

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

Madhavi S. Agrawal and Bruce F. Bowden*

School of Pharmacy and Molecular Sciences, James Cook University, Townsville 4811, Qld., Australia
Tel. +61 747 814533, Fax +61 747 816078, e-mail bruce.bowden@jcu.edu.au

* Author to whom correspondence should be addressed.

Received: 22 October 2004 / Accepted: 14 March 2005 / Published: 14 March 2005

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

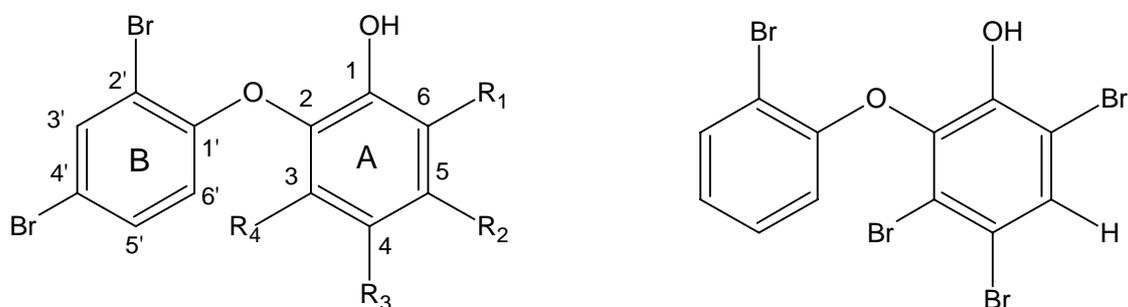
It is well documented that the marine sponge *Dysidea herbacea* occurs in two general chemotypes: one produces sesquiterpenes (usually furanosesquiterpenes) and polychlorinated amino acid derivatives, while the other produces only polybrominated diphenyl ethers [1]. The production of the chlorinated 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.



	R ₁	R ₂	R ₃	R ₄
(1)	Br	H	Br	Br
(2)	Br	Br	H	Br
(3)	H	Br	Br	Br
(4)	Br	Br	Br	H

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 δ 7.74, but at δ 7.42 for (**3**) [10,11], δ 7.01 for (**4**) [7] and δ 7.55 for (**2**) [10] (Table 1).

The ^1H NMR shifts for the protons on the B ring of (**1**) (δ 6.40, 7.29 and 7.78) were quite similar to those reported for (**2**) (δ 6.41, 7.28 and 7.78) and (**3**) (δ 6.41, 7.29 and 7.79), but the shifts reported for H5' and H6' for (**4**) (δ 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 (δ 7.74) and ^{13}C NMR shifts observed in the A ring (Table 1) were in good agreement with ^1H NMR data (δ 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.

Acknowledgements

We wish to thank Dr. John Hooper from the Queensland museum for taxonomy and Mr. Rick Willis from A.I.M.S. for mass spectrometry.

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 CDCl_3 at 300 MHz and ^{13}C NMR spectra at 75.5 MHz on a Varian Mercury NMR using residual solvent peaks for calibration. Merck t.l.c. grade silica gel 5-40 μ (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 $\text{C}_{12}\text{H}_4^{79}\text{Br}_3^{81}\text{Br}_2\text{O}_2$ $[\text{M}-\text{H}]^-$: Calcd 578.6093; Found 578.6097.

References

- [1] Blunt, J.W.; Copp, B.R.; Munro, M.H.G.; Northcote, P.T.; Princep, M.R. Marine Natural Products. *Nat. Prod. Rep.* 2004, **21**, 1-49 and earlier reviews in that series, including Faulkner, D.J. Marine Natural Products. *Nat. Prod. Rep.* 2002, **19**, 1-49 and earlier reviews in that series.
- [2] Faulkner, D.J.; Unson, M.D.; Bewley, C.A. The chemistry of some sponges and their symbionts. *Pure Appl. Chem.* 1994, **66**, 1983-1990.
- [3] Unson, M.D.; Faulkner, D.J. Cyanobacterial symbiont biosynthesis of chlorinated metabolites from *Dysidea herbacea* (Porifera). *Experientia* 1993, **49**, 349.
- [4] Unson, M.D.; Holland, N.D.; Faulkner, D.J. A brominated secondary metabolite synthesized by the bacterial symbiont of a marine sponge and accumulation of the crystalline metabolite in the sponge tissue. *Mar. Biol.* 1994, **119**, 1-11.
- [5] Thacker, R.W.; Starnes, S. Host specificity of the symbiotic cyanobacterium *Oscillatoria spongelliae* in marine sponges *Dysidea* spp. *Mar. Biol.* 2003, **142**, 643-648.
- [6] Vetter, W.; Stoll, E.; Garson, M.J.; Farhey, S.J.; Gaus, C.; Muller, J.F. Sponge halogenated natural products found at parts-per-million levels in marine mammals. *Environ. Toxic. Chem.* 2002, **21**, 2014-2019.
- [7] (a) Marsh, G.; Stenutz, R.; Bergman, A. Synthesis of hydroxylated and methoxylated polybrominated diphenyl ethers – natural products and potential polybrominated diphenyl ether metabolites. *Eur. J. Org. Chem.* 2003, 2566-2576.

- (b) Marsh, G.; Athanasiadou, M.; Bergman, A.; Jakobsson, E.; Asplund, L. Hydroxylated and methoxylated polybrominated diphenyl ethers in salmon plasma: synthesis and identification. *Organohalogen Compounds* 2001, **52**(Dioxin 2001), 62-66.
- (c) Asplund, L.; Malmvaern, A.; Marsh, G.; Athanasiadou, M.; Bergman, A.; Kautsky, L. Hydroxylated brominated diphenyl ethers in salmon (*Salmo salar*), blue mussels (*Mytilus edulis*) and the red algae (*Ceramium tenuicorne*) from the Baltic Sea – natural production in Baltic Sea biota. *Organohalogen Compounds* 2001, **52**(Dioxin 2001), 67-70.
- [8] Popov, A.M.; Stekhova, S.I.; Utkina, N.K.; Rebachuk, N.M. Antimicrobial and cytotoxic activity of sesquiterpenequinones and brominated diphenyl ethers isolated from marine sponges. *Pharm. Chem.* 1999, **33**, 15-16.
- [9] Fu, X.; Schmitz, F.J.; Govindan, M.; Abbas, S.A.; Hanson, K.M.; Horton, P.A.; Crews, P.; Laney, M.; Schatzman, R.C. Enzyme inhibitors: new and known polybrominated phenols and diphenyl ethers from four Indo-Pacific *Dysidea* sponges. *J. Nat. Prod.* 1995, **58**, 1384-1391.
- [10] Bowden, B.F.; Towerzey, L.; Junk, P.C. A new brominated diphenyl ether from the marine sponge *Dysidea herbacea*. *Aust. J. Chem.* 2000, **53**, 299-301.
- [11] Carté, B.; Faulkner, D.J. Polybrominated diphenyl ethers from *Dysidea chlorea* and *Phyllospongia foliascens*. *Tetrahedron* 1981, **37**, 2335-2339.
- [12] Handayani, D.; Edrada, R.A.; Proksch, P.; Wray, V.; Witte, L.; Van Soest, R.W.M.; Kunzmann, A.; Soedarsono. Four new bioactive polybrominated diphenyl ethers of the sponge *Dysidea herbacea* from West Sumatra, Indonesia. *J. Nat. Prod.* 1997, **60**, 1313-1316.

Sample availability: Not available.