Adociasulfate-9, a New Hexaprenoid Hydroquinone from the Great Barrier Reef Sponge *Adocia aculeata*

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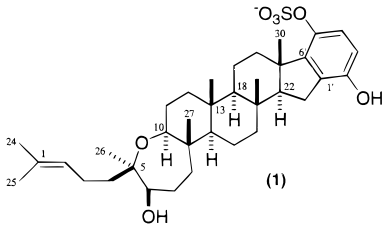
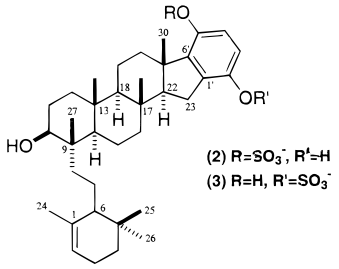
Adociasulfate-9 (**1**) and the known adociasulfate-5 (**2**) were isolated from an extract of the Great Barrier Reef Sponge *Adocia aculeata*. Structure elucidation by 1D and 2D NMR spectroscopy revealed that **1** is a novel hexaprenoid hydroquinone.

Compounds isolated from marine sponges of the *Adocia* genus (order Haplosclerida, family Chalinidae) have shown a range of biological activities. These include the kinesin inhibitor adociasulfate-2[1,2] and the HIV-inhibitory protein adociavirin,[3] and we recently reported the isolation and structure elucidation of three hexaprenoid hydroquinones, adociasulfates-1, -7, and -8, all of which inhibited proton pump activity.[4] In the search for similar compounds a subsequent investigation of a sample of *Adocia aculeata* collected from Cormorant Pass, North Great Barrier Reef, led to the isolation of a new triterpene hydroquinone sulfate, adociasulfate-9 (**1**), and the known adociasulfate-5 (**2**).[2] In this paper we report the isolation and structure elucidation of adociasulfate-9 and adociasulfate-5.





The MeOH extract of the freeze-dried sample of *Adocia aculeata* was chromatographed on Sephadex LH-20 (100% MeOH). Fractions containing **1** and **2** were further separated by C_{18} reversed-phase HPLC using MeOH and aqueous NaCl to yield adociasulfate-9 (6.0 mg) and adociasulfate-5 (7.7 mg).

The ^{1}H NMR spectrum of adociasulfate-9 (**1**) (see Table 1 for NMR data) revealed seven methyl singlets (δ 1.61, 1.54, 1.04, 0.99, 0.96, 0.82, 0.73), two heteroatom-bonded protons (δ 8.67, 4.54), an olefinic proton (δ 5.07), and two ortho coupled aromatic protons (δ 6.98, 6.38). The ^{13}C NMR spectrum of adociasulfate-9 (**1**) showed the resonances of 35 carbon atoms, including seven unsaturated, three oxygen-bonded, and seven methyl carbon atoms. Multiple HMBC correlations revealed an additional quaternary carbon atom resonating at 142.0 ppm not observed in the 1D NMR spectrum. The negative ion high-resolution electrospray mass spectrum, [(−)-HRESMS], showed a molecular ion at *m*/*z* 629.3531, which supported a molecular formula of $C_{36}H_{54}O_7S$ (calcd *m*/*z* 629.3517). The 10 units of unsaturation together with the ^{1}H and ^{13}C NMR spectra suggested that adociasulfate-9 (**1**) contained a tetrasubstituted aromatic ring, one double bond, and five other rings.

The majority of the carbon framework of **1** was elucidated from HMBC correlations. HMBC correlations from the phenolic OH (δ 8.67) to C1′, C2′, and C3′ confirmed the assignment of the hydroxy group at C2′ and, hence, the sulfate group at C5′ of the aromatic ring. The attachment of the aromatic ring to C23 was based on correlations from H23a (δ 2.31) and H23b (δ 2.45) to C1′, C2′, and C6′. Further correlations were observed from these protons to C22 and from H23b to C21. Supporting the fusion of the aromatic ring with the five-membered ring (ring E) was the HMBC correlation from the C30 methyl protons (δ 0.99) to C6′. Further correlations from these methyl protons to C21 and C22 established the five-membered ring. An additional HMBC correlation from the C30 methyl protons to C20 was observed and, along with HMBC correlations from the methyl protons on C29 (δ 0.96) to C17, C18, and C22, established part of the D ring and supported the fusion with the C ring. HSQC−TOCSY correlations from the C18 methine proton (δ 0.83) to C19 and C20 established ring D. HMBC correlations from the methyl protons (δ 0.82) on C28 to C13, C14, and C18 established the majority of the C ring and supported the fusion with ring B. HSQC−TOCSY correlations from the C14 methine proton (δ 0.78) to C14 and C15 allowed the complete assignment of ring C. The assignment of ring B was supported by HMBC correlations from the methyl protons on C28 to the methylene C12, and from the methyl protons on C27 (δ 0.73) to carbons C9, C10, and C14, along with a HMBC correlation from the H10 methine proton (δ 3.44) to C11. HSQC−TOCSY correlations from the C10 methine proton (δ 3.44) to C12 confirmed the C10−C11−C12 connectivity and allowed the complete assignment of ring B. A further HMBC correlation was observed from H10 to C9 and, along with a HMBC correlation from the methyl protons on C27 to C8, confirmed the A/B ring fusion. Crucial contributions to the structure elucidation of the seven-membered ring (ring A) were the HMBC correlations from the methyl protons H26 (δ 1.04) to the C4 methylene, the oxygen-bonded quaternary C5 (79.0 ppm), and the oxygen-bonded methine C6 (73.4 ppm). A HMBC correlation from H10 to C5 confirmed the ether linkage between C5 and C10.

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Table 1. NMR Data for Adociasulfate-9 (**1**)[a]

position	^{13}C (δ)	^{1}H (δ, mult., J in Hz)	$^{2}J_{CH}$ and $^{3}J_{CH}$	COSY	ROESY[b]	HSQC−TOCSY[b]
1	130.1					
2	124.9	5.07 (dd, 7.5, 6.8)	C3, C24, C25	H3a, H3b	H3a, H4a, H4b, H25	C3, C4
3	22.5	1.87 (m)	C1, C2, C4	H2, H3b, H4a, H4b	H2	C2, C4
		1.97 (m)	C1, C2, C4	H2, H3a, H4a, H4b	H26	C2, C4
4	41.0	1.17 (m)	C2, C3, C5, C6, C26	H3a, H3b	H2	C2, C3
		1.30 (m)	C2, C3, C5, C6, C26	H3a, H3b	H2	C2, C3
5	79.0					
6	73.4	3.56 (br dd, 6.2, 4.2)	C4, C7, C8	H7a, 6-OH	6-OH, H26	C7, C8
7	25.7	1.52 (m)	C5, C6, C9	H6, H7b		C6, C8
		1.77 (m)		H7a, H8b		C6, C8
8	35.1	1.29 (m)				C6, C7
		1.46 (m)		H7b		C6, C7
9	40.5					
10	75.5	3.44 (m)	C5, C8, C9, C11, C27	H11a, H11b	H14, H26	C11, C12
11	26.8	1.26 (m)		H10, H12a		C10, C12
		1.41 (m)		H10, H12a		C10, C12
12	38.2	0.88 (m)		H11a, H11b, H12b		C10, C11
		1.60 (m)		H12a		C10, C11
13	36.3					
14	57.0	0.78 (m)	C13, C16	H15a, H15b	H10	C15, C16
15	17.1	1.39 (m)		H14, H16a		C14, C16
		1.48 (m)		H14, H16a		C14, C16
16	41.7	1.03 (m)		H15a, H15b		C14, C15
		1.58 (m)				C14, C15
17	36.7					
18	61.0	0.83 (m)		H19a	H22	C19, C20
19	17.8	1.41 (m)		H18		C18, C20
		1.48 (m)				C18, C20
20	37.2	1.55 (m)		H20b		C18, C19
		2.40 (m)	C18, C22	H20a, H19b		C18, C19
21	46.4					
22	63.9	1.59 (m)		H23a, H23b	H18, 2′-OH, H23b	C23
23	24.8	2.31 (dd, 13.5, 13.5)	C22, C1′, C2′, C6′	H22, H23b	H29, H30	C22
		2.45 (dd, 13.5, 6.0)	C21, C22, C1′, C2′, C6′	H22, H23a	H22, 2′-OH	C22
24	17.3	1.54 (s)	C1, C2, C25		H26	
25	25.3	1.61 (s)	C1, C2, C24		H2	
26	18.6	1.04 (s)	C4, C5, C6		H3b, H24, H10, H6	
27	13.5	0.73 (s)	C8, C9, C10, C14		H28	
28	16.0	0.82 (s)	C12, C13, C14, C18		H27, H29	
29	17.0	0.96 (s)	C16, C17, C18, C22		H23a, H28	
30	20.5	0.99 (s)	C20, C21, C22, C6′		H23a	
1′	127.9					
2′	149.1					
3′	112.4	6.38 (d, 9.0)	C1′, C2′, C5′	H4′	H4′	C4′
4′	119.3	6.98 (d, 9.0)	C2′, C5′, C6′	H3′	H3′	C3′
5′	142.0					
6′	144.9					
6-OH		4.54 (d, 4.2)	C5, C6, C7	H6	H6	
2′-OH		8.67 (s)	C1′, C2′, C3′		H22, H23b	

[a] Spectra recorded in DMSO-d_6 at 30 °C. [b] Mixing time = 500 ms.

HMBC correlations from the hydroxy methine H6 (δ 3.56) to C7 and C8 completed the assignment of the ring. A series of HMBC correlations from the hydroxy proton 6-OH (δ 4.54) to C5, C6, and C7 further supported the assignment of the A ring and completed the assignment of the pentacyclic ring system. HMBC correlations were used to establish the terminal isoprene unit of the molecule. Methyl protons on C24 (δ 1.54) and C25 (δ 1.61) both showed correlations to C1 (130.1 ppm) and C2 (124.9 ppm) and each showed a third correlation to the other, which indicated that these methyl groups were geminal. As these methyl protons appeared as singlets in the ^{1}H NMR spectrum, it was apparent that they were attached to the quaternary carbon C1 (130.1 ppm). HMBC correlations from H2 (δ 5.07) to C24, C25, and C3 confirmed the assignment of the isoprene unit. COSY correlations between H2 and the methylene protons H3a (δ 1.87) and H3b (δ 1.97) as well as a HMBC correlation from the protons on C3 to C2 supported the assignment of the isoprene unit. The attachment of the isoprene unit to the pentacyclic ring system was established through a HMBC correlation from H3 to C4, and COSY correlations between protons H3a and the methylene protons H4a (δ 1.17) and H4b (δ 1.30) thus completing the assignment of the molecule.

ROESY data established an all trans stereochemistry across ring junctions. Correlations were observed from methyl protons H28 to H27 and H29. A further correlation from methyl protons H29 to the methylene H23a, which also correlated to methyl protons H30, confirmed that all of the methyl groups were on the α face of the molecule. The relative stereochemistry around the seven-membered ring was established through correlations from the hydroxy methine protons H6 and H10. Both protons showed correlations to the methyl protons H26, indicating that they were on the same face of the molecule. A correlation from H10 to H14 confirmed that both hydroxy methine protons and the methyl group H26 were on the opposite β face of the molecule.

Adociasulfate-5 (**2**) showed a molecular ion in the (−)-HRESMS at m/z 613.3593 consistent with the molecular formula $C_{36}H_{54}O_6S$ (calcd m/z 613.3568), the same formula as adociasulfate-7.[4] Comparison of 1D NMR spectra (see Table 2 for NMR data) of this compound with those of adociasulfate-7 (**3**) confirmed that they were structurally similar. The ^{1}H NMR spectrum showed a pair of ortho coupled doublets at δ 6.98 and 6.38 (J = 8.4 Hz), an olefinic proton at δ 5.19, a hydroxy doublet at δ 4.04 (J = 5.4 Hz), and seven methyl groups, one of which (δ 1.62) was

Table 2. NMR Data for Adociasulfate-5 (**2**)[a] and Adociasulfate-7 (**3**)[4]

	adociasulfate-5		adociasulfate-7	
position	^{13}C (δ)	^{1}H (δ, mult., *J* in Hz)	^{13}C (δ)	^{1}H (δ, mult., *J* in Hz)
1	136.7		136.4	
2	119.0	5.19 (s)	119.2	5.21 (s)
3	22.6	1.86 (brs)	22.5	1.88 (brs)
4	31.0	1.04 (m)	31.0	1.07 (m)
		1.37 (m)		1.39 (m)
5	32.3		32.2	
6	49.5	1.26 (m)	49.4	1.28 (m)
7	23.5	0.97 (m)	23.4	1.02 (m)
		1.33 (m)		1.38 (m)
8	37.6	1.09 (m)	37.5	1.13 (m)
		1.60 (m)		1.61 (m)
9	40.9		40.9	
10	71.0	3.27 (m)	70.8	3.28 (m)
11	27.2	1.49 (m)	27.2	1.52 (m)
12	37.9	0.86 (m)	37.8	0.89 (m)
		1.60 (m)		1.61 (m)
13	36.6		36.5	
14	49.5	0.90 (m)	49.4	0.93 (m)
15	17.2	1.02 (m)	17.2	1.01 (m)
		1.33 (m)		1.35 (m)
16	41.3	0.99 (m)	41.2	1.03 (m)
		1.56 (m)		1.59 (m)
17	36.9		36.8	
18	61.3	0.84 (m)	61.2	0.85 (m)
19	17.9	1.46 (m)	17.9	1.48 (m)
		1.52 (m)		1.56 (m)
20	37.2	1.54 (m)	37.2	1.56 (m)
		2.41 (m)		2.41 (m)
21	46.6		46.5	
22	64.1	1.49 (m)	64.0	1.49 (m)
23	24.9	2.27 (dd, 13.2, 13.2)	24.9	2.32 (dd, 13.2, 13.2)
		2.41 (dd, 6.0, 13.2)		2.45 (dd, 6.0, 13.2)
24	23.1	1.62 (brs)	23.0	1.63 (brs)
25	27.6	0.88 (s)	27.5	0.90 (s)
26	27.4	0.80 (s)	27.4	0.81 (s)
27	17.4	0.67 (s)	17.4	0.68 (s)
28	16.6	0.83 (s)	16.5	0.84 (s)
29	17.1	0.96 (s)	17.0	0.97 (s)
30	20.6	0.99 (s)	20.5	1.01 (s)
1′	128.0		136.4	
2′	149.2		144.8	
3′	112.5	6.38 (d, 8.4)	118.8	6.99 (d, 9.0)
4′	119.4	6.98 (d, 8.4)	112.3	6.36 (d, 9.0)
5′	141.4		148.8	
6′	145.0		141.3	
10-OH		4.04 (d, 5.4)		4.04 (d, 5.2)
2′-OH		8.68 (s)		

[a] Spectra recorded in DMSO-d_6 at 30 °C.

attached to a double bond. An HMBC experiment showed that the only difference between adociasulfate-5 (**2**) and the previously isolated adociasulfate-7 (**3**) was the positioning of the aromatic substituents. Important HMBC correlations for **2** were observed from the phenolic OH (δ 8.68) to C1′ (128.0 ppm), C2′ (149.2 ppm), and C3′ (112.5 ppm); these correlations, along with the chemical shifts of these carbon atoms, supported the attachment of the OH to the carbon atom resonating at 149.2 ppm (C2′) and hence the sulfate at position C5′ (141.4 ppm). An HMBC correlation from the C30 methyl protons (δ 0.99) to C6′ (145.0 ppm) confirmed that the carbon bearing the OH was at least five bonds distant from the methyl, and hence structure **2** was assigned as the previously reported adociasulfate-5.[2]

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Varian Unity INOVA at 599.926 MHz for ^{1}H and 149.98 MHz for ^{13}C. ^{1}H and ^{13}C chemical shifts were referenced to the solvent peak (DMSO-d_6) δ 2.49 and 39.51 ppm, respectively. Standard parameters were used for 1D and 2D NMR spectra obtained, which included ^{1}H, ^{13}C, gradient COSY, HMQC, HSQC−TOCSY, HMBC, and ROESY. UV spectra were recorded on a GBC 916 UV−vis spectrophotometer and IR spectra were recorded on a Perkin−Elmer 1725X FT-IR spectrophotometer. Optical rotations were measured on a JASCO P-1020 polarimeter. HRESIMS were measured on a Bruker BioAPEX 47e mass spectrometer. Sephadex LH-20 (400-mm length × 40-mm i.d) (Pharmacia Biotech) was used for gel permeation chromatography. A Rainin 3-μm C_{18} microsorb (50-mm length × 10-mm i.d.) HPLC column was used for semipreparative chromatography. A Waters 600 pump with a 996 PDA detector was used for semipreparative HPLC separations. Omnisolv MeOH (EM Science) and Milli-Q H_2O were used for chromatography, AnalaR NaCl (BDH) was used for HPLC.

Animal Material. The sponge sample *Adocia aculeata* Pulitzer-Finali, 1982 (phylum Porifera, class Demospongiae, order Haplosclerida, family Chalinidae) was collected by hand using scuba at Cormorant Pass, North Great Barrier Reef, Australia, at a depth of 30 m. A voucher sample (G304365) is lodged at the Queensland Museum, Brisbane, Australia.

Extraction and Isolation. The freeze-dried sponge material (6.96 g) was ground and exhaustively extracted with MeOH to afford 3.56 g of crude extract. The MeOH crude extract was fractionated on Sephadex LH-20 (100% MeOH). Fractions containing **1** and **2** were combined (117 mg) and separated by semipreparative C_{18} HPLC using a linear gradient elution from 65% MeOH−35% 0.2M NaCl to 100% MeOH over 10 min, with adociasulfate-5 (**2**, 7.7 mg, 0.11% dry wt.) eluting at 5.5 min and adociasulfate-9 (**1**, 6.0 mg, 0.08% dry wt) eluting at 7.25 min.

Adociasulfate-9 (**1**): white powder (6.0 mg, 0.08% dry wt); $[\alpha]^{25}_{D}$ +6.9°(*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 215.7 nm (8.45), 272 (7.24); IR ν_{max} (film) 3416, 2931, 1632, 1485, 1235, 1052 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; HRESMS *m*/*z* 629.3531 (calcd for $C_{36}H_{53}O_7S$, 629.3517).

Adociasulfate-5 (**2**): white powder (7.7 mg, 0.11% dry wt); $[\alpha]^{25}_{D}$ −49.3°(*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ϵ) 210.5 nm (8.95), 272 (7.33); IR ν_{max} (film) 3417, 2932, 1642, 1485, 1235, 1053 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; HRESMS *m*/*z* 613.3593 (calcd for $C_{36}H_{53}O_6S$, 613.3568).

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Supporting Information Available: ^{1}H NMR and ^{13}C NMR spectra for all title compounds described in the Experimental Section. This material is available free of charge via the Internet at http://pubs.acs.org.

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