Adociasulfates 1−6, Inhibitors of Kinesin Motor Proteins from the Sponge *Haliclona* (aka *Adocia*) sp.

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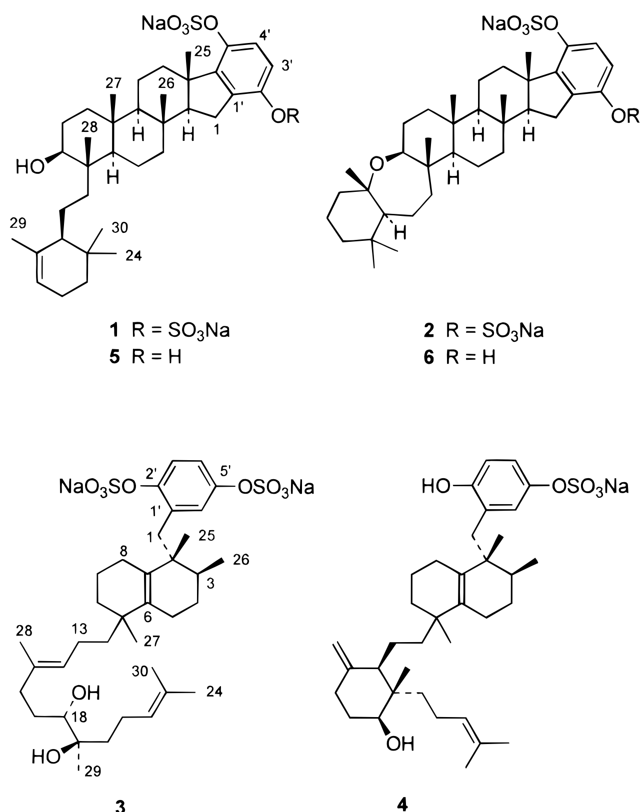
Adociasulfates 1−6 (**1**−**6**) were isolated from an extract of the Palauan sponge *Haliclona* (aka *Adocia*) sp. that inhibited the transport of stabilized microtubules by the motor protein kinesin, which was immobilized on a microscope slide. The structures of adociasulfates 1−6, the relative stereochemistry of adociasulfates 1, 2, 5, and 6, and the relative stereochemistry of subunits of adociasulfates 3 and 4 were determined by interpretation of spectroscopic data. In a quantitative assay that measures ATP hydrolysis by kinesin, adociasulfates 2 and 6 were the most active.

Kinesins comprise a superfamily of eukaryotic motor proteins used to transport a large variety of cargoes along microtubule tracks. They are important targets for inhibition because they are involved in many dynamic microtubule-mediated events, including cell division and the transport of vesicles and organelles.[3,4] As part of our program to study marine natural products that affect basic cellular processes, we screened both the aqueous and organic extracts of 134 sponges from Palau, Western Caroline Islands, for their ability to disrupt the transport of stabilized microtubules by kinesin motor proteins, which were immobilized on a microscope slide. This initial screening gave positive results for 18 extracts, of which the crude aqueous extract of *Haliclona* (aka *Adocia*) sp. appeared the most promising. The extract was chromatographed on TSK HW-40 solid support to obtain adociasulfates 1 (**1**, 0.09% yield) and 2 (**2**, 0.132% yield), as the major active metabolites. Adociasulfate 2 (**2**) was shown to be the first specific inhibitor of kinesin motor proteins.[5] We subsequently isolated adociasulfates 3 (**3**, 0.076% yield), 4 (**4**, 0.032% yield), 5 (**5**, 0.012% yield), and 6 (**6**, 0.012% yield). In this paper, we report the structural elucidation of adociasulfates 1−6 (**1**−**6**) together with a comparative study of their inhibition of motor proteins.

Adociasulfate 1 (**1**) was isolated as an amorphous white solid. The molecular formula, $C_{36}H_{52}O_9S_2Na_2$, which was determined by high-resolution mass measurement of the $[M - Na]^-$ ion, suggested the presence of two sulfate ester groups. The IR spectrum contained bands at 3425 (hydroxyl), 1635 (aromatic), and 1230 (sulfate) cm^{-1}. The ^{13}C NMR spectrum (Table 1) contained eight signals in the aromatic region, due to a tetrasubstituted aromatic ring and a trisubstituted olefin. These data required a hexacyclic alcohol with one aromatic ring. The ^1H NMR spectrum (Table 1) contained two aromatic proton signals at δ 7.30 (d, 1 H, J = 8.5 Hz) and 7.10 (d, 1 H, J = 8.5 Hz) that were directly coupled to ^{13}C signals at δ 120.2 and 120.6, respectively.



The ^{13}C chemical shifts, together with the three-bond HMBC correlations, defined the aromatic ring as a 2,3-disubstituted hydroquinone disulfate. The benzylic proton signals at δ 2.82 (dd, 1 H, J = 15, 6 Hz) and 2.61 (dd, 1 H, J = 15, 13.5 Hz) were coupled to a methine signal at

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Table 1. ^{1}H (300 MHz, MeOH-d_4) and ^{13}C (100 MHz, MeOH-d_4) NMR Data for 1 and 2

	1				2		
C no.	δ_C	δ_H	mult, J (Hz)	HMBC (**1** and **2**)	δ_C	δ_H	mult, J (Hz)
1	27.1	2.82	dd, 15, 6	C-2, C-3, C-1′, C-6′	27.6	2.86	dd, 15, 6
		2.61	dd, 15, 13.5	C-2, C-6′		2.66	dd, 15, 13.5
2	65.6	1.71	dd, 13.5, 6		65.6	1.76	dd, 13.5, 6
3	48.6				48.6		
4	38.8	2.56	br d, 13		39.0	2.59	br d, 13
		1.75	m			1.82	m
5	18.7	1.38	m, 2 H		19.1	1.58	m, 2 H
6	63.2	0.91	m		63.2	0.97	m
7	38.6				38.6		
8	42.9	1.62	m		43.5	1.72	m
		1.06	m			1.18	m
9	19.6	1.63	m, 2 H		19.7	1.72	m, 2 H
10	51.5	0.94	m		59.3	0.88	m
11	38.3				38.5		
12	39.6	1.66	m		40.3	1.76	m
		1.04	m			1.04	m
13	28.3	1.64	m		28.5	1.72	m
		1.58	m			1.40	m
14	73.6	3.42	dd, 11, 5	C-27	75.4	3.60	dd, 11, 5
15	42.5				43.0		
16	39.1	1.67	m, 2 H		46.6	1.81	m, 2 H
17	25.3	1.35	m		21.2	1.54	m
		1.02	m			1.48	m
18	51.3	1.28	m		55.1	1.54	m
19	138.2				79.5		
20	120.6	5.22	br s		39.5	1.52	m, 2 H
21	24.0	1.90	m, 2 H		22.5	1.60	m, 2 H
22	32.5	1.41	m		42.3	1.38	m
		1.04	m			1.20	m
23	33.6				36.8		
24	28.0	0.82	s, 3 H	C-18, C-22, C-23, C-30	21.7	0.83	s, 3 H
25	21.3	1.12	s, 3 H	C-2, C-3, C-4, C-6′	21.4	1.17	s, 3 H
26	17.9	1.02	s, 3 H	C-2, C-6, C-7, C-8	18.0	1.09	s, 3 H
27	17.3	0.88	s, 3 H	C-6, C-10, C-11, C-12	17.0	0.94	s, 3 H
28	17.9	0.75	s, 3 H	C-10, C-14, C-15, C-16	21.7	0.83	s, 3 H
29	24.0	1.64	s, 3 H	C-18, C-19, C-20	23.6	1.17	s, 3 H
30	28.4	0.91	s, 3 H	C-18, C-22, C-23, C-29	33.6	0.97	s, 3 H
1′	138.3				138.5		
2′	146.3				146.5		
3′	120.6	7.10	d, 8.5	C-1′, C-5′	121.1	7.10	d, 9
4′	120.2	7.30	d, 8.5	C-2′, C-6′	120.4	7.33	d, 9
5′	146.8				146.9		
6′	147.1				147.3		

δ 1.71 (dd, 1 H, J = 13.5, 6 Hz), with no further correlations. The HMBC correlations from the H-1 signal at δ 2.82 to C-2, C-3, and two aromatic signals, together with the HMBC correlations from Me-25 to C-2, C-3, C-4, and one of the aromatic signals, defined the five-membered ring attached to the aromatic ring. The backbone of the pentacyclic ring system was elucidated from a series of HMBC correlations from the methyl signals, all of which were singlets in the ^{1}H NMR spectrum, to the methine ring junction carbon signals (Me-25/C-2/Me-26/C-6/Me-27/C-10/Me-28). As expected, Me-25, Me-26, and Me-27 each showed additional correlations to both fully substituted and methylene carbon signals while Me-28 showed correlations to a fully substituted carbon and to a carbon signal at δ 73.6 assigned to the hydroxymethine group. The remaining ring contained the trisubstituted olefin and three methyl groups. The ^{1}H NMR chemical shifts coupled with the HMBC experiment indicated that one methyl group was vinylic and the other two formed a *gem*-dimethyl group. Since all three methyl proton signals were correlated to C-18, this strongly suggested that we were dealing with a normal polyisoprenoid containing a 6-substituted 1,5,5-trimethylcyclohexene ring system. All remaining spectral data were appropriate for the proposed structure of adociasulfate 1 (**1**), the stereochemistry of which was derived from that of adociasulfate 2 (**2**). All attempts to determine the absolute stereochemistry of adociasulfate 1 (**1**) by forming Mosher's esters at the 14-hydroxyl group were unsuccessful.

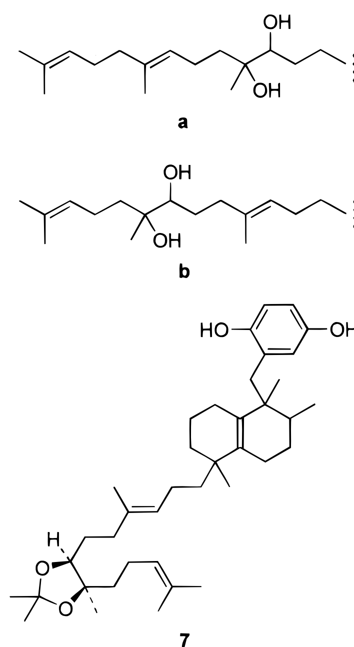
Adociasulfate 2 (**2**), which was isolated as an amorphous white solid, had the same molecular formula as adociasulfate 1 (**1**), $C_{36}H_{52}O_9S_2Na_2$. The IR spectrum was similar to that of **1** except that it lacked the hydroxyl band. The major difference in the ^{13}C NMR spectrum (Table 1) was that there were only six signals in the aromatic/olefinic region and that there was an additional signal in the region assigned to carbon atoms bearing oxygen. In the ^{1}H NMR spectrum, there were no signals in the olefinic region and the vinyl methyl signal had moved upfield. From these data, we proposed that adociasulfate 2 (**2**) was simply the cyclic ether derived by cyclization of adociasulfate 1 (**1**). A detailed analysis of the spectral data resulted in the assignments listed in Table 1. The stereochemistry of **2** was determined from a ROESY experiment that showed correlations between the axial methyl groups from Me-25 to Me-28 as expected for the all *trans-anti-trans* ring system. The coupling constants of H-14 at δ 3.60 (dd, 1 H, J = 11, 5 Hz) required H-14 to be axial. A strong NOE correlation between H-14 and H-18 indicated that both protons were on the α-face of the compound. Finally, there was a strong correlation between the two axial methyl signals, Me-29 and Me-30. These data clearly defined the relative stereochemistry of adociasulfate 2 (**2**).

Adociasulfate 3 (**3**) was isolated as an opaque glass. The molecular formula, $C_{36}H_{54}O_{10}S_2Na_2$, which was determined by high-resolution mass measurement of the $[M + Na]^+$ ion at m/z 779.2898, again suggested the presence of two sulfate groups. The major bands in the IR spectrum were not significantly different from those of adociasulfate 1 (**1**). The ^{13}C NMR spectrum (Table 2) contained 12 signals in the olefinic/aromatic region that were due to one aromatic ring and three olefins. Since the molecular formula required nine unsaturation equivalents, adociasulfate 3 (**3**) had to contain two additional rings. The ^{1}H NMR spectrum (Table 2) contained three signals in the aromatic region at δ 7.38 (d, 1 H, J = 8.5 Hz), 7.22 (d, 1 H, J = 2.5 Hz), and 7.11 (dd, 1 H, J = 8.5, 2.5 Hz) that were assigned to a monoalkylated hydroquinone disulfate. Interpretation of the HMQC and HMBC data allowed the assignment of signals at δ 150.3 (C-2′), 149.9 (C-5′), 135.3 (C-1′), 125.8 (C-6′), 123.1 (C-3′), and 120.6 (C-4′) to the aromatic ring. Two olefinic proton signals at δ 5.17 (t, 1 H, J = 7 Hz) and 5.12 (t, 1 H, J = 7 Hz) were assigned to trisubstituted olefins on an alkyl chain. The signal at δ 5.12 showed a direct correlation to a signal at δ 126.2, which in turn showed three-bond correlations to methyl signals at δ 1.61 (br s, 3 H) and 1.66 (br s, 3 H), indicative of a terminal dimethylvinyl group. The signal at δ 5.17 showed a direct correlation to a signal at δ 126.9, which in turn showed

Table 2. **^{1}H (300 MHz, MeOH-d_4) and ^{13}C (100 MHz, MeOH-d_4) NMR Data for 3**

C no.	δ_C	δ_H	mult, J (Hz)	HMBC
1	37.8	2.81	d, 14.5	C-1′, C-2, C-2′, C-3, C-6′,
		3.08	d, 14.5	C-7, C-25
2	43.5			
3	34.9	1.74		C-26
4	27.9	1.84	m	
		1.91	m	
5	23.6	1.94	m, 2H	
6	135.8			
7	136.0			
8	30.5	1.28	m, 2H	
9	21.2	1.64	m	
		1.60	m	
10	36.8	1.64	m	
		1.30	m	
11	39.0			
12	42.0	1.19	m	
		1.43	m	
13	24.4	1.96	m	
		1.76	m	
14	126.9	5.17	t, 7	
15	135.5			
16	38.1	1.98	m, 2H	
17	27.6	2.15	m	
		1.36	m	
18	78.1	3.26	under MeOH	
19	75.6			
20	39.7	1.50	m	
		1.42	m	
21	23.1	2.02	m, 2H	
22	126.2	5.12	t, 7	
23	132.0			
24	25.9	1.66	br s, 3 H	C-22, C-23, C-30
25	22.8	0.92	s, 3 H	C-1, C-2, C-3, C-7
26	16.8	0.83	d, 6.5, 3 H	C-2, C-3, C-4
27	28.2	1.05	s, 3 H	C-6, C-10, C-11, C-12
28	16.3	1.59	br s, 3 H	C-14, C-15, C-16
29	22.1	1.09	s, 3 H	C-18, C-19, C-20
30	17.9	1.61	br s, 3 H	C-22, C-23, C-24
1′	135.3			
2′	150.3			
3′	123.1	7.38	d, 8.5	C-1′, C-2′, C-5′
4′	120.6	7.11	dd, 8.5, 2.5	C-2′, C-5′, C-6′
5′	149.9			
6′	125.8	7.22	d, 2.5	C-2′, C-4′, C-5′

a three-bond correlation to a methyl signal at δ 1.59 (br s, 3 H) and required the presence of a methyl-substituted trisubstituted olefin. Having assigned signals at δ 132.0 and 135.5 to the fully substituted olefinic carbons of the trisubstituted olefins, we were left with two downfield signals at δ 135.8 and 136.0 that were assigned to a fully substituted olefin located at the bicyclic ring junction. ^{1}H NMR signals at δ 3.08 (d, 1 H, J = 14.5 Hz) and 2.81 (d, 1 H, J = 14.5 Hz), which showed HMBC correlations to three aromatic ring carbons (C-1′, C-2′, and C-6′), the olefinic carbon signal at δ 136.0 (C-7), the methyl carbon at δ 22.8 (C-25), a methine signal at δ 34.9 (C-3), and a fully substituted carbon signal at δ 43.5 (C-2) were assigned to an isolated methylene group between the aromatic ring and the bicyclic ring system. The corresponding carbon signal at δ 37.8 (C-1) was correlated with the methyl signal at δ 0.92 (s, 3 H), which was in turn correlated to C-2, C-3, and C-7. HMBC correlations between the Me-26 signal at δ 0.83 (d, 3 H, J = 6.5 Hz) and C-2, C-3, and a methylene signal at δ 27.9 (C-4) placed the methyl group at C-3. The methyl signal at δ 1.05 (s, 3 H) showed HMBC correlations to the olefinic signal at δ 135.8 (C-6), methylene signals at δ 42.0 (C-12) and δ 36.8 (C-10), and a fully substituted carbon signal at δ 39.0 (C-11). On the basis of biosynthetic considerations, we proposed that **3** contained a decalin ring system with a double bond at the ring junction and that the alkyl chain was attached at C-11. The remaining methyl signal at δ 1.09 (δ_C 22.1) was correlated to signals at δ 78.1 (–CH–), 75.6 (–C–), and 39.7 (–CH_2–), which were assigned to a –CH_2CMe(OH)CH(OH)– moiety. Again using biosynthetic considerations, two alkyl chains **a** and **b** could be proposed. Although chain **a** was originally preferred on the basis of a biosynthetic argument that allows **3** to be derived from the same epoxide as **1** and **2**, analysis of the mass spectrum using MS/MS resulted in the identification of a fragmentation pathway from m/z 733 $[M - Na]^-$ to 606 $[M - Na - C_8H_{15}O]^-$ to 562 $[M - Na - C_{10}H_{19}O_2]^-$ and from m/z 733 to 650 $[M - Na - C_6H_{11}]^-$, which clearly favored chain **b**. The presence of chain **b** was confirmed using a 1D TOCSY experiment that revealed coupling from H-18 at δ 3.26 to H-14 at δ5.17. All other data were in accord with the planar structure assigned to adociasulfate 3 (**3**).



The relative stereochemistry at C-18 and C-19 was determined by analysis of the ROESY spectrum of the acetonide **7**, which was formed by treating the diol **3** with 2,2-dimethoxypropane in DMF containing pyridinium *p*-toluenesulfonate. The acetonide functionality gave rise to two new methyl signals in the ^{1}H NMR spectrum at δ 1.36 and 1.29 that both showed HMBC correlations to the ketal carbon signal at δ_C 107.9 (C-31). Examination of the ROESY data for **7** showed that both the H-18 methine proton signal at δ 3.70 and the C-29 methyl signal at 1.18 were correlated to the same acetonide methyl signal at δ 1.29 (H-33), proving that H-18 and methyl-29 were on the same face of the acetonide ring. The relative stereochemistry at C-2 and C-3 was determined by analysis of ROESY correlations as described for adociasulfate 4 (**4**).

Adociasulfate 4 (**4**), which was obtained as an amorphous white solid after lyophilization, is a monosulfate that has the molecular formula $C_{36}H_{53}O_6SNa$. The ^{1}H NMR spectrum (Table 3) contained three aromatic signals at δ 7.06 (d, 1 H, J = 3 Hz, H-6′), 6.94 (dd, 1 H, J = 8.4, 3 Hz, H-4′), and 6.64 (d, 1 H, J = 8.4 Hz, H-3′) that

Table 3. ^{1}H (300 MHz, MeOH-d_4) and ^{13}C (100 MHz, MeOH-d_4) NMR Data for 4

C no.	δ_C	δ_H	mult, J (Hz)	HMBC
1	37.3	2.61	d, 12	C-1′, C-2, C-2′, C-3, C-6′, C-7, C-25
		2.89	d, 12	
2	43.5			
3	35.0	1.77	m	C-2
4	27.7	2.09	m, 2H	
5	23.5	1.93	m, 2H	C-4
6	135.4			
7	136.1			
8	27.9	1.84	m	
		1.91		
9	20.7	1.23	m	
		1.43	m	
10	37.5	1.12	m, 2H	C-11
11	38.9			
12	41.3	1.31	m	
		1.96	m	
13	21.3	1.61	m, 2H	
14	48.7	1.75	m	C-15, C-16, C-18, C-19, C-28, C-29
15	149.6			
16	35.1	1.98	m	C-14, C-15, C-17, C-18, C-28
		2.30	dt, 13, 4	
17	33.2	1.52	m	
		1.78	m	
18	73.4	3.62	dd, 12, 6	C-14, C-17, C-19, C-20, C-29
19	44.2			
20	37.0	1.24	m	
		1.30	m	
21	22.6	2.04	m	
		1.90	m	
22	126.4	5.07	t, 4.5	C-24, C-30
23	131.8			
24	26.1	1.67	s, 3 H	C-22, C-23, C-30
25	22.7	0.91	s, 3 H	C-1, C-2, C-3, C-7
26	17.0	0.82	d, 6.9, 3 H	C-2, C-3, C-4
27	28.7	1.03	s, 3 H	C-6, C-10, C-11, C-12
28	108.7	4.58	br s	C-14, C-16
		4.79	br s	
29	16.5	0.64	s, 3 H	C-14, C-18, C-19, C-20
30	18.2	1.62	s, 3 H	C-22, C-23, C-24
1′	128.4			
2′	154.6			
3′	115.7	6.64	d, 8.4	C-1, C-1′, C-2′, C-4′, C-5′
4′	121.0	6.94	dd, 8.4, 3	C-2′, C-5′, C-6′
5′	146.1			
6′	126.2	7.06	d, 3	C-1, C-2′, C-4′, C-5′,

were assigned to a 1-alkyl-2,5-hydroquinone-5-sulfate. The ^{13}C NMR spectrum (Table 3) contained 12 downfield signals, of which those at δ 154.6 (C-2′), 146.1 (C-5′), 128.4 (C-1′), 126.2 (C-6′), 121.0 (C-4′), and 115.7 (C-3′) were assigned to the aromatic ring. The other six were due to three olefins. Since the molecular formula required 10 unsaturation equivalents, adociasulfate 4 (**4**) had to contain three additional rings. Comparison of the spectral data of **4** with those of **3** allowed us to identify a terminal dimethylvinyl group and a decalin ring system with identical unsaturation across the ring junction and methyl and benzylic substitution patterns. The additional ring must therefore be in the side chain between C-13 and C-21. The most significant changes in the ^{1}H NMR spectrum were the presence of methylene signals at δ 4.79 (br s, 1 H, obscured by H_2O in methanol-d_4) and 4.58 (br s, 1 H), both of which showed three-bond correlations in the HMBC spectrum to a methine carbon signal at 48.7 (C-14). A methyl signal at δ 0.64 (s, 3 H) also showed a three-bond correlation to the signal at δ 48.7 and to signals at δ 73.4 (C-18), 44.2 (C-19), and 37.0 (C-20). We therefore proposed that **4** contained a six-membered ring formed by cyclization of **3** between C-14 and C-19. The relative stereochemistry of the six-membered ring was established using a ROESY experiment. The H-18 signal at δ 3.62 (dd, 1 H, J = 12, 6 Hz) was axial and showed NOE correlations to H-14$_{ax}$ at δ 1.75 and H-16$_{ax}$ at δ 1.98, which was geminally coupled to H-16$_{eq}$ at δ 2.30 (dt, 1 H, J = 13, 4 Hz) that in turn showed a NOE correlation to the olefinic signal at δ 4.79. The methyl signal at δ 0.64 showed NOE correlations to signals at δ 1.24 (H-20) and 1.52 (H-17$_{ax}$), which was coupled to signals at δ 3.62, 2.30, 1.98, and 1.78 (H-17$_{eq}$). The ROESY correlations thus defined the relative stereochemistry about the six-membered ring. Analysis of the ROESY correlations between Me-25, Me-26, and the H-1 signal at δ 2.89 in adociasulfate 4 (**4**) indicated that Me-25 was axial while Me-26 was equatorial. The 6,7-olefin flattens the bicyclic ring system and prevents correlations between the substituents at C-2 and those at C-11.

Adociasulfates 5 (**5**) and 6 (**6**) are monosulfates of adociasulfates 1 (**1**) and 2 (**2**), respectively. Both have the same molecular formula, $C_{36}H_{53}O_6SNa$, which was determined by high-resolution mass measurement of the $[M - Na]^-$ ion. The major differences in the NMR spectral data of **5** and **6**, when compared with those of **1** and **2**, were observed in the aromatic regions of the spectra. In the ^{1}H NMR spectra of both **5** and **6**, the proton on the carbon adjacent to the phenol occurred at δ 6.45 as opposed to δ 7.10 for the same proton in the spectra of both **1** and **2**. The position of the phenol was defined by interpretation of the HMQC and HMBC data: the key correlations for both **5** and **6** were the three-bond couplings between the C-6′ signals at δ 147.5 and both the CH_3-25 signal at δ 1.12 and the aromatic H-4′ signal at δ 7.13, which is adjacent to the sulfate group. All other spectral data support the structural assignments of adociasulfates 5 (**5**) and 6 (**6**).

The adociasulfates are new members of a relatively rare, but consistently bioactive, group of sulfated hexaprenoid hydroquinones. The Red Sea sponge *Toxiclona toxius* yielded a series of inhibitors of HIV-1 reverse transcriptase, of which toxiusol was the most active.[6] A similar compound, akaterpin, which was isolated from a *Callyspongia* sp., was an inhibitor of phosphatidylinositol-specific phospholipase C.[7] The adociasulfates inhibit the motor protein kinesin by a mechanism that targets its motor domain and mimics the activity of the microtubule.[5]

Motor proteins are powered by conversion of ATP to ADP, releasing inorganic phosphate. On addition of microtubules to a solution containing kinesin and ATP, the rate of ATP hydrolysis increases ca. 1000-fold. Motor protein inhibition can therefore be measured by using a simple colorimetric phosphatase assay,[8] which was modified to use EnzCheck (360 nm) in place of malachite green as an indicator to measure inorganic phosphate release. The IC_{50} values are the concentration of each inhibitor required to decrease the microtubule-stimulated ATPase activity of kinesins by 50%. As shown in Table 4, adociasulfates 2 (**2**) and 6 (**6**) are the most active, with

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Table 4. Inhibiton of ATPase Activity in Kinesin by Adociasulfates 1−6 (1−6)

compound	IC_{50} (mM)	compound	IC_{50} (mM)
adociasulfate 1 (**1**)	12.5	adociasulfate 4 (**4**)	15
adociasulfate 2 (**2**)	6	adociasulfate 5 (**5**)	8
adociasulfate 3 (**3**)	10	adociasulfate 6 (**6**)	6

the disulfate **2** and the monosulfate **6** having essentially identical efficacy.

It is striking that removal of one of the sulfates does not disturb inhibitory activity, while alterations in the hydrophobic ring system result in substantial changes. Perhaps the ring system makes multiple contacts, while at least one of the sulfates has no contact with the kinesin−microtubule interface. Further work, focused on modifications of the ring system, may give more potent compounds and may perhaps give rise to compounds that will have differential inhibitory activity upon different types of kinesin.[3] Research is in progress to remove the sulfate groups and obtain the corresponding hydroquinones that, unlike adociasulfate 2, could cross cell membranes and, if active, might have useful therapeutic properties.

Experimental Section

Collection, Extraction and Isolation. The sponge *Haliclona (*aka *Adocia)* sp. was collected by hand using scuba at a depth of 7−15 m at Turtle Island Basin, Palau, in June 1995. The specimen (95−100) was immediately frozen and kept at −20 °C until extraction. The wet sponge (500 g) was extracted twice with MeOH and once with DCM. These extracts were combined and reduced in vacuo to an aqueous suspension, which was partitioned against DCM. The organic phase was further partitioned between hexane and MeOH. The water-soluble material (13.38 g) was chromatographed on TSK HW40 gel in 50% aqueous MeOH to obtain, in order of elution, adociasulfate 3 (**3**, 0.076% wet weight), adociasulfate 1 (**1**, 0.09% wet weight), and adociasulfate 2 (**2**, 0.132% wet weight). The MeOH partition (416 mg) was subjected to C_{18} flash chromatography with a solvent gradient from water to MeOH to acetonitrile. Fractions were combined on the basis of their ^{1}H NMR spectra and activity. Furthur separation by HPLC on a C_{18} support using 55:45 H_2O/MeCN as eluant yielded, in order of elution, adociasulfate 5 (**5**, 0.012% wet weight), adociasulfate 4 (**4**, 0.032% wet weight), and adociasulfate 6 (**6**, 0.012% wet weight).

Adociasulfate 1: amorphous white solid; $[\alpha]_D$ −15.0° (*c* 0.1, MeOH); UV (MeOH) λ_{max} 265 nm (ϵ 540); IR (AgCl) ν_{max} 3425, 2930, 1635, 1230, 1055, 970, 895 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD),see Table 1; ^{13}C NMR (100 MHz, CD_3OD), see Table 1; HRFABMS *m*/*z* 715.2970 (M − Na)$^-$ (calcd for $C_{36}H_{52}O_9S_2$-Na 715.2951).

Adociasulfate 2: amorphous white solid; $[\alpha]_D$ −10.9° (*c* 0.15, MeOH); UV (MeOH) λ_{max} 265 nm (ϵ 415); IR (AgCl) ν_{max} 2920, 1640, 1250, 1035 cm^{-1}, ^{1}H NMR (300 MHz, CD_3OD), see Table 1; ^{13}C NMR (100 MHz, CD_3OD), see Table 1; HRFABMS *m*/*z* 715.2934 (M − Na)$^-$ (calcd for $C_{36}H_{52}O_9S_2$Na, 715.2951).

Adociasulfate 3: opaque glass; $[\alpha]_D$ −6.25° (*c* 0.08, MeOH); UV (MeOH) λ_{max} 270 nm (ϵ 868); IR (AgCl) ν_{max} 3460, 2930, 1635, 1245, 1045, 850 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD), see Table 2; ^{13}C NMR (100 MHz, CD_3OD), see Table 2; HRFABMS *m*/*z* 779.2898 (M + Na)$^+$ (calcd for $C_{36}H_{54}O_{10}S_2Na_3$ 779.2851.

Adociasulfate 4: white amorphous solid; $[\alpha]_D$ −12.1° (*c* 1.17, MeOH); UV (MeOH) λ_{max} 281.0 nm (ϵ 1730); IR (AgCl) ν_{max} 3450, 2930, 1645, 1230, 1050, 965, 890, 830 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD), see Table 3; ^{13}C NMR (100 MHz, CD_3-OD), see Table 3; HRFABMS *m*/*z* 613.3567 (M − Na)$^-$ (calcd for $C_{36}H_{53}O_6S$ 613.3580).

Adociasulfate 5: white amorphous solid; $[\alpha]_D$ −38.7° (*c* 0.33, MeOH); UV (MeOH) λ_{max} 277 nm (ϵ 1475); IR (AgCl) ν_{max} 3425, 2940, 1645, 1240, 1015, 820 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 7.13 (d, 1 H, *J* = 6.6 Hz, H-4′), 6.45 (d, 1 H, *J* = 6.6 Hz, H-3′), 5.25 (s, 1 H, H-20), 3.24 (dd, 1 H, *J* = 6, 12 Hz, H-14), 2.58 (m, 1 H, H-4), 2.57 (dd, 1 H, *J* = 6, 13.5 Hz, H-1), 2.52 (m, 1 H, H-4), 2.44 (t, 1 H, *J* = 13.5 Hz, H-1), 1.92 (m, 1 H, H-21), 1.74 (m, 1 H, H-12), 1.72 (m, 1 H, H-16), 1.70 (m, 1 H, H-8), 1.68 (m, 1 H, H-2), 1.68 (m, 1 H, H-12), 1.68 (m, 1 H, H-16), 1.67 (s, 3 H, H-29), 1.64 (m, 1 H, H-8), 1.62 (m, 2 H, H-9), 1.62 (m, 1 H, H-21), 1.60 (m, 1 H, H-13), 1.58 (m, 1 H, H-13), 1.52 (m, 1 H, H-22), 1.38 (m, 2 H, H-5), 1.33 (m, 1 H, H-18), 1.13 (m, 1 H, H-22), 1.12 (s, 3 H, H-25), 1.06 (s, 3 H, H-26), 1.03 (m, 2 H, H-17), 0.97 (m, 1 H, H-10), 0.95 (s, 3 H, H-30), 0.92 (s, 3 H, H-27), 0.92 (m, 1 H, H-6), 0.86 (s, 3 H, H-24), 0.77 (s, 3 H, H-28); ^{13}C NMR (100 MHz, CD_3OD) δ 151.6 (C-2′), 147.5 (C-6′), 142.5 (C-5′), 138.3 (C-19), 130.5 (C-1′), 120.9 (C-4′), 120.7 (C-20), 114.2 (C-3′), 73.7 (C-14), 66.0 (C-2), 63.5 (C-6), 51.6 (C-10), 51.4 (C-18), 48.6 (C-3), 43.1 (C-8), 42.6 (C-15), 39.7 (C-12), 39.2 (C-16), 38.9 (C-11), 38.7 (C-7), 38.4 (C-4), 33.5 (C-23), 32.6 (C-22), 28.6 (C-30), 28.5 (C-13), 28.1 (C-24), 26.2 (C-1), 25.4 (C-17), 24.2 (C-21), 24.1 (C-29), 21.6 (C-25), 19.7 (C-9), 18.8 (C-5), 18.1 (C-26), 18,1 (C-28), 17.4 (C-27); HRFABMS *m*/*z* 613.3572 (M − Na)$^-$ (calcd for $C_{36}H_{53}O_6S$ 613.3580).

Adociasulfate 6: white amorphous solid; $[\alpha]_D$ −12.3 (*c* 0.76, MeOH); UV (MeOH) λ_{max} 276 nm (ϵ 2525); IR (AgCl) ν_{max} 3420, 2930, 1210, 1050 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 7.13 (d, 1 H, *J* = 8.7 Hz, H-4′), 6.45 (d, 1 H, *J* = 8.7 Hz, H-3′), 3.55 (dd, 1 H, *J* = 10.7, 4.2 Hz, H-14), 2.56 (dd, 1 H, *J* = 12.8, 7.8 Hz, H-1), 2.44 (t, 1 H, *J* = 12.3 Hz, H-1), 1.78 (m, 1 H, H-16), 1.76 (m, 2 H, H-4), 1.73 (m, 1 H, H-12), 1.72 (m, 1 H, H-16), 1.71 (m, 1 H, H-8), 1.69 (m, 1 H, H-9), 1.68 (m, 1 H, H-2), 1.66 (m, 1 H, H-12), 1.65 (m, 1 H, H-8), 1.65 (m, 1 H, H-13), 1.63 (m, 1 H, H-9), 1.58 (m, 2 H, H-5), 1.57 (m, 2 H, H-21), 1.48 (m, 1 H, H-17), 1.48 (m, 1 H, H-18), 1.45 (m, 2 H, H-20), 1.42 (m, 1 H, H-17), 1.36 (m, 1 H, H-22), 1.32 (m, 1 H, H-13), 1.30 (m, 1 H, H-22), 1.14 (s, 3 H, H-29), 1.12 (s, 3 H, H-25), 1.06 (s, 3 H, H-26), 0.94 (s, 3 H, H-30), 0.91 (s, 3 H, H-27), 0.90 (m, 1 H, H-6), 0.83 (m, 1 H, H-10), 0.79 (s, 3 H, H-24), 0.79 (s, 3 H, H-28); ^{13}C NMR (100 MHz, CD_3OD) δ 151.6 (C-2′), 147.5 (C-6′), 142.2 (C-5′), 130.5 (C-1′), 120.9 (C-4′), 114.1 (C-3′), 79.5 (C-19), 75.3 (C-14), 66.1 (C-2), 63.2 (C-6), 59.4 (C-10), 55.1 (C-18), 48.6 (C-3), 46.7 (C-16), 43.7 (C-8), 42.9 (C-15), 42.3 (C-22), 40.3 (C-12), 39.5 (C-20), 38.9 (C-4), 38.5 (C-7), 38.4 (C-11), 36.7 (C-23), 33.7 (C-30), 28.4 (C-13), 26.6 (C-1), 23.6 (C-29), 22.4 (C-21), 21.7 (C-24), 21.5 (C-25), 21.2 (C-17), 19.7 (C-9), 19.1 (C-5), 18.1 (C-26), 17.1 (C-27), 15.2 (C-28); HRFABMS *m*/*z* 613.3584 (M − Na)$^-$ (calcd for $C_{36}H_{53}O_6S$ 613.3580).

Preparation of Acetonide 7. Adociasulfate 3 (**3**, 4 mg) was added to a solution of dry DMF (400 μL) containing 2,2-dimethoxypropane (1 mL) and a catalytic amount of pyridinium *p*-toluenesulfonate. The reaction was stirred overnight and then dried. The solid was partitioned between H_2O and EtOAc twice and the organic layer dried over Na_2SO_4 to obtain the acetonide **7** (4.1 mg, 98% yield): white powder; ^{1}H NMR (300 MHz, CD_3OD) δ 6.56 (d, 1H, *J* = 2.6 Hz, H-6′), 6.54 (d, 1H, *J* = 8.4 Hz, H-3′), 6.43 (dd, 1H, *J* = 8.1, 2.4 Hz, H-4′), 5.16 (t, 1H, *J* = 7.4 Hz, H-14), 5.11 (t, 1H, *J* = 6.7 Hz, H-22), 3.70 (q, 1H, *J* = 4.3 Hz, H-18), 2.87 (d, 1H, *J* = 14.1, H-1), 2.58 (d, 1H, *J* = 14.1, H-1), 2.01 (m,1H, H-16), 1.97 (m, 1H, H-16), 1.92 (m, 2H, H-21), 1.92 (m, 2H, H-5), 1.77 (m, 1H, H-3), 1.66 (s, 3H, H-24), 1.64 (m, 1H, H-10), 1.61 (m, 2H, H-9), 1.60 (s, 3H, H-30), 1.59 (s, 3H, H-28), 1.53 (m, 1H, H-17), 1.51 (m, 2H, H-12), 1.49 (m, 1H, H-17), 1.37 (m, 1H, H-10), 1.37 (m, 1H, H-4), 1.36 (s, 3H, H-32), 1.33 (m, 1H, H-4), 1.30 (m, 2H, H-20), 1. 29 (m, 2H, H-8), 1.29 (s, 3H, H-33), 1.29 (m, 2H, H-13), 1.18 (s, 3H, H-29), 1.03 (s, 3H, H-27), 0.91 (s, 3H, H-25), 0.83 (d, 3H, *J* = 6.7 Hz, H-26); ^{13}C NMR (100 MHz, CD_3OD) δ 150.6 (C-2′), 150.4 (C-5′), 136.3 (C-7), 135.0 (C-6), 134.6 (C-15), 132.3 (C-23), 128.8 (C-1′), 127.5 (C-14), 125.9 (C-22), 119.6 (C-6′), 116.5 (C-3′), 114.2 (C-4′), 107.9 (C-31), 84.9 (C-18), 83.2 (C-19), 43.5 (C-2), 42.0 (C-12), 39.0 (C-11), 38.2 (C-1), 37.9 (C-16), 36.8 (C-10), 36.4 (C-20), 35.1 (C-3), 30.9 (C-8), 28.9 (C-32), 28.4 (C-27), 27.9 (C-17), 27.7 (C-4), 27.3 (C-33), 26.0 (C-24), 24.4 (C-13), 23.3 (C-29), 23.2 (C-5), 23.0 (C-21), 22.7 (C-25), 21.2 (C-9), 17.8 (C-30), 16.8 (C-26), 16.1 (C-28); HRFABMS *m*/*z* 615.4365 (M + Na)$^+$ (calcd for $C_{39}H_{60}O_4Na$ 615.4389).

ATPase Assay. The ATPase assay was performed using the EnzCheck ATPase kit (Molecular Probes). The assays were performed in 50 μL microcuvettes in an Ultraspec spectrophotometer (Pharmacia), and the progress of the reaction was monitored by absorbance increase at 360 nm. Microtubules (1.7 mM final), kinesin (K5-351, 0.11 mM final), inhibitor (or DMSO blank at 5% final), and the EnzCheck components (purine nucleotide phosphorylase and MESG substrate) were premixed in the cuvette in a reaction buffer (40 mM PIPES pH 6.8, 5 mM paclitaxel, 1 mM EGTA, 5 mM $MgCl_2$). The reaction was initiated by addition of MgATP (1 mM final).

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra for adociasulfates 1−6 (**1**−**6**) and acetonide **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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