

Marine Sponge *Dysidea herbacea* revisited: Another Brominated Diphenyl Ether

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Abstract: A pentabrominated phenolic diphenyl ether (**1**) that has not previously been reported from marine sources has been isolated from *Dysidea herbacea* collected at Pelorus Island, Great Barrier Reef, Australia. The structure was determined by comparison of NMR data with those of known structurally-related metabolites. NMR spectral assignments for (**1**) are discussed in context with those of three previously reported isomeric pentabrominated phenolic diphenyl ethers.

Keywords: Brominated diphenyl ether, marine sponge, *Dysidea herbacea*.

Introduction

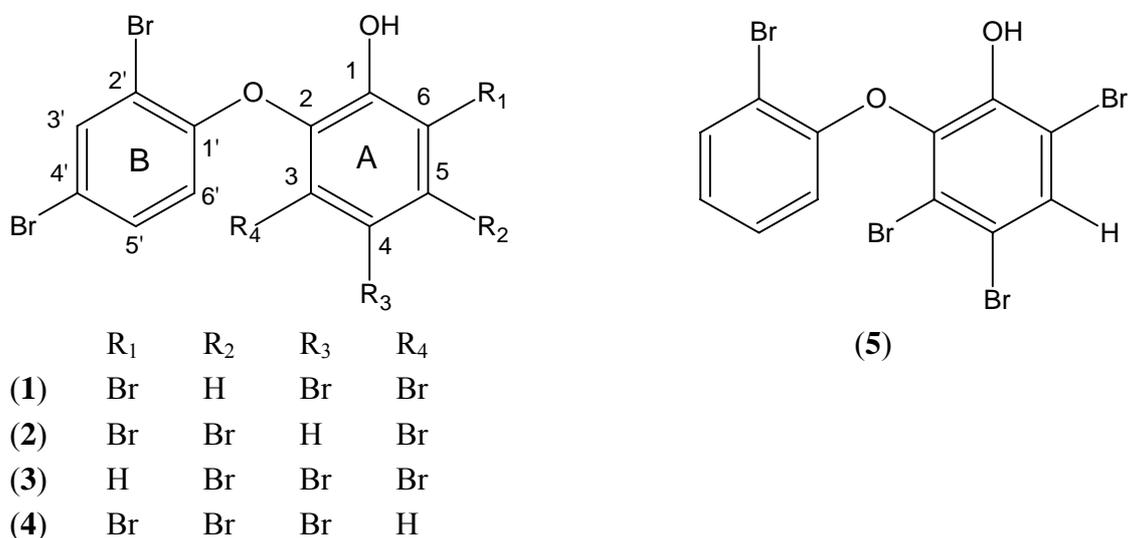
It is well documented that the marine sponge *Dysidea herbacea* occurs in two general chemotypes: one produces sesquiterpenes (usually furanosesquiterpenes) and polychlorinated amino acid derivatives, while the other produces only polybrominated diphenyl ethers [1]. The production of the chlorinated metabolites and the polybrominated diphenyl ethers has been reported to be due to the filamentous cyanobacterium *Oscillatoria spongelliae* [2-4]. Recently, 16S-rDNA studies on *Oscillatoria* strains isolated from *Dysidea* species that exhibited different colourmorph and growth characteristics indicated that each species of *Dysidea* hosted a distinct strain of *Oscillatoria*, which was interpreted to imply a high degree of host specificity and possible coevolution between the

symbiotic bacterium and its host sponge [5]. Recent studies have also reported detection of polybrominated diphenyl ethers in higher trophic groups such as fish, turtles, birds and even marine mammals [6,7a-c], implying that these compounds are bioaccumulated in nature, and may persist in significant concentrations in such higher trophic organisms.

As a group, the polybrominated diphenyl ethers exhibit a wide range of activities in bioassays, ranging from antibacterial activity (against *S. aureus* and *T. mentagrophytes*), to cytotoxicity (Ehrlich ascite tumor cells) [8]. This cytotoxicity is exhibited by inhibition of a range of enzymes that are implicated in tumor development, such as inosine monophosphate dehydrogenase, guanosine monophosphate synthetase and 15-lipoxygenase [9]. We previously reported the isolation of the pentabrominated diphenyl ether (**2**) from samples of *Dysidea herbacea* collected from Cattle Bay, Orpheus Island [10]. We now report the characterisation of the last remaining isomer of this particular group of pentabrominated phenolic diphenyl ethers. It was obtained as a minor metabolite from a *Dysidea herbacea* sample, that also contained (**2**) as its major metabolite, collected from Pelorus Island.

Results and Discussion

The ^1H NMR spectrum of the crude dichloromethane extract from a *Dysidea herbacea* sample collected from -11m at Pelorus Island in December 2003 contained, in addition to the signals characteristic of the pentabrominated diphenyl ether (**2**) [10], a singlet at $\delta 7.74$ indicative of the presence of a minor metabolite. The brominated diphenyl ether fraction was isolated by vacuum liquid chromatography, and the major and minor metabolites were separated by reverse-phase HPLC.



The minor metabolite (**1**) was found by high-resolution electrospray mass spectrometry (negative ion mode) to have the same molecular formula as (**2**), $\text{C}_{12}\text{H}_5\text{Br}_5\text{O}_2$. The ^1H and ^{13}C NMR spectra were consistent with a phenolic diphenyl ether that contained a tribrominated phenolic A-ring, and a dibrominated B-ring. This meant that the A ring substitution pattern was isomeric with that of (**2**), but

two (**3** and **4**) of the other three positional isomers for the sole hydrogen atom on the A-ring had already been reported from *Dysidea* and *Phyllospongia* samples [9,11]. The ^1H NMR signal for the sole hydrogen atom on ring A for (**1**) resonated at $\delta 7.74$, but at $\delta 7.42$ for (**3**) [10,11], $\delta 7.01$ for (**4**) [7] and $\delta 7.55$ for (**2**) [10] (Table 1).

The ^1H NMR shifts for the protons on the B ring of (**1**) ($\delta 6.40$, 7.29 and 7.78) were quite similar to those reported for (**2**) ($\delta 6.41$, 7.28 and 7.78) and (**3**) ($\delta 6.41$, 7.29 and 7.79), but the shifts reported for $\text{H}5'$ and $\text{H}6'$ for (**4**) ($\delta 7.45$ and 6.89 resp. in CDCl_3 [7], 7.4 and 6.82 resp. in CCl_4 [11]) were significantly different. The structure of (**1**) was clearly 1-hydroxy-3,4,6,2',4'-pentabromodiphenyl ether, and indeed the observed ^1H NMR ($\delta 7.74$) and ^{13}C NMR shifts observed in the A ring (Table 1) were in good agreement with ^1H NMR data ($\delta 7.75$) and ^{13}C NMR data (Table 1) reported for 1-hydroxy-3,4,6,2'-tetrabromodiphenyl ether (**5**) [12].

During our isolation and structural elucidation of this metabolite, a report of the synthesis of 1-hydroxy-3,4,6,2',4'-pentabromodiphenyl ether was published as a full paper, elaborating on results that had previously been presented at the Dioxin 2001 meeting [7a,7b,7c]. However, only ^1H NMR data was presented in those reports. The reported ^1H NMR data is in agreement with that observed for (**1**).

Table 1. ^1H and ^{13}C NMR Assignments in CDCl_3 for 1-4 and comparison with the ring-A ^{13}C data for 5; No ^{13}C NMR data has to date been published for 4.

	1		5 [12]	2 [10]		3 [10]		4 [7]
C #	$\delta^1\text{H}$, mult., J(Hz)	$\delta^{13}\text{C}$	$\delta^{13}\text{C}$	$\delta^1\text{H}$, mult., J(Hz)	$\delta^{13}\text{C}$	$\delta^1\text{H}$, mult., J(Hz)	$\delta^{13}\text{C}$	$\delta^1\text{H}$, mult., J(Hz)
1		146.4	146.7		148.1		148.9	
2		140.5	141.0		138.7		139.9	
3		120.1	120.2		116.7		113.6	7.01, s
4		116.1	116.1	7.55, s	128.0		119.3	
5	7.74, s	132.8	132.8		122.2		122.9	
6		110.1	109.8		113.4	7.42, s	120.8	
1'		151.9			152.1		151.8	
2'		112.6			112.6		112.8	
3'	7.78, d, 2.4	136.1		7.78, d, 2.2	136.1	7.79, d, 2.2	136.4	7.81, d, 2.4
4'		115.7			115.8		116.3	
5'	7.29, dd, 8.8, 2.4	131.4		7.28, dd, 8.8, 2.2	131.3	7.29, dd, 8.8, 2.2	131.6	7.45, dd, 8.6, 2.4
6'	6.40, d, 8.8	115.9		6.41, d, 8.8	115.8	6.41, d, 8.8	115.8	6.89, d, 8.6

Conclusions

The pentabrominated phenolic diphenyl ether (1) that has not previously been reported from marine sources has been isolated from *Dysidea herbacea* collected at Pelorus Island, Great Barrier Reef, Australia. This means that all 4 positional isomers of diphenyl ethers that contain a 2,4-dibrominated

B- ring and a 1-hydroxytribrominated A-ring with the ether linkage at the 2-position have now been reported from marine sponges.

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Experimental

General

IR spectra were determined on a Nicolet Nexus 670 infrared spectrometer. Mass spectral data were determined on a Bruker BioAPEX 47e mass spectrometer operating in negative ion electrospray mode at the Australian Institute of Marine Science, Cape Ferguson. ¹H NMR spectra were measured in CDCl₃ at 300 MHz and ¹³C NMR spectra at 75.5 MHz on a Varian Mercury NMR using residual solvent peaks for calibration. Merck t.l.c. grade silica gel 5-40μ (type 60) was used for column chromatography. HPLC purification was carried out on a Hewlett-Packard C18 column (10 x 250 mm), monitored with a GBC diode array detector. The metabolite ratio was determined by integration at 292 nm. All solvents used were freshly distilled.

Animal material

The sponge *Dysidea herbacea* was collected by hand using scuba (from -11m) near Pelorus Island (18° 34' S; 146° 29' E) in the central section of the Great Barrier Reef Marine Park, Australia. The sample was frozen immediately after collection and kept frozen until used. A taxonomic sample (registered sample No.G25097) is lodged with the Museum of Tropical Queensland, Flinders Street, Townsville, Qld. 4810.

Extraction and Purification.

The freeze-dried sponge (10.89 g) was successively extracted with dichloromethane (3 × 100 ml). The solvent was removed on a rotary evaporator to afford a crude extract (0.448g) which was rapidly chromatographed on silica gel under vacuum using a stepwise gradient from hexane to

dichloromethane to ethyl acetate. A mixture of the diphenyl ethers (**1** and **2**) (22.8mg, 0.21%) was eluted in the dichloromethane/hexane 1:9 and 1:4 fractions as a crystalline solid. This material was separated by reverse phase HPLC by elution with acetonitrile / 1% aqueous ammonium acetate (9:1) at a flow rate of 1.5 ml/min. The acetonitrile was removed from fractions that contained the metabolites **1** (retention time 3.23 min) and **2** (retention time 2.85 min) and each was transferred to a separating funnel and extracted with dichloromethane. Removal of the dichloromethane solvent afforded the minor metabolite **1** and the major metabolite **2** in a ratio of 1:10 (based on integrated peak areas).

Spectral Data

1-Hydroxy-3,4,6,2',4'-pentabromodiphenyl ether (**1**)

^1H and ^{13}C NMR (CDCl_3): see Table 1.

IR (Chloroform) cm^{-1} : 3515, 3019, 2927, 2854, 1727, 1466, 1392, 1299, 1257, 1091, 1043, 932, 871, 807.

UV (EtOH) nm: 211 (ϵ 27193), 292 (ϵ 2379), sh 317 (ϵ 1022)

HRESMS (negative ion mode) for $\text{C}_{12}\text{H}_4^{79}\text{Br}_3^{81}\text{Br}_2\text{O}_2$ $[\text{M-H}]^-$: Calcd 578.6093; Found 578.6097.

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Sample availability: Not available.