Echinosulfonic Acids A−C and Echinosulfone A: Novel Bromoindole Sulfonic Acids and a Sulfone from a Southern Australian Marine Sponge, *Echinodictyum*

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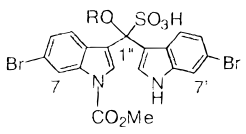
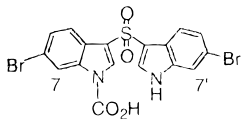
The crude EtOH extract of an *Echinodictyum* sp. collected during trawling operations in the Great Australian Bight, Australia, displayed antibacterial and antiparasitic properties. Bioassay-directed fractionation yielded three novel sulfonic acids, the echinosulfonic acids A to C (**1**−**3**), and a new sulfone, echinosulfone A (**4**). Structures were assigned to these compounds on the basis of detailed spectroscopic analysis. It was determined that echinosulfonic acids A−C (**1**−**3**) and echinosulfone A (**4**) contributed to the antibacterial but not antiparasitic activity of the crude extract.

In our search for bioactive metabolites from southern Australian marine organisms we frequently encounter extracts that inhibit the growth of test microbes. In recent years we have extended our biological screening regime to include assays that target antiparasitic agents with potential application in the field of animal health.

This bioassay-directed fractionation procedure has proved very effective at targeting active agents[1,2] and on occasion has revealed multiple classes of bioactive metabolites in the same extract. In this report we describe the results of our investigations into an *Echinodictyum* sp. The EtOH extract of this sponge displayed both antibacterial and antiparasitic activity. Fractionation yielded an unprecedented class of marine metabolite, the echinosulfonic acids A−C (**1**−**3**) and echinosulfone A (**4**), which contribute to the antibacterial but not antiparasitic activity of the crude extract.



echinosulfonic acid A (**1**) R = Et
echinosulfonic acid B (**2**) R = Me
echinosulfonic acid C (**3**) R = H



echinosulfone A (**4**)

Results and Discussion

The crude EtOH extract of an *Echinodictyum* sp. obtained during trawling operations in the Great Australian Bight, Australia, displayed growth inhibitory properties against the bacteria *Serratia marcescens, Micrococcus luteus*, and *Staphylococcus aureus*. This material was also active in assays designed to measure anthelmintic activity against both endo and exo parasites of significance in the area of animal health. The decanted EtOH extract was concentrated in vacuo and subjected to solvent partitioning, after which the n-BuOH-soluble material was eluted through Sephadex LH-20 and further resolved by ODS HPLC into four novel bromoindole dimers (**1**−**4**). Although compounds **1**−**4** were responsible for the antibacterial activity of the crude extract, they were not responsible for the antiparasitic properties. The identity of the antiparasitic agent is currently under investigation and will be reported at a later date. Quite by chance, we also noted the pH indicator characteristics of compounds **1**−**4**. Solutions of each of these compounds underwent a *reversible* color change from pale yellow to purple at low pH (∼0.5−1). Likewise, during handling these same compounds were observed to take on a pale purple coloration when stored dry on glass surfaces, reverting to yellow when redissolved in solvents. Assignment of structures to these new marine metabolites was achieved as follows.

Echinosulfonic acid A (**1**) was isolated as a stable orange oil with a molecular formula (M − H, $C_{21}H_{17}N_2O_6SBr_2$, Δ 10.5 mmu) requiring 13−15 DBE depending on the oxidation state of sulfur. Initial examination of the NMR data (DMSO-d_6, see Table 1) revealed a highly aromatic compound that featured a deshielded exchangeable proton (δ 11.27), as well as ethoxy (^{1}H, δ 1.07, t; δ 3.24, q; ^{13}C, δ 15.4, q; δ 59.6, t) and methyl ester (^{1}H, δ 3.66, s; ^{13}C, δ 52.0, q; δ 171.5, s) moieties. The presence of an exchangeable proton and an ester functionality were supported by characteristic absorbances in the IR spectrum (3410 and 1740 cm^{-1}, respectively).

More detailed analysis of the DQCOSY and ^{1}H−^{13}C gHMBC NMR data for **1** revealed two isolated *bromoindole* spin systems linked via C3 and C3′ to a common deshielded sp^3 quaternary carbon (^{13}C: δ 79.9, s) designated C1″. That one *bromoindole* subunit (N1 to C7a) was substituted at N1 by the methyl ester moiety was apparent from the absence of a corresponding NH resonance in the ^{1}H NMR spectrum, together with a ^{1}H−^{13}C gHMBC NMR correlation between H2 and the ester carbonyl carbon. Assignment of the bromine regiochemistry for the indole system N1 to C7a was not trivial. Previous experience with dimeric marine bromoindoles had alerted us to the very similiar ^{1}H and ^{13}C NMR chemical shifts for C5 versus C6 regioisomers.[3] This regiochemical issue was resolved by measurement of a ^{1}H−^{15}N gHMBC correlation between H7 (^{1}H: δ 7.94, d, 1.7 Hz) and N1, which provided conclusive evidence for C6 bromine substitution. Assignment of the bromine regiochemistry for the indole system N1′ to C7a′ was achieved both by observation of a NOESY correlation between 1′ NH and C7′ (^{1}H: δ 7.56, d, 1.5 Hz), and a ^{1}H−^{15}N gHMBC correlation between H7′ and N1′. Furthermore, a ^{1}H−^{13}C gHMBC correlation between the oxymethylene protons (δ 3.24) and C1″ (79.9 ppm) placed the ethoxy moiety at C1″. The structure arguments presented above account for all but the elements of SO_3H from the

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Table 1. NMR (400 MHz, DMSO-d_6) Data for Echinosulfonic Acid A (**1**)

				gHMBC		
no.	^{13}C	^{1}H δ [m, J (Hz)])	DQ-COSY	^{1}H−^{13}C	^{1}H−^{15}N	NOESY
1						
2	127.5	7.44 (s)		-CO_2CH_3, C3, C3a, C7a, C1″	N1	-CO_2CH_3
3	114.0					
3a	125.6					
4	122.0	7.29 (d, 8.6)	H5	C5, C6, C7a		
5	122.4	7.06 (dd, 8.6, 1.7)	H4, H7	C4, C7		
6	114.5					
7	116.0	7.94 (d, 1.7)	H5	C3a, C5, C6, C7a	N1	
7a	135.6					
1′		11.27 (br s)	H2′	C2′, C3′, C3a′, C7a′		H7′
2′	126.1	7.36 (d, 2.4)	H1′	C3′, C3a′, C1″	N1′	-CO_2CH_3
3′	113.9					
3a′	124.5					
4′	122.0	7.33 (d, 8.8)	H5′	C3′, C7a′		
5′	121.7	7.01 (dd, 8.8, 1.5)	H4′, H7′	C3a′, C7′		
6′	113.6					
7′	114.1	7.56 (d, 1.5)	H5′		N1′	H1′
7a′	137.3					
1″	79.9					
-CO_2CH_3	171.5					
-CO_2CH_3	52.0	3.66 (s)		-CO_2CH_3		H2, H2′
-OCH_2CH_3	59.6	3.24 (m)	-OCH$_2CH_3$	-OCH$_2CH_3$, C1″		
-OCH$_2CH_3$	15.4	1.07 (t)	-OCH_2CH_3	-OCH_2CH_3		

[a] ^{13}C NMR assignments are supported by a DEPT 135 NMR experiment.

molecular formula, and a single point of attachment at C1″. Thus echinosulfonic acid A (**1**) must have the novel sulfonic acid structure as shown. The assigned structure explains the ease with which echinosulfonic acid A (**1**) generated negative (M − H) rather than positive (M + H) mode ESI mass spectra. Although **1** incorporates a chiral center C1″ (evidenced by the diastereotopic nature of the ethoxy methylene protons) the absence of a measurable optical rotation and the presence of an ethoxy unit suggests that **1** is a racemic artifact of the isolation process, generated by solvolysis during long-term storage in EtOH. This analysis appears all the more probable when considered in conjunction with the structures assigned to echinosulfonic acid B (**2**) and C (**3**).

Echinosulfonic acid B (**2**) was isolated as a stable orange oil with a molecular formula (M − H, $C_{20}H_{15}N_2O_6SBr_2$, Δ 0.8 mmu) consistent with a homologue of echinosulfonic acid A (**1**). The NMR data (DMSO-d_6) for **1** and **2** were very similar, with the only significant difference being that **2** possessed a methoxy (^{1}H, δ 3.04, s; ^{13}C, δ 80.3, d) substituent at C1″, as opposed to the ethoxy substituent in **1**. ^{1}H NMR analysis of the crude EtOH extract revealed that the methoxy moiety was present prior to contact with MeOH, confirming that **2** was indeed a natural product. Despite this, the use of MeOH during isolation does open the possibility for solvolysis of either echinosulfonic acid A (**1**) or B (**2**), leading to racemic echinosulfonic acid B (**2**). Lack of a measurable optical rotation confirmed that the isolated sample of **2** was most likely racemic, either as a consequence of biosynthesis or subsequent solvolysis.

Echinosulfonic acid C (**3**) was isolated as a stable orange oil with a molecular formula (M − H, $C_{19}H_{13}N_2O_6SBr_2$, Δ 5.7 mmu) homologous to both echinosulfonic acids A (**1**) and B (**2**). The NMR data (DMSO-d_6) for **3** was very similar with that for **1** and **2** with the only significant difference being the absence of either methoxy or ethoxy resonances. Given the molecular formula and observation that C1″ remained deshielded (δ 73.6) by attachment to oxygen, echinosulfonic acid C (**3**) was assigned the structure as shown. Since the isolation process involved chromatography with *aqueous* MeOH we cannot exclude the possibility that echinosulfonic acid C (**3**) is a solvolysis artifact of echinosulfonic acid A (**1**) and B (**2**), although the comparable yields of **2** and **3** suggest that the latter occurs as a natural product.

Echinosulfone A (**4**) was isolated as a stable orange oil with a molecular formula (M − H, $C_{17}H_9N_2O_6SBr_2$, Δ 0.6 mmu) corresponding to 13−15 DBE depending on the oxidation state of sulfur. Examination of the NMR data (DMSO-d_6, see Table 2) for **4** revealed an aromatic compound with none of the pendant methoxy, ethoxy and methyl ester functionalities so apparent in the echinosulfonic acids A to C (**1**−**3**). The NMR data for **4** did reveal two isolated bromoindole spin systems, one substituted on N by a carboxylic acid (^{13}C, δ 183.7, s; IR, 2900 and 1740 cm^{-1}). As with the echinosulfonic acids, the C6 and C6′ bromine regiochemistry for the bromoindole subunits was established by both ^{1}H−^{13}C and ^{1}H−^{15}N gHMBC NMR experiments. In major departure from the echinosulfonic acids, **4** lacked C1″, requiring that the bromoindole subunits be connected via C3 and C3′ to a common sulfone functionality. The proposed sulfone functionality was also supported by characteristic absorptions in the IR spectrum (1420, 1250, and 1130 cm^{-1}).[4]

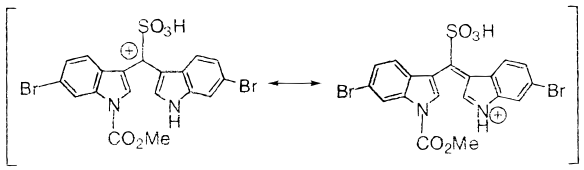
Thus, echinosulfonic acids A−C (**1**−**3**) are homologous sulfonic acids that are capable of interconversion via solvolysis. Although all three echinosulfonic acids are present in the crude EtOH extract, it is our belief that prolonged storage in EtOH has resulted in partial solvolysis of the natural products **2** and **3** into **1**. Consistent with this proposal is the observation that storage of the pure echinosulfonic acids in MeOH resulted in slow conversion of **1** and **3** into **2**. When treated with acid (HCl in MeOH) on a preparative scale (1−5 mg) **1** underwent the expected color change but also experienced an irreversible structure change. Attempts to isolate and identify the products of this decomposition have so far proved inconclusive. The pH indicator characteristics of the echinosulfonic acids may reflect the acid catalyzed formation of a stabilized carbocation such as **5**, the very same carbocation that would be the reactive intermediate in the proposed S_N1 solvolysis linking **1**, **2**, and **3**. The pH sensitivity of **4** is less obvious,

Table 2. NMR (400 MHz, DMSO-d_6) Data for Echinosulfone A (**4**)

				gHMBC	
no.	^{13}C δ	^{1}H δ [m, J (Hz)]	COSY	^{1}H−^{13}C	^{1}H−^{15}N[a]
1					
2	132.7	8.08 (s)		-CO_2H, C3, C3a, C7a	N1
3	115.4				
3a	126.2				
4	122.9	8.13 (d, 8.6)	H5	C7, C7a	
5	124.4	7.37 (dd, 8.6, 1.9)	H4, H7	C3a, C7	
6	115.6				
7	116.4	8.02 (d, 1.9)	H5	C3a, C5, C6, C7a	
7a	135.6				
1′		12.00 (br s)			
2′	132.9	8.15 (br s)		C3, C3a′	N1′
3′	114.4				
3a′	125.3				
4′	122.9	8.14 (d, 8.5)	H5′	C3′, C3a′, C7a′	
5′	123.9	7.32 (dd, 8.5, 1.7)	H4′, H7′	C3a′, C7′	
6′	115.1				
7′	114.7	7.70 (d, 1.7)	H5′	C3a′, C6′	
7a′	137.5				
-CO_2H	183.7				

[a] Detection of the gHMBC correlation from H2 to N1-CO_2H required a longer relaxation delay time (4 s rather than the standard value of 2.5 s).

and the lack of available material precluded further investigation of this phenomenum.



(**5**)

The echinosulfonic acids A−C (**1**−**3**) and echinosulfone A (**4**) are unprecedented marine natural products. While the sulfonic acid functionality has been reported in a small number of marine natural products, these are dominated by metabolites featuring primary sulfonic acids biosynthetically related to taurine, together with a few sulfonoglycolipids. This contrasts with the echinosulfonic acids A to C (**1**−**3**) which incorporate a tertiary sulfonic acid. Likewise, while a taurine-derived sulfone moiety does feature in a few marine natural products, the biosynthetic origins of the sulfone moiety in echinosulfone A (**4**) does not appear to involve taurine. The echinosulfonic acids A−C (**1**−**3**) and echinosulfone A (**4**) belong to a new class of structurally novel marine metabolite.

Experimental Section

General Experimental Procedures. For general experimental details, see ref 5.

Collection, Extraction and Isolation. A specimen of *Echinodictyum* sp. (Museum of Victoria Registry Number F79983) was collected at a depth of 65 m during trawling operations in the Great Australian Bight in July 1995. [This specimen of *Echinodictyum* was a stalked growth form, flabelliform, biplanar-lamellate 10−20 mm thick; color on deck beige-orange; beige in EtOH; texture spongy, fibrous, and harsh; surface transparent, porous, optically smooth and detachable; oscules conspicuous and lateral on lamellae; megascleres oxeas (fusiform 190−200 × 8−12 μm); acanthostyles (100−120 μm); no microscleres; ectosome an almost continuous palisade of brushes of oxeas; choanosome an irregular plumo-reticulate network of fibers cored by multispicular tracts of oxeas and echinated by acanthostyles, becoming more plumose extra-axially. Spicules scattered interstitially in dense, granular collagen.] The frozen sponge was transported to the laboratory where it was thawed, diced, steeped in EtOH and stored at −18 °C for 3 years. The decanted crude EtOH extract was concentrated in vacuo and sequentially subjected to gel permeation chromatography (elution through Sephadex LH-20 with MeOH) and C_{18} reversed-phase HPLC (2 mL/min elution with either 30 or 50% H_2O/MeOH through a 5-μm Phenomenex C_{18} Ultracarb 250 × 10 mm column) to yield four novel metabolites: **1** (80 mg, 2.4%), **2** (14.8 mg, 0.44%), **3** (12.1 mg, 0.36%), and **4** (7.8 mg, 0.23%). Note that percentage yields are calculated against the mass of crude EtOH extract.

Echinosulfonic acid A (1): orange oil; IR (film) ν_{max} 3410, 1740, 1540, 1240, 1050 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 223 (42 000), 276 (8000) nm; ^{1}H NMR data (DMSO-d_6, 400 MHz) see Table 1; ^{13}C NMR data (DMSO-d_6, 100 MHz) see Table 1; ESIMS (20 kV) m/z 583, 585, 587 (1:2:1, M − H); HRESIMS 582.9207 ($C_{21}H_{17}N_2O_6S^{79}Br_2$ requires 582.9192).

Echinosulfonic acid B (2): orange oil; IR (film) ν_{max} 3420, 1730, 1540, 1240, 1050 cm^{-1}; UV(MeOH) λ_{max} (ϵ) 223 (39 000), 276 (8000) nm; ^{1}H NMR data (DMSO-d_6, 400 MHz) δ 11.37 (br s, H1), 7.90 (d, J = 1.5 Hz, H7), 7.55 (d, J = 1.7 Hz, H7′), 7.40 (s, H2), 7.36 (d, J = 2.2 Hz, H2′), 7.26 (d, J = 8.6 Hz, H4′), 7.24 (d, J = 8.6 Hz, H4), 7.06 (dd, J = 8.6, 1.7 Hz, H5), 7.00 (dd, J = 8.6, 1.5 Hz, H5′), 3.65 (s, -CO_2CH_3), 3.04 (s, C1″-OCH_3); ^{13}C NMR data (DMSO-d_6, 100 MHz) δ 171.3 (s, -CO_2-CH_3), 137.3 (s, C7a′), 135.6 (s, C7a), 127.8 (d, C2), 126.4 (d, C2′), 125.6 (s, C3a), 124.5 (s, C3a′), 122.5 (d, C5), 122.0 (d, C4′), 121.9 (d, C4), 121.8 (s, C5′), 116.0 (d, C7), 114.5 (s, C6), 114.1 (d, C7′), 114.0 (s, C3), 113.2 (d, C3′), 113.1 (s, C6′), 80.3 (s, C1″), 52.1 (q, -CO_2CH_3), 51.9 (q, -OCH_3); ESIMS (20 kV) m/z 569, 571, 573, (1:2:1, M − H); HRESIMS 568.9027 ($C_{20}H_{15}N_2O_6S^{79}Br_2$ requires 568.9035).

Echinosulfonic acid C (3): orange oil; IR (film) ν_{max} 3420, 1730, 1460, 1260, 1130 cm^{-1}; UV (MeOH) λ_{max} (ϵ), 222 (47 000), 276 (8800) nm; ^{1}H NMR (DMSO-d_6) δ 11.25 (br s, H1), 7.91 (d J = 1.8 Hz, H7), 7.55 (d, J = 1.7 Hz, H7′), 7.34 (d, J = 8.6 Hz, H4′), 7.28 (d, J = 8.4 Hz, H4), 7.23 (s, H2), 7.16 (d, J = 2.2 Hz, H2′), 7.07 (dd, J = 8.4, 1.8 Hz, H5), 7.01 (dd, J = 8.6, 1.7 Hz, H5′), 3.65 (s, -CO_2CH_3); ^{13}C NMR (DMSO-d_6) δ 173.5 (s, -CO_2CH_3), 137.3 (s, C7a′), 135.7 (s, C7a), 126.5 (d, C2), 125.6 (d, C2′), 124.6 (s, C3a), 124.4 (s, C3a′), 122.1 (d, C5), 122.0 (d, C4′), 121.9 (d, C4), 121.2 (d, C5′), 117.0 (d, C7), 116.8 (s, C6), 115.9 (d, C7′), 113.6 (s, C6′), 114.1 (s, C3), 113.8 (d, C3′), 73.6 (s, C1″), 51.9 (q, -CO_2CH_3); ESIMS (60 kV) m/z 555, 557, 559 (1:2:1, M − H); HRESIMS 554.8822 ($C_{19}H_{13}N_2O_6S^{79}Br_2$ requires 554.8879).

Echinosulfone A (4): orange oil; IR (film) ν_{max} 3420, 2900, 1740, 1420, 1250, 1130 cm^{-1}; UV (MeOH) λ_{max} (ϵ) 221 (42 000),

250 (sh, 14 000), 280 (17 000), 320 (sh, 9000) nm; ^{1}H NMR (DMSO-d_6, 400 MHz), see Table 2; ^{13}C NMR (DMSO-d_6, 100 MHz), see Table 2; ESIMS (20 kV) *m*/*z* 495, 497, 499 (1:2:1, M − H); HRESIMS 494.8674 ($C_{17}H_9N_2O_6S^{79}Br_2$ requires 494.8668).

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Supporting Information Available: ^{1}H NMR, ^{13}C NMR, and low-resolution ESIMS data for echinosulfonic acids A−C and echinosulfone A. This material is available free of charge via the Internet at http://pubs.acs.org.

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