Pergamon

Tetrahedron 56 (2000) 8429–8432

TETRAHEDRON

Adociasulfate 10, A New Merohexaprenoid Sulfate from the Sponge *Haliclona* (aka *Adocia*) sp.

Christine L. Blackburn and D. John Faulkner*

Marine Research Division, Scripps Institution of Oceanography, University of California at San Diego, La Jolla, CA 92093-0212, USA

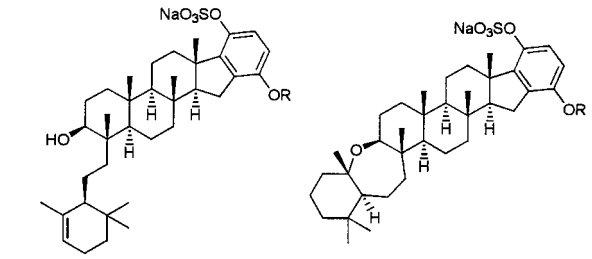
Received 17 July 2000; accepted 29 August 2000

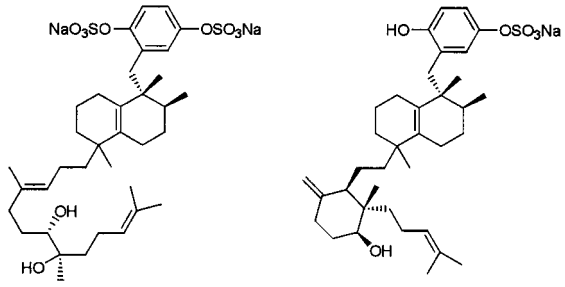
Abstract—The meroterpenoid adociasulfate 10 was isolated from the Palauan sponge *Haliclona* (aka *Adocia*) sp., together with the previously reported metabolites adociasulfates 1–6. Unlike other adociasulfates, which contain mono- or di-sulfated hydroquinones, adociasulfate 10 contains an unusual glycolic acid residue in place of one of the sulfate groups. Adociasulfate 10 is an inhibitor of the kinesin family of microtubule motor proteins with an IC_{50} of 7 μM.

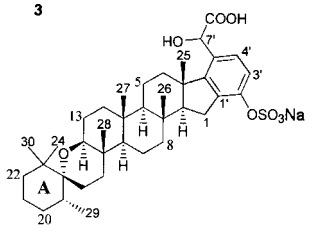
Introduction

Triterpene hydroquinone sulfates from marine sources possess a wide range of biological activities. The toxicols and toxiusol from the sponge *Toxiclona toxius* were reported to inhibit HIV reverse transcriptase,[1,2] and the shaagrockols from the same sponge were originally identified as anti-fungal compounds.[2,3] Akaterpin from the sponge *Callyspongia* sp. is a phosphatidylinositol-specific phospholipase C inhibitor.[4] Adociasulfates 1–6 (**1**–**6**) were the first reported kinesin motor protein inhibitors.[5] In addition, adociasulfate 1 (**1**) and two similar compounds, adociasulfates 7 and 8, from the sponge *Adocia* sp., were isolated as inhibitors of H^+-ATPase proton pump activity.[6]

In a screen for natural products that inhibit kinesin motor protein activity, the crude extract of *Haliclona* (aka *Adocia*) sp., which contained adociasulfates 1–6 (**1**–**6**) and 10 (**7**), was most active.[5] The kinesin superfamily of proteins is of special interest because it is responsible for transport of vesicles and organelles within the cell, as well as being an active participant in cell division.[7,8] Adociasulfate 2 (**2**) was the first known specific inhibitor of the kinesin family and is believed to inhibit motor protein activity by binding at or near the microtubule binding site.[9] It is interesting to note that substitution of one sulfate group by the glycolic acid moiety of adociasulfate 10 (**7**) does not significantly reduce its inhibition of kinesin motors.







Results and Discussion

The sponge *Haliclona* (aka *Adocia*) sp. was collected using SCUBA (−15 m) at Turtle Island Basin, in Palau in 1995.

Keywords: sponge; marine natural product; kinesin motor protein.
* Corresponding author. Tel.: +1-858-534-4259; fax: +1-858-534-2997; e-mail: jfaulkner@ucsd.edu

0040–4020/00/$ - see front matter
PII: S0040-4020(00)00786-9

Table 1. NMR data (300 MHz, MeOH-d_4) for adociasulfate 10 (**7**) (* not observed)

C#	δ_C	δ_H	mult, J (Hz)	HMBC	ROESY
1	26.5	2.81	dd, 13, 6	C-2, C-3, C-1′, C-2′, C-6′	H-1b
		2.62	t, 13		H-1a, H-25, H-26
2	65.2	1.80	d, 6	C-1, C-3, C-4, C-6, C-7, C-25, C-26	H-6
3	50.2				
4	40.2	2.44	d, 12	C-2, C-3, C-5, C-6, C-25	H-25
		2.10	td, 12, 4		
5	19.9	1.67	m, 2H		
6	62.6	1.00	m		H-2, H10
7	38.9				
8	43.3	1.72	m	C-6, C-10	
		1.15	m		
9	17.9	1.52	m, 2H		H-27, H-28
10	58.5	0.82	m	C-27, C-28	H-6, H-14
11	39.0				
12	40.4	1.76	m		
		1.03	m		
13	25.7	1.56	m		
		1.40	m		
14	78.7	3.15	bd, 11	C-28	H-10, H-29
15	37.0				
16	36.6	1.64	m		
		1.26	m		
17	24.7	1.92	td, 13, 5	C-18	H-17b, H-24, H-28, H-30
		1.50	m		H-17a
18	78.7				
19	*	2.33	bs		
20	31.3	1.54	m, 2H		
21	30.6	1.29	m, 2H		
22	38.6	1.38	m		
		1.24	m		
23	40.4				
24	25.3	1.01	s, 3H	C-18, C-22, C23, C30	H-28, H-30
25	22.6	1.18	s, 3H	C-2, C-3, C-4, C-6′	H-26
26	18.0	1.07	s, 3H	C-2, C-6, C-7, C-8	H-25, H-27
27	18.1	0.95	s, 3H	C-6, C-10, C-11, C-12	H-26, H-28
28	13.4	0.87	s, 3H	C-10, C-14, C-15, C-16	H-27, H-24
29	18.0	1.04	d, 7, 3H	C-18, C-20	H-14
30	27.0	0.93	s, 3H	C-18, C-22, C-23, C-24	H-24
1′	136.6				
2′	149.7				
3′	120.4	7.13	d, 9	C-1′, C-2′, C-5′, C-6′	H-4′
4′	127.6	7.08	d, 9	C-3, C-2′, C-6′, C-7′	H-3′
5′	134.5				
6′	155.8				
7′	71.1	5.30	s	C-4′, C-5′, C-6′, C-8′	H-4a, H-25
8′	179.8				

The water soluble material from a methanolic extract was chromatographed on TSK HW-40 in 50% aq. MeOH to yield the previously reported metabolites, adociasulfates 1–6 (**1**–**6**), and the new compound adociasulfate 10 (**7**).

Adociasulfate 10 (**7**) has a molecular formula of $C_{38}H_{55}O_8SNa$, which was established from HRFABMS and NMR data (Table 1). The molecular formula suggested the presence of a sulfate ester that was subsequently confirmed by the observation of peaks resulting from the loss of 23 amu (Na) and 103 amu (SO_3Na) in the negative ion ESIMS. The formula required eleven degrees of unsaturation, four of which were due to the aromatic ring and one to a carbonyl group, suggesting the presence of six additional rings. The proton signals at δ 7.08 (d, 1H, J=9 Hz) and 7.13 (d, 1H, J=9 Hz) combined with the ^{13}C data, showed that **7** contained a sulfated 1,2,3,4-tetrasubstituted aromatic ring which was defined using 3-bond HMBC correlations. The presence of the glycolic acid and its position at C-5′ were established by HMBC correlations from the proton signal at δ 5.30 (H-7′, δ_C 71.1) to the aromatic C-4′, C-5′ and C-6′ signals as well as to the carbonyl signal at δ 179.8.

Two geminally coupled proton signals at δ 2.62 (t, 1H, J=13 Hz) and 2.81 (dd, 1H, J=13, 6 Hz) showed HMBC correlations to the C-1′, C-2′ and C-6′ aromatic ring carbon signals, as well as to a methine signal at δ 65.2 (C-2) and a quaternary carbon signal at δ 50.2 (C-3). The HMBC cross-peaks for Me-25 also showed a correlation to the aromatic ring (C-6′), as well as to C-2 and C-3, thereby completing the assignment of the five membered ring attached to the aromatic ring. The structure of the ring system between C-2 and C-17 was determined from a contiguous series of HMBC correlations between the CH_3 signals and intervening ring junction methine carbons (Me-25/C-2/Me-26/C-6/Me-27/C-10/Me-28). Each of these methyl signals also showed a 2-bond correlation to a fully substituted carbon

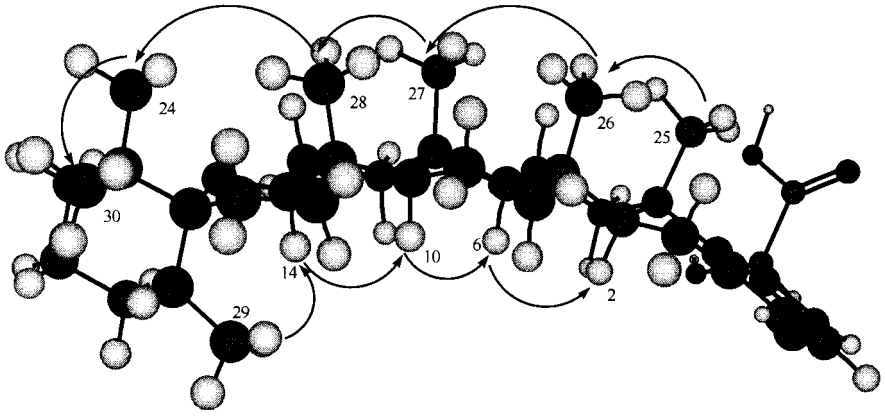


Figure 1. 3-Dimensional projection of AS 10 (**7**) showing key ROESY correlations (arrows).

signal and a 3-bond correlation to a methylene signal. Signals for the remaining methylene groups at C-5, C-9, C-13 and C-17 were assigned using the HSQC-TOCSY experiment.

The Me-28 signal showed a three-bond HMBC correlation to a signal at δ 78.7 due to C-14, an oxygen bearing methine carbon. We were initially confused by the observation that in the HMBC spectra the proton signals for Me-24, Me-29 and Me-30 also showed correlations to the carbon signal at δ 78.7. However, on close examination of the DEPT and HMQC experiments, it was determined that there are two overlapping carbons at δ 78.7, one due to a quaternary carbon and one due to the C-14 methine. Thus, Me-24, Me-29 and Me-30 are correlated to C-18, the spirocarbon that completes the ether linkage.

The structure of ring A was assigned using the 2- and 3-bond couplings from Me-24 and Me-30 to carbons C-18, C-22 and C-23 as well as from Me-29 to C-18 and C-20. The HSQC-TOCSY experiment was again essential in assigning the ^{1}H and ^{13}C signals for the C-21 methylene. The carbon signal for C-19 was never observed, possibly due to conformational mobility in ring A. A broad proton signal at δ 2.33 was assigned to H-19 on the basis of coupling with Me-29 in the COSY spectrum. This assignment was confirmed by irradiation at δ 2.33 which decoupled the Me-29 signal at δ 1.04. However, irradiation of the Me-29 signal failed to resolve the residual couplings for H-19, which was still observed as a broad, albeit sharpened, singlet.

The relative stereochemistry was determined using a ROESY experiment that showed correlations between the axial methyls as indicated in Fig. 1, ending with Me-30 coming out of the page. The stereochemistry at C-18 was confirmed by the NOE correlations between Me-24 and Me-28, and Me-29 and H-14. A series of NOE correlations linked H-14 (δ 3.15) to the axial bridgehead methine protons (H-14/H-10/H-6/H-2). Due to the lack of through-space correlations to the broad H-19 signal, the relative stereochemistry of C-19 was tentatively assigned with the aid of modeling experiments.

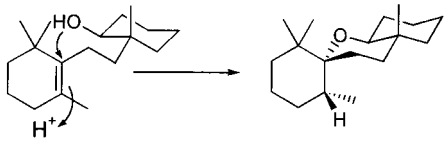


Figure 2. Hypothetical biosynthetic precursor leading to the proposed stereochemistry.

Molecular modeling experiments reflect a conformational flexibility about the spiro ring junction (Fig. 1). The conformation illustrated is the lowest energy conformation generated by PC Model™. The ROESY correlations are in agreement with this model, which might also be predicted by assuming that the ether linkage was formed by the *trans* addition across the 18, 19-double bond of a hypothetical biosynthetic precursor (Fig. 2). In addition, one might also predict that Me-29 would prefer to be in an equatorial conformation to avoid the 1–3 diaxial interaction with Me-30. Low temperature NMR experiments showed no sharpening of the H-19 proton signal and no evidence for a preferred conformation of ring A.

Adociasulfate 10 (**7**) was tested for its effect on the kinesin family of microtubule motors, a group of proteins that transport cargo along the microtubules within the cell. Adociasulfate 10 (**7**) had an IC_{50} of 7 μM, which is almost identical to the activity of adociasulfate 2 (**2**), IC_{50}=6 μM.[5,9] It has been shown that at least one sulfate group is necessary for the activity of the adociasulfates,[10] but it appears that the presence of the glycolic acid, in lieu of a sulfate, does not significantly effect the activity.

Experimental

General

All solvents were distilled prior to use. The ^{1}H NMR, gCOSY, gHMBC, gHMQC, gROESY, and gHSQC-TOCSY experiments were all recorded on a Varian Inova 300 MHz spectrometer. The ^{13}C and DEPT experiments were recorded on a Varian Gemini 400 MHz spectrometer. All NMR data were recorded in CD_3OD. The HRFABMS measurement was obtained from the UC Riverside Regional Mass Spectrometry Facility.

Animal material

The sponge *Haliclona* (aka *Adocia*) sp. (95–100) was collected by hand using SCUBA at a depth of −15 m at Turtle Island Basin, Palau, in June 1995. The sponge was frozen immediately after collection and was kept at −20°C until extracted.

Extraction and isolation

The wet sponge (500 g) was extracted with MeOH (2×500 mL) and DCM (500 mL). These combined extracts were dried under reduced pressure until an aqueous slurry remained. The slurry was then partitioned between H_2O and EtOAc. The water soluble material (13.38 g) was chromatographed on TSK HW-40 gel in 50% aq. MeOH to yield, in order of elution, adociasulfate 3 (**3**), adociasulfate 1 (**1**), adociasulfate 2 (**2**), adociasulfate 10 (**7**, 47.2 mg, 0.009% wet weight), adociasulfate 5 (**5**), adociasulfate 4 (**4**), and adociasulfate 6 (**6**).

Adociasulfate 10 (**7**): white solid; $[\alpha]_D=-30.2°$ (*c* 0.57, MeOH); UV (MeOH) λ_{max} 265 nm (ϵ 977.8); IR (AgCl) ν_{max} 3420, 2930, 1635, 1230, 1045 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) see Table 1; ^{13}C NMR (100 MHz, CD_3OD) see Table 1; HRFABMS *m/z* 671.3643 $[M-Na]^-$ (calcd for $C_{38}H_{55}O_8S$, 671.3618).

Acknowledgements

We thank Mary Kay Harper for the identification of the sponge and Roman Sakowicz, in the laboratory of Lawrence S. B. Goldstein, for performing the kinesin motor protein assay. We also thank the Republic of Palau and the State of Koror for marine research permits. This research was sponsored by grants from Sea Grant (R/MP-72) and the National Institutes of Health (CA 49084).

References

1. Isaacs, S.; Hizi, A.; Kashman, Y. *Tetrahedron* **1993**, *49*, 4275–4282.
2. Loya, S.; Tal, R.; Hizi, A.; Isaacs, S.; Kashman, Y.; Loya, Y. *J. Nat. Prod.* **1993**, *56*, 2120–2125.
3. Isaacs, S.; Kashman, Y. *Tetrahedron Lett.* **1992**, *33*, 2227–2230.
4. Fukami, A.; Ikeda, Y.; Kondo, S.; Takeuchi, T.; Furuya, S.; Hirabayashi, Y.; Shimoike, K.; Hosaka, S.; Wantanabe, Y.; Umezawa, K. *Tetrahedron Lett.* **1997**, *38*, 1201–1202.
5. Blackburn, C. L.; Hopmann, C.; Sakowicz, R.; Berdelis, M. S.; Goldstein, L. S. B.; Faulkner, D. J. *J. Org. Chem.* **1999**, *64*, 5565–5570.
6. Kalaitzis, J. A.; Leone, P. A.; Harris, L.; Butler, M. S.; Ngo, A.; Hooper, J. N. A.; Quinn, R. J. *J. Org. Chem.* **1999**, *64*, 5571–5574.
7. Barton, N. R.; Goldstein, L. S. B. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 1735–1742.
8. Hirokawa, N. *Science* **1998**, *279*, 519–526.
9. Sakowicz, R.; Berdelis, M. S.; Ray, K.; Blackburn, C. L.; Hopmann, C.; Faulkner, D. J.; Goldstein, L. S. B. *Science* **1998**, *280*, 292–295.
10. Unpublished results from this laboratory.