Acanthosulfate, a Sulfated Hydroxyhydroquinone Sesterterpenoid from the Sponge *Acanthodendrilla* sp.

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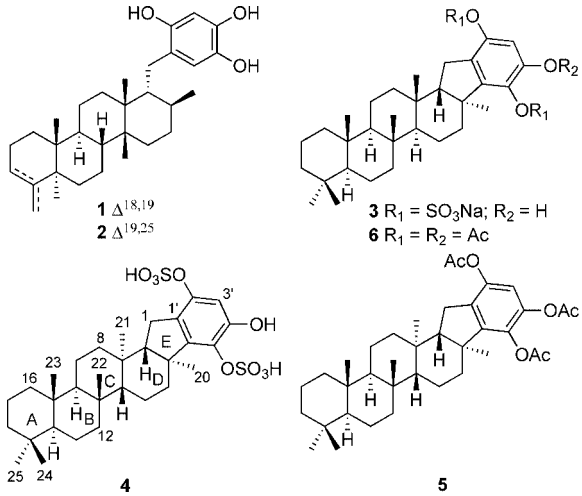
The marine sponge *Acanthodendrilla* sp. contains the proteasome inhibitor acanthosulfate (**4**), a disulfated merosesterterpene having a scalarane-type skeleton. The structure of acanthosulfate (**4**), which possesses an unusual configuration, was elucidated by interpretation of spectroscopic data.

Sponges of the order Dictyoceratida are a rich source of sesterterpenoids possessing the scalarane carbon skeleton,[1] although examples of the merosesterterpenes are relatively rare.[1] Among the merosesterterpenoids are toxistylides A (**1**) and B (**2**) from the sponge Microciona toxistyla[2] and disidein (**3**) from the sponge Dysidea pallescens.[3]



The ubiquitin−proteasome proteolytic pathway is a central player in the regulation of several diverse cellular processes such as selective protein degradation, cell growth, and apoptosis. To date a number of natural and synthetic compounds have been discovered which are able to inhibit proteasomal activity that have found application as molecular probes as well as potential therapeutic agents for the treatment of various human diseases.[4] In the course of screening the crude extracts of marine invertebrates for proteasome inhibition, activity was found in the crude extracts of the Philippines sponge *Acanthodendrilla* sp. A bioassay-directed investigation of this sponge led to the isolation of a disulfated hydroxyhydroquinone sesterterpene, acanthosulfate (**4**). Acanthosulfate was found to have the same planar structure as disidein (**3**), but had different configuration at several sterogenic centers.

The sponge *Acanthodendrilla* sp. was collected by hand using scuba at a depth of 5–27 m near Boracay Island in the Philippines and was kept frozen until extraction. The methanolic extract was fractionated on HP-20 to obtain acanthosulfate (**4**, 0.23% yield). HP-20 poly(styrene-divinylbenzene) resin is a macroporous, cross-linked polymer that lacks any polar sites. Thus it has good utility for the separation of medium-polar biologically active compounds from water-soluble salts and carbohydrates and nonpolar fats and steroids.[5]

Acanthosulfate (**4**) was obtain as a white solid, $[\alpha]^{20}_D$ −35.2. The molecular formula, $C_{31}H_{46}O_9S_2$, was established by high-resolution mass measurement of the $[M - SO_3H]^-$ ion at *m/z* 545.2935 (Δ 0.6 mmu). In the ESIMS the presence of an $[M - H]^-$ ion at *m/z* 625 and fragment ions at *m/z* 545 and 463 suggested the presence of two sulfate groups. A strong S=O stretching band at 1210 cm^{-1} in the IR spectrum also indicated the presence of sulfate groups. An initial analysis of the ^{13}C and DEPT spectra revealed the presence of six methyl groups, 10 methylenes, four methines, five quaternary carbons, and six aromatic carbon resonances, only one of which was protonated (Table 1). A signal at δ 9.21 in the ^1H NMR spectrum was assigned to a phenolic hydroxy group. The aromatic ring accounted for four of the nine double-bond equivalents required by the molecular formula and indicated that acanthosulfate (**4**) contained five additional rings.

A major portion of the backbone of the pentacyclic terpene skeleton was established using the HMBC correlations from the six methyl signals, all of which were singlets in the ^1H NMR, to intervening ring junction methine carbons (Me-20/C-2/Me-21/C-6/Me-22/C-10). Owing to overlap of Me-23 and Me-22 in the ^1H NMR spectrum, the connectivity between Me-23 and C-14 was assigned using the HMBC correlation between H-14 at δ 0.82 and C-23 at δ 16.9. Additional correlations from each methyl signal to both a quaternary carbon and a methylene carbon allowed the assignments of all carbons two and three bonds removed from the ring junction methyl groups. The remaining methylene groups at C-1, C-5, C-9, and C-17 were assigned by using HSQC-TOCSY and COSY correlations. Correlations from H_2-18 signals at δ 1.32 and 1.11 to C-16 and C-17 at δ 40.3 and 18.6, respectively, established the C-16/C-17/C-18 connectivity, and similar HSQC-TOCSY and COSY correlations defined the C-4/C-5/C-6, C-8/C-9/C-10, and C-12/C-13/C-14 connectivities. The COSY spectrum revealed coupling between H-2 at δ 1.41 and H_2-1 at δ 2.53 and 2.34. HMBC correlations from H-2 to the aromatic C-1′ at δ 125.7 and 146.8 (C-6′) together with correlations from H-1a at δ 2.53 to C-1′, C-2′, and C-6′ established the connection of C-1 to C-1′. An additional correlation from Me-20 to the C-6′ signal connected C-3 to C-6′ and thereby created a five-membered ring adjacent to the aromatic ring.

The ^{13}C NMR chemical shifts of the aromatic carbons together with the HMBC correlations from the proton resonance at δ 6.54 (H-3′) suggested a disulfated trihydroxyphenyl ring. HMBC correlations from the phenolic hydroxy signal at δ 9.21 to C-3′, C-4′, and C-5′ established the presence of a hydroxy group at C-4′ and suggested the sulfate groups were at C-2′ and C-5′. Support for

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Table 1. NMR Data for Acanthosulfate **4** (DMSO-d_6)

C#	δ_C (DEPT)	δ_H, mult (J in Hz)	HMBC	ROESY
1a	25.4 (CH_2)	2.53 dd,(14.0, 6.0)	C-1′, C-2′, C-6′, C-2, C-3	H-2
1b		2.34 t (14.0)		Me-20
2	65.3 (CH)	1.41 m	C-6′, C-1′, C-1, C-3, C-6 C-7, C-20, C-21	H-1a, H-6
3	47.7 (C)			
4a	37.4 (CH_2)	2.37 m		H-4b, Me-20
4b		1.56 m		H-4a
5	19.6 (CH_2)	1.53 m		
6	56.0 (CH)	0.98 m	C-12, C-21	H-2, Me-22
7	36.1 (C)			
8a	39.0 (CH_2)	1.49 m		
8b		1.36 m		
9a	16.1 (CH_2)	1.52 m		
9b		1.22 m		Me-22
10	50.8 (CH)	1.66 d (13.0)	C-9, C-11, C-12, C-22, C-23	H-14, Me-21
11	37.2 (C)			
12a	36.0 (CH_2)	1.54 m	C-22	
12b		1.33 m	C-22	Me-20, Me-22
13a	19.7 (CH_2)	1.52 m		
13b		1.39 m		
14	57.3 (CH)	0.82 m	C-23	H-10
15	37.9 (C)			
16a	40.3 (CH_2)	1.60 m		
16b		0.79 m		
17a	18.6 (CH_2)	1.59 m		
17b		1.38 m		
18a	42.3 (CH_2)	1.32 m		
18b		1.11 m		
19	33.4 (C)			
20	20.8 (CH_3)	1.00 s	C-6′, C-2, C-3, C-4	H-1b, H-4a, Me-21
21	21.4 (CH_3)	1.18 s	C-8, C-7, C-6, C-2	H-10, Me-20
22	23.6 (CH_3)	0.90 s	C-12, C-11, C-10, C-6	H-6, H-9b, H-12b, Me-23
23	16.9 (CH_3)	0.79 s	C-10, C-15, C-16	Me-22, Me-24
24	21.4 (CH_3)	0.78 s	C-14, C-18, C-19, C-25	Me-23
25	33.4 (CH_3)	0.81 s	C-14, C-18, C-19, C-24	
1′	125.7 (C)			
2′	146.1 (C)			
3′	108.20 (C)	6.54 s	C-1′, C-2′, C-4′, C-5′	
4′	148.20 (C)			
5′	132.80 (C)			
6′	146.8 (C)			
4′-OH		9.21s	C-3′, C-4′, C-5′	

this assignment was provided by a ^{13}C NMR experiment carried out in the presence of a 1:1 mixture of H_2O/D_2O, which allows the identification of carbon atoms two and three bonds away from an exchangeable proton due to the deuterium isotope effect. Carbon C-4′ showed a large apparent splitting of 0.4 ppm indicating a short-range (two-bond) deuterium isotope effect consistent with a phenolic hydroxy group at this position. The C-3′ and C-5′ signals showed small apparent splittings of 0.02 ppm, indicative of a long-range (three-bond) deuterium isotope effect. The planar structure of acanthosulfate (**4**) is probably identical to that of disidein (**3**), although the position of the sulfate groups on disidein was never unambiguously determined.

Comparison of the ^{13}C NMR spectrum of acanthosulfate (**4**) with that of the disidein triacetate (**6**) showed significant differences in the chemical shifts of C-2, C-6, C-10, Me-21, and Me-22, which suggested a configurational difference in the sesterterpene portion of the molecule. The relative configuration of **4** was determined by NOE correlations observed in a ROESY experiment (Figure 1). The NOE correlations observed between Me-22 and Me-23 and between H-10 and H-14 established a *trans*−*anti*−*trans* relationship between rings A, B, and C. An NOE observed between Me-22 and H-6 suggested a *cis* relationship between these two groups. A long-range NOE correlation between H-10 and Me-21 was consistent with a *cis* relationship and suggested that ring C was in a boat conformation. This stereochemistry is consistent with a downfield shift of Me-22 from δ 18.1 in the disidein triacetate (**6**) to δ 23.6 in **4** and an upfield shift of H-6 from δ 1.80 to 0.98. The *anti*-periplanar relationship of Me-21 and H-2 was assigned from the NOE correlations observed between H-6 and H-2 and between Me-21 and Me-20. Finally, correlations observed between H-2 and H-1a and between Me-20 and H-1b established the *trans*-fused nature of the five-membered ring. To further confirm the proposed difference in the relative configurations, acanthosulfate (**4**) was converted into the corresponding triacetate **5**, to allow a direct comparison of NMR data with those reported for disidein triacetate (**6**).[6] Hydrolysis of **4** using *p*-toluenesulfonic acid followed by acetylation of the crude product mixture with acetic anhydride in pyridine yielded the triacetate derivative **5**. The high-resolution mass measurement of the $[M + Na]^+$ ion at m/z 615.3655 (Δ –0.1 mmu) confirmed that the product was the expected triacetate. Analysis of the NMR data allowed most proton and carbon signals to be assigned by direct comparison of their chemical shifts with those of **4**. The NMR data also contained signals due to three acetate groups, and the chemical shifts in the aromatic ring carbons

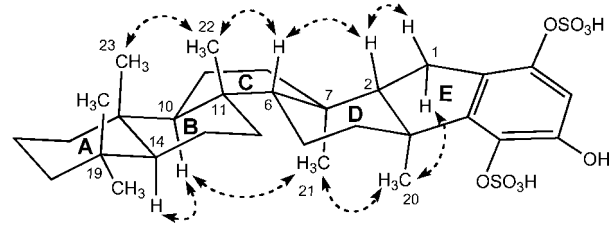

Figure 1. Selected NOE correlations observed for acanthosulfate (**4**), which establish the relative configuration of the stereogenic centers of the A, B, C, D, and E rings.

were consistent with a 1,2,4-triacetate. A comparison of the spectroscopic data of **5** with those of disidein triacetate revealed ^{13}C NMR chemical shift differences and NOE enhancements consistent with the proposed stereochemistry of **4** and **5**.

Acanthosulfate (**4**) is the first reported merosesterterpene possessing a scalarane-type skeleton with a *trans*−*antitrans*−*syn*−*anti* configuration. The only other scalaranes that were reported to have an abnormal configuration are coscinalactone and coscinafuran, isolated from the marine sponge *Coscinoderma mathewsi*,[7] which possess the *trans*−*antisyn*−*trans*−*antitrans* configuration. Compound **4** was evaluated for inhibition of the proteasome function, the enzyme responsible for the degradation of endogenous proteins. The compound showed an IC_{50} value of 4.5 μM, however lacked selectivity and potency in the BMS Oncology Diverse Cell Panel (ODCA) to be considered for *in vivo* evaluation.[8]

Experimental Section

General Experimental Procedures. The ^1H, ^{13}C, and DEPT spectra were recorded on a Varian Gemini 400 MHz spectrometer. All 2D NMR experiments were performed on a Varian Inova 300 MHz NMR spectrometer. All chemical shifts were referenced to the residual solvent peak (DMSO-d_6: ^1H, δ 2.39: ^{13}C, δ 39.5; $CDCl_3$: ^1H, δ 7.25: ^{13}C, δ 77.0). Optical rotations were measured on a Rudolph Research Autopol III polarimeter (*c* g/100 mL) at 589 nm. IR spectra were recorded on a Perkin-Elmer 1600 FTIR spectrophotometer. UV spectra were recorded on a Lambda 3B instrument. ESIMS spectra were recorded using a Finnigan LCQ mass spectrometer. The high-resolution MALDI Fourier transform mass spectrum was recorded on an Ionspec FTMS mass spectrometer at the Scripps Research Institute. The HRFABMS measurement performed on a VG ZAB2SE was obtained from the UC Riverside Regional Facility.

Biological Material. The sponge *Acanthodendrilla* sp. (order Dendroceratida, family Dictyodendrillidae) was collected using scuba at a depth of 5−27 m at Boracay Island, Philippines, in 1998 and was immediately frozen and stored at −20 °C until extraction. The sponge was identified by John Hooper and Mary Kay Harper and is believed to belong to the genus *Acanthodendrilla*, possibly *A. australis* (Bergquist, 1995). A voucher specimen has been deposited in the Scripps Institution of Oceanography Benthic Invertebrate Collection (#P1191).

Extraction and Purification. The sponge (150 g wet wt) was extracted with MeOH (2 × 200 mL) for 24 h. The second and then the first extracts were passed through a column of HP-20 (2.5 × 15 cm). The combined eluents was repassed through the column. Finally the eluent was diluted with H_2O (800 mL) and passed again through the column. The column was eluted with 300 mL fractions of (1) H_2O, (2) 25% Me_2CO/H_2O, (3) 50% Me_2CO/H_2O, (4) 75% Me_2CO/H_2O, and (6) Me_2CO. Concentration of fraction 3 yielded 350 mg of **4**.

Acanthosulfate (4): white solid; $[\alpha]^{20}_D$ −35.2 (*c* 0.09, MeOH); IR (KBr) ν_{max} 3385, 2940, 2850, 1640, 1210, 1135, 1105, 1045, 1000 cm^{-1}; UV (MeOH) λ_{max} 210 nm (ϵ 33 300), 220 (ϵ 24 388), 280 (ϵ 6017); ^1H and ^{13}C NMR (400 MHz, DMSO-d_6) see Table 1; HRFABMS *m/z* 545.2935 $[M - SO_3H]^-$, calcd for $C_{31}H_{45}O_6S$, 545.2931.

Acid Hydrolysis and Acetylation of 4. To a solution of **4** (50 mg) in Me_2CO (1.5 mL) was added excess *p*TsOH·H_2O. After 1 h the reaction was quenched with H_2O (500 μL) and passed through a column of HP-20ss (1 × 2 cm). The eluent was diluted with H_2O and repassed through the column. The column was then eluted with 10 mL fractions of (1) 50% Me_2CO/H_2O, and (2) Me_2CO. The Me_2CO fraction was concentrated to dryness and dissolved in pyridine (1.5 mL) and Ac_2O (2 mL). The solution was stirred at room temperature for 24 h and then quenched with H_2O (1 mL). The crude reaction mixture was passed through a column of HP-20ss (1 × 2 cm). The eluent was diluted with H_2O (5 mL) and repassed through the column. The column was then eluted with 10 mL fractions of (1) 20% Me_2CO/H_2O, (2) 40% Me_2CO/H_2O, (3) 60% Me_2CO/H_2O, (5) 80% Me_2CO/H_2O, and (6) Me_2CO. Fractions 5 and 6 (30 mg) were concentrated to dryness and chromatographed on Si gel using a gradient from hexanes to EtOAc eluants to obtain triacetate **5** (17.8 mg, 36% yield).

Triacetate 5: white solid; $[\alpha]^{20}_D$ −35.6 (*c* 0.40, CH_2Cl_2); IR (KBr) ν_{max} 2988, 2930, 2860, 1771, 1468, 1450, 1363, 1194, 1165, 1089, 1013 cm^{-1}; UV (ACN) λ_{max} 204 nm (ϵ 20 128), 216 (ϵ 10 122), 266 (ϵ 846); ^1H NMR data (400 MHz, $CDCl_3$) δ 6.78 (1H, s, H-3′), 2.48 (2H, 2.48, H_2-1), 2.27 (3H, s, OAc), 2.27 (3H, s, OAc), 2.22 (3H, s, OAc), 2.10 (1H, m, H-4a), 1.70 (1H, m, H-2), 1.68 (1H, m, H-10), 1.64, (1H, m, H-16a), 1.62 (1H, m, H-17a), 1.60 (1H, m, H-4b), 1.59 (1H, m, H-5a), 1.59 (2H, m, H-13), 1.58 (1H, m, H-9a), 1.55 (1H, m, H-12), 1.53 (1H, m, H-8a), 1.42 (1H, m, H-17b), 1.40 (1H, m, H-8b), 1.40 (1H, m, H-5b), 1.36 (1H, m, H-18a), 1.36 (1H, m, H-12b), 1.26 (1H, m, H-9b), 1.22 (3H, s, H-21), 1.15 (1H, m, H-18b), 1.07 (3H, s, H-20), 1.05 (1H, m, H-6), 0.90 (3H, s, H-22), 0.85 (1H, m, H-14), 0.83 (3H, s, H-25), 0.82 (1H, m, H-16b), 0.81 (3H, s, H-23), 0.80 (3H, s, H-24); ^{13}C NMR data (100 MHz, $CDCl_3$) δ 168.3 (OAc), 168.3 (OAc), 167.9 (OAc), 147.0 (C-6′), 144.1(C-2′), 140.8 (C-4′), 134.7 (C-5′), 133.5 (C-1′), 114.3 (C-3′), 64.8 (C-2), 57.5 (C-14), 55.7 (C-6), 51.0 (C-10), 47.8 (C-3), 42.5 (C-18), 40.4 (C-16), 38.9 (C-8), 38.0 (C-15), 37.3 (C-11), 36.9 (C-4), 36.3 (C-12), 36.1 (C-7), 33.5 (C-19), 33.4 (C-25), 26.1 (C-1), 23.5 (C-22), 21.2 (C-24), 21.2 (C-21), 21.0 (OAc), 20.8 (C-20), 20.8 (OAc), 20.5 (OAc), 19.8 (C-13), 19.8 (C-5), 18.8 (C-17), 16.9 (C-23), 16.1 (C-9); MALDI-FTMS *m/z* 615.3655 $[M + Na]^+$, calcd for $C_{37}H_{52}O_6Na$ 615.3656.

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Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

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