Two New Minor Polybrominated Dibenzo-*p*-dioxins from the Marine Sponge *Dysidea dendyi*

Natalia K. Utkina,*,† Vladimir A. Denisenko,† Marina V. Virovaya,‡ Olga V. Scholokova,‡ and Nina G. Prokof'eva†

Pacific Institute of Bioorganic Chemistry of the Russian Academy of Sciences, 690022 Vladivostok, Russia, and Department of Bioorganic Chemistry and Biotechnology, Far Eastern State University, 690000 Vladivostok, Russia

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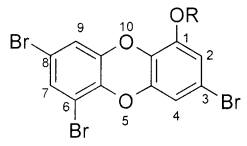
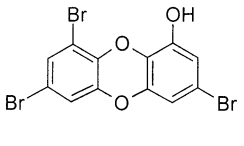
Two new minor tribromodibenzo-*p*-dioxins, spongiadioxin C (**1**) and its methyl ether (**2**), were isolated from an Australian marine sponge *Dysidea dendyi*, together with the known minor metabolites methyl ethers of spongiadioxins A (**4**) and B (**6**) and polybrominated diphenyl ethers (**7**−**9**). The structures of the new compounds were established by 1D and 2D NMR spectroscopy and confirmed by synthesis of **2** from diphenyl ether **9**. All isolated compounds inhibited the cell division of fertilized sea urchin eggs.

Tropical marine sponges of the genus *Dysidea* (order Dendroceratida, family Dysideidae) contain a series of polybrominated diphenyl ethers, whose production in some *Dysidea* species is due to the cyanobacterium *Oscillatoria spongeliae*.[1] Previous work on the sponge *Dysidea dendyi* (*Lendenfeld, 1889*) (order Dendroceratida, family Dysideidae) has yielded two major metabolites, spongiadioxins A (**3**) and B (**5**), bearing a dibenzo-*p*-dioxin structure.[2] Three polybromodibenzo-*p*-dioxins have been isolated in minute amounts from the marine sponge T*edania ignis*.[3]

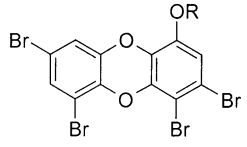
On further examination of the marine sponge *D. dendyi*, we have now found and report here seven minor metabolites which include two new polybrominated dibenzo-*p*-dioxins, spongiadioxin C (**1**) and its methyl ether (**2**), and known polybrominated dibenzo-*p*-dioxins **4** and **6**, and polybrominated diphenyl ethers **7**−**9**.



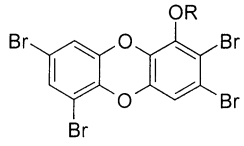
1 R = H
2 R = Me



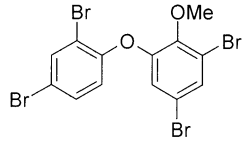
1a



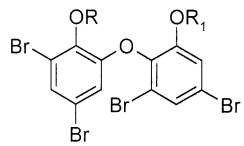
3 R = H
4 R = Me



5 R = H
6 R = Me



7



8 R = R_1 = Me
9 R = H, R_1 = Me

The hexane-soluble fraction from the $CHCl_3$ extract of the freeze-dried sponge was separated by vacuum flash chromatography on Si gel to give, in order of elution, seven compounds, **6**, **7**, **2**, **8**, **4**, **9**, and **1**. Dibenzo-*p*-dioxins **4** and **6** were identified by comparison of their spectral data and melting points with those for products of methylation of **3** and **5**,[2] but this is the first report of these compounds as natural products. Diphenyl ether **7** has been isolated from the green alga *Cladophora fascicularis*[4] and synthesized,[5] but this is the first report of its isolation from a sponge. Compounds **7**−**9** were identfied by comparison of their spectral data with published values.[4−7]

The new compound **1** exhibited a cluster of peaks in EIMS at *m*/*z* 440, 438, 436, and 434 characteristic for tribrominated compounds. The molecular formula of **1** was found to be $C_{12}H_5O_3Br_3$ by microanalysis. The IR spectrum of **1** revealed the presence of a hydroxyl group (3524 cm^{-1}) that was supported by formation of monomethyl ether **2**. The ^1H NMR spectrum of **1** contained signals of two pairs of *meta*-situated aromatic protons. The ^{13}C NMR spectrum (Table 1) exhibited 12 carbon signals of two aromatic rings. The molecular formula and the presence of only one hydroxyl group in compound **1** indicated a dibenzo-*p*-dioxin skeleton as in **3** and **5**.[2] The sequence of substitution in each ring was determined by HMQC and HMBC experiments and from long-range $^{13}C-^1H$ coupling constants measured in gated-decoupling experiments. The values for *ortho*, *meta*, and *para* $^{13}C-^1H$ coupling constants in **1** were consistent with long-range $^{13}C-^1H$ coupling constants in polybrominated dibenzo-*p*-dioxins[2] and polybrominated diphenyl ethers.[6] The data obtained, however, could not distinguish between the possible structures **1** and **1a**. Chemical shifts and $^{13}C-^1H$ coupling constants of six signals of C-5a to C-9a of **1** were essentially identical with those of compounds **3** and **5**,[2] indicating that this ring has two bromine atoms at the 6,8-position rather than 7,9-position, as in the possible isomer **1a**. To confirm the 6,8-position of the two bromine atoms in **1**, we carried out dehydrobromination of diphenyl ether **9** and compared the obtained product with the sample prepared by methylation of **1**. The product of dehydrobromination exhibited a melting point and ^1H NMR and EIMS spectra identical with those for the product of methylation of **1**. The mixed melting point of the two compounds showed no depression, and hence structure **1** was confirmed.

The second new compound was **2**, whose formula $C_{13}H_7O_3Br_3$ and ^1H NMR spectrum showed that it was the OMe analogue of **1**. The natural compound **2** exhibited a melting

* To whom correspondence should be addressed. Fax: (4232) 31 4050. E-mail: utkinan@mail.ru.
† Pacific Institute of Bioorganic Chemistry.
‡ Far Eastern State University.

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Table 1. ^{13}C NMR Data and $^{1}H-^{13}C$ Correlations for **1** and **2** (DMSO-d_6, 75 MHz)

	1		**2**	
C	δ_C (mult, J_{CH} in Hz)	HMBC	δ_C (mult, J_{CH} in Hz)	HMBC
1	146.8 (dd, 3.1;1.0)		148.3 (dd, 3.2; 0.9)	
2	115.6 (dd, 170.3; 6.0)	1, 3, 4, 10a	112.2 (dd, 168.7; 6.1)	1, 3, 4, 10a
3	114.7 (t, 4.8)		115.2 (t, 5.2)	
4	109.7 (dd, 171.2; 6.5)	2, 3, 4a, 10a	111.5 (dd, 171.4; 6.1)	2, 3, 4a, 10a
4a	142.3 (dd, 4.5; 1.1)		141.8 (dd, 4.0; 0.8)	
10a	129.2 (dd, 7.6; 7.0)		129.8 (dd, 7.9; 6.8)	
5a	138.3 (dd, 8.0; 7.2)		138.2 (dd, 8.1; 7.2)	
6	110.2 (dd, 3.9; 1.4)		110.2 (dd, 4.0; 1.5)	
7	129.5 (dd, 175.3; 6.5)	5a, 6, 8, 9	129.7 (dd, 175.4; 6.5)	5a, 6, 8, 9
8	115.4 (t, 4.5)		115.6 (t, 4.6)	
9	118.9 (dd, 171.0; 6.1)	5a, 7, 8, 9a	119.0 (dd, 171.0; 6.5)	5a, 7, 8, 9a
9a	142.8 (dd, 4.3; 1.3)		142.6 (dd, 4.6; 1.2)	
OCH_3			56.6 (q, 146.0)	1

point and ^{1}H NMR and mass spectra identical with those for the sample prepared by methylation of **1** and for the product of dehydrobromination of diphenyl ether **9**, and hence structure **2** was confirmed. The carbon signals of **2** (Table 1) were assigned from HMQC, HMBC, and gated-decoupling experiments.

All isolated compounds were tested for the inhibition of cell division of the fertilized eggs of the sea urchin *Strongylocentrotus intermedius*. Among spongiadioxins **1**, **3**, and **5** with a free hydroxyl group, compound **5** demonstrated the most toxic effect (IC_{50} 5.7, 4.8, and 1.1 μM, respectively). It is known that the number and position of halogen atoms in polyhalogenated dibenzo-*p*-dioxins influence toxicity, and 2,3,7,8-tetrasubstituted dioxins are most potent.[8] The biological activities of spongiadioxins **1**, **3**, and **5** are consistent with this hypothesis. Diphenyl ether **9** exhibited toxicity (IC_{50} 4.7 μM) comparable with **3**. Methylation of hydroxyl groups in all compounds leads to a significant decrease in cytostatic effects. The IC_{50}'s for **2**, **4**, **6**, **7**, and **8** are 166, 141, 94, 145, and 137 μM, respectively.

Experimental Section

General Experimental Procedures. For general experimental details, see ref 2. Chemical shifts was referenced to the residual solvent signal (DMSO-d_6: $\delta_H = 2.50$, $\delta_C = 39.6$ ppm; $CDCl_3$: $\delta_H = 7.26$ ppm). HMBC spectra were optimized for 10 Hz coupling. Si gel ICN (63−100, 60 Å, ICN Biomedicals, Germany) was used for vacuum flash chromatography.

Animal Material. Collection and description of the sponge were published previously.[2]

Extraction and Isolation. The freeze-dried sponge *D. dendyi* (100 g) was extracted at room temperature with $CHCl_3$ (3 × 500 mL). The combined $CHCl_3$ extracts were concentrated under reduced pressure to yield a green gum. This material was partitioned into hexane solubles (0.9 g) and hexane insolubles (8.6 g). The hexane-soluble material was fractionated by vacuum flash chromatography over Si gel using hexane to yield 16 fractions with UV-absorbing compounds. Fractions 1−3 contained a 1:3 mixture of **6** and **7**, which was separated on Sorbfil plates in hexane to obtain **6** (7 mg, 0.007%) and **7** (21 mg, 0.021%). Slow solvent evaporation from fractions 4−6 gave pure **2** (18 mg, 0.018%). Fractions 7−12 yielded **8** (250 mg, 0.25%). Fraction 13 contained a 1:1 mixture of **8** and **4**, which was separated on Sorbfil plates in hexane−$CHCl_3$ (9:1) to obtain **4** (1 mg, 0.001%). Fraction 15 contained a 1:1 mixture of **9** and **1**, which was separated on Sorbfil plates in hexane−acetone (4:1) to obtain **9** (5 mg, 0.005%). The last fraction 16 yielded **1** (100 mg, 0.1%). The hexane-insoluble fraction contained a 3:1 mixture of major metabolites **3** and **5**, which were separated as described previously[2] to obtain **3** (5 g, 5.0%) and **5** (1.6 g, 1.6%).

Spongiadioxin C (1): colorless needles ($CHCl_3$); mp 203−205 °C; UV (EtOH) λ_{max} (log ϵ) 241 (3.97), 295 (2.88) nm; IR ($CHCl_3$) ν_{max} 3524, 1602, 1571, 1493, 1468, 1422, 1322, 1256, 1074 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.43 (1H, d, J = 2.4 Hz, H-7), 7.18 (1H, d, J = 2.4 Hz, H-9), 6.73 (1H, d, J = 2.1 Hz, H-2), 6.64 (1H, d, J = 2.1 Hz, H-4); ^{13}C NMR, see Table 1; EIMS *m/z* 440 (32), 438 (97), 436 (100), 434 (34) [M^+], 360 (7), 358 (15), 356 (8) [$M - Br]^+$; *anal.* C 32.94%, H 1.17%, Br 54.93%, calcd for $C_{12}H_5O_3Br_3$, C 32.99%, H 1.15%, Br 54.87%.

Compound 2: colorless needles ($CHCl_3$); mp 205−207 °C; UV (EtOH) λ_{max} (log ϵ) 239 (3.97), 294 (2.86) nm; IR ($CHCl_3$) ν_{max} 1616, 1573, 1498, 1467, 1447, 1419, 1325, 1301, 1257, 1106 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.50 (1H, d, J = 2.2 Hz, H-7), 7.39 (1H, d, J = 2.2 Hz, H-9), 7.01 (1H, d, J = 2.2 Hz, H-2), 6.87 (1H, d, J = 2.2 Hz, H-4), 3.83 (3H, s, OCH_3); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.27 (1H, d, J = 2.2 Hz, H-7), 7.05 (1H, d, J = 2.2 Hz, H-9), 6.79 (1H, d, J = 2.1 Hz, H-2), 6.72 (1H, d, J = 2.1 Hz, H-4), 3.87 (3H, s, OCH_3); ^{13}C NMR, see Table 1; EIMS *m/z* 454 (32), 452 (98), 450 (100), 448 (34) [M^+], 374 (19), 372 (40), 370 (20) [$M - Br]^+$.

Methylation of 1. A mixture of 8 mg of **1**, 2 mL of MeI, and 100 mg of K_2CO_3 in 3 mL of dry acetone was stirred at room temperature for 20 h. Excess K_2CO_3 was filtered off, the solvent was evaporated, and the residue was partitioned between water (5 mL) and $CHCl_3$ (3 × 15 mL). The combined organic extracts were washed with water (2 × 5 mL) and dried over Na_2SO_4, and the solvent was evaporated to obtain ether **2** (7 mg), which exibited mp, ^{1}H NMR, and EIMS similar to those of the natural compound **2**.

Synthesis of 2 from Diphenyl Ether 9. A mixture of diphenyl ether **9** (5 mg), KOH (1 mg), Cu_2CO_3 (5 mg), and dry pyridine (2 mL) was refluxed for 15 h. After evaporation of the solvent the mixture was subjected to flash chromatography over Si gel using hexane. The hexane fraction was chromatographed on Sorbfil plates in hexane−$CHCl_3$ (1:1) to yield dioxin **2** (3 mg, 71%), which exhibited mp, ^{1}H NMR, and EIMS similar to those of the natural compound **2**.

Bioassay. Compounds **1**−**9** were tested for the inhibition of cell division of the fertilized eggs of the sea urchin *Strongylocentrotus intermedius* as previously described.[9]

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