ requires 549.2886, Δ +1.6 mmu); ^1H and ^{13}C NMR data, see Tables 1 and 2.

Brine Shrimp Toxicity Assay Procedure. Brine shrimp toxicity assay was based on an established 96-well plate protocol.[14]

Cytotoxicity Testing. Cytotoxicity assays were performed by Instituto Biomar S. A., Madrid, Spain.

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Supporting Information Available: Copies of COSY, HMQC/HETCOR, HMBC, and NOESY NMR spectra of compounds **1**, **2**, **4**, **5** and **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

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