Pergamon

Tetrahedron Letters 42 (2001) 6557–6560

TETRAHEDRON LETTERS

Isolation and structures of hedathiosulfonic acids A and B, novel thiosulfonic acids from the deep-sea urchin *Echinocardium cordatum*

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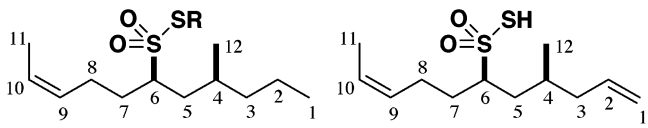
Received 20 April 2001; revised 21 July 2001; accepted 23 July 2001

Abstract—Hedathiosulfonic acids A and B were isolated from the deep-sea urchin *Echinocardium cordatum*, and were determined to be novel 6-undecanethiosulfonic acids by 2D NMR, HRMS, and methylation reaction. Hedathiosulfonic acids exhibited acute toxicity.

In our continuing search for marine bioactive compounds, we have reported the isolation and structural determination of pinnatoxins, halichlorine, haterumalides and pinnamine.[1] To discover new bioactive compounds, we have directed our attention to the deep-sea invertebrates as sources for such compounds. As a result, we have recently isolated hedathiosulfonic acids, which have a thiosulfonic acid functionality. We report here the isolation and structure determination of hedathiosulfonic acids A (**1**) and B (**2**) from the deposit-feeding deep-sea heart urchin *Echinocardium cordatum*.

The aqueous 80% EtOH extract of the deep-sea urchin *E. cordatum*, collected off the Heda coast of the Izu Peninsula, was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction was subjected to fractionation guided by acute toxicity against mice using column chromatography (ODS, aqueous 60% MeOH) and reversed-phase HPLC (ODS, 50% MeOH) to give hedathiosulfonic acids A (**1**, 0.0039% yield based on wet wt) and B (**2**, 0.0028% yield based on wet wt) as colorless oils.[2] Hedathiosulfonic acids A (**1**) and B (**2**) exhibited low acute toxicity against mice, with LD_{99}s of 0.39 and 0.36 g/kg, respectively.



1: R = H
3: R = Me

2

The fluorescent X-ray analysis of hedathiosulfonic acid A (**1**) suggested the presence of two sulfur atoms. The molecular formula of **1** was determined to be $C_{12}H_{24}O_2S_2$ by HRFABMS (m/z 309.0951, calcd for $C_{12}H_{23}Na_2O_2S_2$, 309.0935). The NMR data for **1** are summarized in Table The ^1H NMR, ^{13}C NMR, and HMQC spectra of **1** showed the presence of three methyl carbons, five methylene carbons, two methine carbons, and two olefinic methine carbons (δ_C 125.3, 131.3 ppm). Based on the chemical shift, it was clarified that one methine carbon was attached to an oxygenated sulfur atom (δ_H 2.86 ppm, δ_C 70.8 ppm). A detailed analysis of COSY spectrum enabled us to elucidate the entire carbon framework (Fig. 1). Furthermore, to determine the functional group (S_2O_2H) at C-6, hedathiosulfonic acid A (**1**) was methylated with Me_2SO_4–Et_3N to give methyl thiosulfonate **3**. The chemical shifts of an additional methyl group (δ_H 2.67 ppm, δ_C 17.5 ppm) in **3** suggested that this methyl carbon was attached to a non-oxygenated sulfur atom. As a result, we determined that hedathiosulfonic acid A (**1**) had a thiosulfonic acid functionality. Finally, the geometry of the C-9 olefin was determined to be 9*Z*

Keywords: *Echinocardium cordatum*; natural thiosulfonic acid; isolation; structure determination; acute toxicity.

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0040-4039/01/$ - see front matter
PII: S0040-4039(01)01314-4

Table 1. NMR data for hedathiosulfonic acid A (**1**) and B (**2**) in CD_3OD

	Hedathiosulfonic acd A (**1**)			Hedathiosulfonic acd B (**2**)	
Atom	$^1H^a$	$^{13}C^b$	Atom	$^1H^a$	$^{13}C^b$
1	0.91 t 3H (7.2)	14.7 q	1a	4.98 br d (17.4)	116.2 t
			1b	5.01 br d (10.1)	
2a	1.32 m	20.9 t	2	5.83 ddt (10.1, 17.4, 7.2)	138.4 d
2b	1.44 m				
3a	1.11 m	40.1 t	3a	1.88 m	
3b	1.39 m		3b	2.19 m	42.1 t
4	1.78 m	31.8 d	4	1.88 m	32.1 d
5a	1.31 m	39.9 t	5a	1.34 ddd (6.3, 6.6, 14.5)	39.5 t
5b	2.11 ddd (5.3, 7.3, 14.3)		5b	2.13 ddd (5.8, 6.7, 14.5)	
6	2.86 dq (5.3, 5.1)	70.8 d	6	2.88 ddt (6.3, 5.8, 5.9)	70.7 d
7a	1.58 m	32.7 t	7a	1.57 m	32.7 t
7b	2.18 m		7b	2.19 m	
8a	2.23 ddt (7.7, 14.5, 7.2)	25.9 t	8a	2.23 ddt (7.2, 14.6, 7.6)	25.8 t
8b	2.30 ddt (8.1, 14.5, 8.0)		8b	2.29 m	
9	5.40 br ddd (7.7, 8.1, 10.9)	131.3 d	9	5.40 dddq (6.7, 7.2, 10.6, 1.7)	131.2 d
10	5.47 br dq (10.9, 6.7)	125.3 d	10	5.47 br dq (10.6, 6.7)	125.3 d
11	1.63 d 3H (6.7)	13.0 q	11	1.63 br d 3H (6.7)	13.1 q
12	0.92 d 3H (6.6)	20.6 q	12	0.93 d 3H (6.2)	20.2 q

[a] Recorded at 800 MHz. Coupling constants (Hz) are in parentheses.
[b] Recorded at 200 MHz. Multiplicity was based on HMQC spectrum.

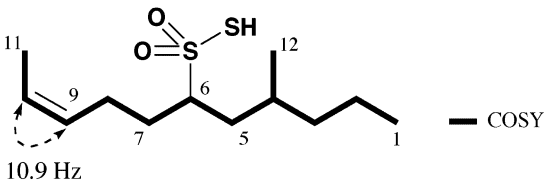

Figure 1. Partial structures of hedathiosulfonic acid A (**1**) based on 2D NMR correlations.

based on the coupling constant (10.9 Hz) between H-9 and H-10. Thus, the gross structure of hedathiosulfonic acid A was determined to be **1**.

The ^1H NMR spectrum of hedathiosulfonic acid B (**2**) resembled that of hedathiosulfonic acid A (**1**), but the presence of three additional vinyl protons (δ_H 4.98, 5.01, 5.83 ppm) and the molecular formula of **2** determined by HRFABMS (m/z 307.0761, calcd for $C_{12}H_{21}Na_2O_2S_2$, 307.0778) suggested that **2** was a dehydro derivative of **1**. The NMR data for **2** are summarized in Table 1. As expected, the gross structure of hedathiosulfonic acid B was determined to be **2** by similar analyses of COSY, HMQC, and HMBC spectra (Fig. 2).

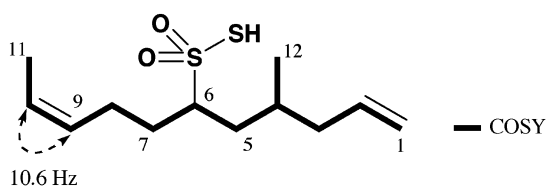

Figure 2. Partial structures of hedathiosulfonic acid B (**2**) based on 2D NMR correlations.

Hedathiosulfonic acid A (**1**) was unstable and gradually decomposed during separation processes. Hedathiosulfonic acid A (**1**) was transformed into sulfonic acid **4**, five-membered cyclic sulfone **5**, and six-membered cyclic sulfone **7** (Fig. 3).[3] The gross structures of **4**, **5**, and **7** were determined using the 2D NMR and the mass spectra. Furthermore, to confirm the position of the hydroxyl group, sulfones **5** and **7** were converted into 3,5-dinitrobenzoate **6** and acetate **8**, respectively. Both the oxymethine protons H-10 in **6** and H-9 in **8** were shifted more than 1 ppm downfield from those in **5** and **7**.[4]

Because the stereochemistry in hedathiosulfonic acid A (**1**) could not be deduced by the spectroscopic analysis, that of the sulfone **7** was determined as follows. As the six-membered sulfone ring part, the magnitude of $J_{8a,9}=10.0$, $J_{8b,9}=3.9$ and $J_{9,10}=10.0$ Hz suggested that H-8a and H-9, H-9 and H-10 were located in *anti* arrangement, respectively. In addition, H-8b and H-9 were located in *gauche* arrangement (Fig. 4). In the NOE experiments (600 MHz) on **7**, irradiation of the signal at H-6 enhanced the signals for H-8a and H-10.

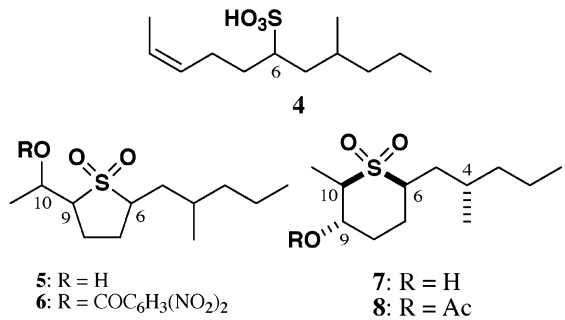

Figure 3. The degraded products of hedathiosulfonic acid A (**1**) and those related compounds.

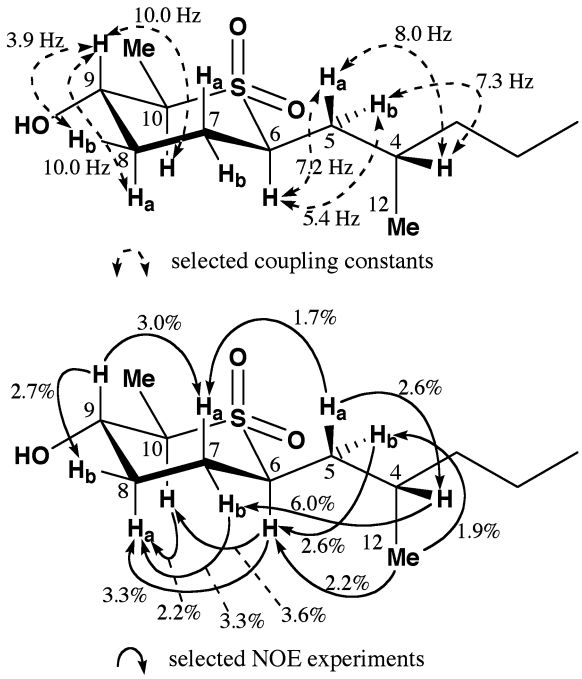

Figure 4. Relative stereochemistry of the six-membered cyclic sulfone **7**.

Irradiation of the signal at H-9 enhanced the signal for H-7a. Irradiation of the signal at H-10 enhanced the signal for H-8a. These results suggested that the protons H-6, H-7a, H-8a, H-9, and H-10 were oriented in *anti* arrangements with respect to the six-membered sulfone ring with the chair conformation. As the alkyl side chain part, the magnitude of $J_{5a,6}=7.2$ and $J_{5b,6}=5.4$ Hz suggested that H-5a and H-6 were in *anti* arrangement and that H-5b and H-6 were in *gauche* arrangement. The stereochemistry of C-4 was not determined clearly because of the subtle difference of the magnitude of $J_{4,5a}=8.0$ and $J_{4,5b}=7.3$ Hz. In the NOE experiments on **7**, irradiation of the signal at H-4 strongly enhanced the signal for H-7b (6.0%). Irradiation of the signal at H-5a enhanced the signal for H-7a. Irradiation of the signal at H-12 enhanced the signal for H-6. These results suggested that the single bond rotation between C-4 and C-5 was restricted in part and that one of the stable conformations of **7** was as shown in Fig. 4. Therefore, the relative stereochemistry in the six-membered cyclic sulfone **7** was suggested to be 4*S**, 6*R**, 9*S** and 10*R**.

The absolute stereochemistry in **7** was determined using the modified Mosher's method (Fig. 5).[5] Treatment of the sulfone **7** with (*R*)- or (*S*)-MTPACl gave (*S*)- or (*R*)-MTPA esters **9** and **10**, respectively. The ^{1}H NMR signals of the two MTPA esters **9** and **10** were assigned on the basis of the 2D NMR spectra, and the $\Delta\delta$ values (δ_S–δ_R, ppm) were then calculated. The results indicated that the absolute stereochemistry of C-9 was 9*S*, and that the absolute stereochemistry in **7** was suggested to be 4*S*, 6*R*, 9*S* and 10*R*. Therefore, the absolute stereochemistry in hedathiosulfonic acid A (**1**) was established to be 4*S* and 6*R*.

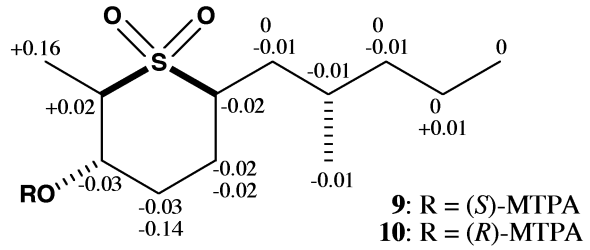

Figure 5. The $\Delta\delta$ values (δ_S–δ_R) for the MTPA esters **9** and **10** in ppm (600 MHz).

In conclusion, hedathiosulfonic acids A (**1**) and B (**2**) were isolated from the deep-sea urchin *E. cordatum*, and were determined to be novel 6-undecanethiosulfonic acids by 2D NMR, HRMS, and methylation reaction. The stereostructure of **1** was elucidated by the spectroscopic analysis of its degradation product **7**. Although many substances that contain sulfur atoms such as sulfonic acids, thiols, and sulfides have been isolated from echinoderms, there have been no previous reports of a thiosulfonic acid like hedathiosulfonic acids.[6] Recently, the genus *Thiothrix*, which can oxidize sulfide, has been reported as symbionts in invertebrates from sulfur-rich habitats.[7] Furthermore, a symbiotic relationship has been demonstrated between *Thiothrix* sp. and *E. cordatum*.[8] Therefore, the genus *Thiothrix*, a symbiont in the deep-sea urchin *E. cordatum*, is probably the actual source of hedathiosulfonic acids.

Acknowledgements

This work was supported in part by Grants-in-Aid for Scientific Research (No. 11558079) and Scientific Research on Priority Areas (A) (No. 12045235) from the Ministry of Education, Culture, Sports, Science, and Technology, Japan. We are indebted to Wako Pure Chemical Industries Ltd., and Banyu Pharmaceutical Co., Ltd. for their financial support. We thank Professors R. Noyori and M. Kitamura (COE, Nagoya University) for allowing us to use the NMR instruments.

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2. Conditions for the isolation of hedathiosulfonic acid A (**1**): Develosil ODS HG-5 (ϕ 20×250 mm); solvent, 50%

MeOH; flow rate, 5.0 mL/min; detection at 205 nm. $[\alpha]_D^{26}$ +2.1° (*c* 0.073, MeOH); IR ($CHCl_3$) 3580–3260 (br), 1180, 1060 cm^{-1}. Conditions for the isolation of hedathiosulfonic acid B (**2**): Develosil ODS HG-5 (ϕ 20× 250 mm); solvent, 50% MeOH; flow rate, 5.0 mL/min; detection at 205 nm. $[\alpha]_D^{26}$ −2.2° (*c* 0.28, MeOH); IR ($CHCl_3$) 3520–3240 (br), 1640, 1180, 1060 cm^{-1}.

3. Studies on this reaction mechanism are in progress, see: Serra, A. C.; da Silva Corrêa, C. M. M. *Tetrahedron Lett*. **1991**, *32*, 6653.
4. Compound **4**: ^1H NMR (CD_3OD) δ 2.72 (1H, H-6); FABMS *m*/*z* 293 $(M-H+2Na)^+$. Compound **5**: ^1H NMR (CD_3OD) δ 4.07 (1H, H-10), 3.10 (1H, H-6), 2.90 (1H, H-9); FABMS *m*/*z* 271 $(M+Na)^+$. Compound **6**: ^1H NMR (CD_3OD) δ 5.59 (1H, H-10), 3.44 (1H, H-9), 3.11 (1H, H-6); FABMS *m*/*z* 465 $(M+Na)^+$. Compound **7**: ^1H NMR (CD_3OD) δ 3.51 (1H, H-9), 3.11 (1H, H-6), 2.93 (1H, H-10); FABMS *m*/*z* 271 $(M+Na)^+$. Compound **8**: ^1H NMR (CD_3OD) δ 4.80 (1H, H-9), 3.30 (1H, H-10), 3.15 (1H, H-6); FABMS *m*/*z* 313 $(M+Na)^+$.
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