Spongiadioxins A and B, Two New Polybrominated Dibenzo-*p*-dioxins from an Australian Marine Sponge *Dysidea dendyi*

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Received July 17, 2000

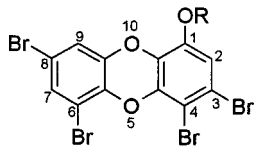
Two new cytotoxic tetrabromodibenzo-*p*-dioxins, spongiadioxins A (**1**) and B (**2**), were isolated from an Australian marine sponge *Dysidea dendyi*. The structures of these compounds were established by 1D and 2D NMR spectroscopy, X-ray analysis of the methyl ether of spongiadioxin A (**3**), and synthesis of the methyl ether of spongiadioxin B (**4**) from diphenyl ether (**9**) isolated from *Dysidea herbacea*.

A number of polybrominated diphenyl ethers have been isolated from a small group of tropical marine sponges, namely, *Dysidea* species (order Dendroceratida, family Dysideidae), which contain a large population of cyanophytes within their tissues.[1−7] Recently, it was shown that the production of polybrominated diphenyl ethers of *Dysidea herbacea* is due to the symbiotic cyanobacterium *Oscillatoria spongeliae*.[8] It was supposed also that cyanobacteria-containing *Dysidea* sponges can produce toxic congeners of polybrominated diphenyl ethers, polybrominated dibenzo-*p*-dioxins.[2] Polyhalogenated dibenzo-*p*-dioxins are byproducts in the synthesis of industrial halogenated aromatic compounds and are known environmental toxins.[9] The dibenzo-*p*-dioxin structure is unusual for natural compounds. To our knowledge, a few naturally occurring dibenzo-*p*-dioxins are isolated from natural sources: aplidioxins from ascidian *Aplidiopsis ocellata*[10] and a new type of phlorotannin bearing a dibenzo-*p*-dioxin skeleton from brown algae.[11−13] In the course of our search for aromatic metabolites from marine sponges we investigated two Australian marine sponges, *Dysidea herbacea (Keller, 1889)* and *Dysidea dendyi (Lendenfeld, 1889)*. We have isolated two new polybrominated dibenzo-*p*-dioxins, spongiadioxins A (**1**) and B (**2**), from the sponge *D. dendyi*. The sample of *D. herbacea* contained the known polybrominated diphenyl ethers **7**, **8**, **9**, and **10**, which were identified by comparison of their spectral data with published values.[3,6] In this report we describe the isolation and structural elucidation of the new compounds **1** and **2**.

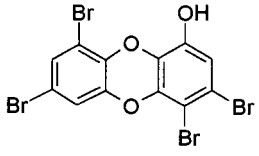
The $CHCl_3$ extract of the freeze-dried sponge *D. dendyi* was separated by flash chromatography on silica gel to give **1** and **2**. The molecular formula of major compound **1** was found to be $C_{12}H_4O_3Br_4$ by microanalysis and EIMS (a cluster of peaks at *m*/*z* 520, 518, 516, 514, 512). The IR spectrum of **1** revealed the presence of a hydroxyl group (3559 cm^{-1}) that was supported by formation of monomethyl ether **3** and monoacetate **5**. The ^1H NMR spectrum of **1** contained signals of two *meta*-situated aromatic protons, a singlet of a single aromatic proton, and one exchangeable proton at δ 10.64. ^{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 the other two oxygen atoms to be in phenoxy linkages forming a dibenzo-*p*-dioxin skeleton. Assignment of the carbon signals of **1** to the corresponding rings was carried out with the help of a COLOC 2D experiment. The sequence of substitution in each ring was determined 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 diphenyl ethers.[3]

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

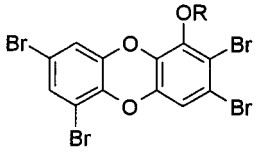
	1	**2**
C	δ_C (mult, J_{CH} in Hz)	δ_C (mult, J_{CH} in Hz)
1	145.7 (d, 3.4)	144.1 (d, 0.8)
2	116.3 (d, 168.8)	109.7 (d, 9.1)
3	117.9 (d, 4.3)	117.9 (d, 4.5)
4	101.3 (d, 9.4)	111.1 (d, 172.5)
4a	140.2 (d, 1.1)	140.9 (d, 4.8)
10a	129.7 (d, 8.5)	129.9 (d, 7.7)
5a	138.4 (dd, 8.5; 7.1)	138.2 (dd, 8.3; 7.1)
6	110.3 (dd, 4.0; 1.4)	110.3 (dd, 4.0; 1.4)
7	129.7 (dd, 175.6; 6.3)	129.8 (dd, 175.1; 6.3)
8	115.9 (t, 4.5)	115.4 (t, 4.5)
9	118.7 (dd, 171.1; 6.0)	118.8 (dd, 171.0; 6.4)
9a	142.4 (dd, 4.5; 1.4)	142.4 (dd, 4.6; 1.4)



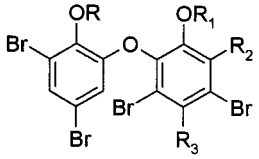
1 R = H
3 R = Me
5 R = Ac



1a



2 R = H
4 R = Me
6 R = Ac



7 R = R_1 = H, R_2 = R_3 = Br
8 R = R_1 = R_3 = H, R_2 = Br
9 R = R_3 = H, R_1 = Me, R_2 = Br
10 R = R_1 = Me, R_2 = R_3 = H

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10.1021/np0003544 CCC: $20.00
Published on Web 01/03/2001

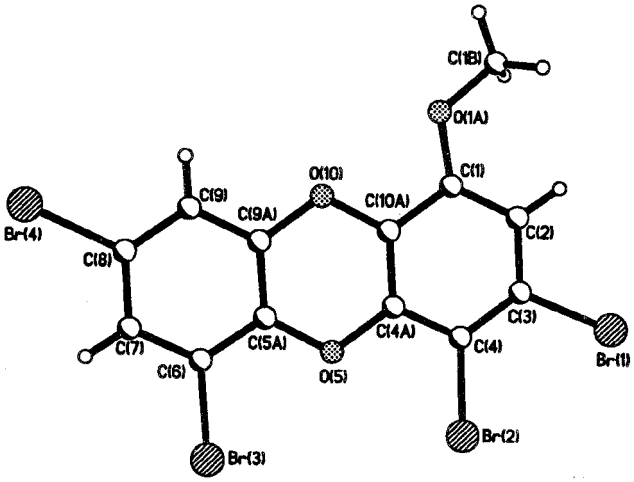

Figure 1. Computer-generated drawing of **3**.

The data obtained, however, allowed for two possible structures, **1** and **1a**. The choice of structure **1** was based on an X-ray analysis of the methyl ether of spongiadioxin A (**3**). Figure 1 presents the final X-ray model of **3**.

Signals in the ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**. The molecular formula determined by EIMS and microanalysis, $C_{12}H_4O_3Br_4$, was the same as that of **1**, indicating that the two compounds are isomers. Compound **2** formed monomethyl ether **4** and monoacetate **6**. The ^{13}C NMR spectrum of **2** (Table 1) contained 12 aromatic signals. Chemical shifts and ^{13}C−^{1}H coupling constants of six signals were essentially identical with those of C-5a to C-9a of compound **1**, indicating that one ring is identical in these compounds. Chemical shifts and values of the long-range ^{13}C−^{1}H coupling constants for the other six carbons established the remaining substitution pattern in **2**. To confirm the 6,8-position of the two bromine atoms in **2**, we carried out dehydrobromination of diphenyl ether **9**, which we have isolated from *D. herbacea*, and compared the obtained product with the methyl ether of spongiadioxin B (**4**). The compound thus prepared exhibited a melting point and ^{1}H NMR and EIMS spectra identical with those for the sample prepared by methylation of **2** with MeI. The mixed melting point of the two compounds showed no depression, and hence structure **2** was confirmed.

X-ray crystallography established the nearly planar tricyclic ring system of **3** as found in eckol.[12] The atoms C-1, C-2, C-3, C-6, C-7, C-8 lie on one side and the atoms C-4, C-4a, O-5, C-5a, C-9, C-9a, O-10, C-10a lie on other side of the mean plane through the 14 ring atoms. In the tricyclic ring system the maximum deviation from the mean plane is 0.045 Å for O-10 and − 0.049 Å for C-2. The deviations of the bromine atoms are no more than 0.06 Å. The methoxy carbon deviates from the mean plane by −0.33 Å.

Spongiadioxins A (**1**) and B (**2**) are cytotoxic against mouse Ehrlich carcinoma cells (ED_{50} 29 and 15.5 μg/mL, respectively). The LD_{50} for **1** and **2** in mouse are more than 150 mg/kg.

Experimental Section

General Experimental Procedures. ^{1}H NMR and ^{13}C NMR spectra (δ in ppm, referenced to the solvent used) were recorded on a Bruker AVANCE DPX-300 MHz NMR spectrometer. EIMS were measured on a LKB-9000S mass spectrometer at 70 eV. IR spectra were recorded on a Bruker Vector-22 FT-IR spectrometer. UV spectra were recorded on a Specord M-40 spectrophotometer. Silica gel L (40/100 μm, Chemapol, Praha, Czechoslovakia) was used for vacuum flash chromatography, Sorbfil plates coated with silica gel F_{254} (Sorbpolimer, Krasnodar, Russia) were used for TLC, and Sephadex LH-20 (Pharmacia Fine Chemicals) was used for column chromatography. All solvents were distilled prior to use. Melting points (uncorrected) were determined on a Boetius apparatus.

Animal Material. The sponge *D. dendyi* was collected by hand using scuba at Scott Reef, Northwest Australia, at a depth of 3 m in November 1990. The sponge *D. herbacea* was collected by hand using scuba at Cockburn Reef, Magra Island, northern part of the Australian Great Barrier Reef, at a depth 10−15 m in December 1990 during the 12th scientific cruise of R/V "Academik Oparin". The specimen of *D. dendyi* was grayish-green in life. The sponge has a thinly bladed shape with numerous flattened small branches. The texture is cartilaginous and friable. The surface is smooth with small conules (up to 0.3 mm). The primary and secondary fibers are cored with sand and spicule debris. Some areas of fiber are devoid of corning material, and their thickness is 30−50 μm. Skeleton meshes are about 250 × 450 μm in size. The greenish interior is assumed to be due to the presence of blue-green algal symbionts. The sponges were freeze-dried and stored in a refrigerator until used. A voucher specimen of *D. dendyi* (012-208) and a voucher specimen of *D. herbacea* (012-333) are held at the Pacific Institute of Bioorganic Chemistry. Taxonomic identification was provided by one of us (V.B.K.).

Extraction and Isolation. The freeze-dried sponge *D. dendyi* (4.8 g) was extracted at room temperature with $CHCl_3$ (2 × 100 mL). The combined $CHCl_3$ extracts (0.46 g) were subjected to flash column chromatography over silica gel using a step gradient of $CHCl_3$ in hexane. Compound **2** was eluted with 10% $CHCl_3$ in hexane, and compound **1** was eluted with 50% $CHCl_3$ in hexane. Fractions eluted with 25% $CHCl_3$ in hexane contained a mixture of **1** and **2**, which was separated by repeated chromatography. Chromatographic procedures gave **1** (241 mg, 5.02%) and **2** (78 mg, 1.62%).

The freeze-dried sponge *D. herbacea* (21 g) was extracted at room temperature with $CHCl_3$ (3 × 200 mL). The combined $CHCl_3$ extracts during slow solvent evaporation gave a pale green powder (120 mg) containing a mixture of **7** and **8**, which was separated by repeated column chromatography on Sephadex LH-20 in $CHCl_3$−MeOH (1:1). Pure **7** (70 mg) was obtained by two recrystallizations from acetonitrile and **8** (42 mg) by crystallization from $CHCl_3$. The $CHCl_3$-soluble material remaining from the filtration of the mixture of **7** and **8** was separated on Sorbfil plates in hexane−EtOAc (10:1) to obtain **9** (5 mg) and **10** (4 mg).

Spongiadioxin A (1): colorless needles ($CHCl_3$); mp 241−242 °C; UV (EtOH) λ_{max} (log ϵ) 242 (3.97), 295 (3.06) nm; IR ($CHCl_3$) ν_{max} 3559, 1574, 1478, 1466, 1430, 1342, 1260, 1072 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.43 (1H, d, J = 2.1 Hz, H-9), 7.17 (1H, d, J = 2.1 Hz, H-7), 6.93 (1H, s, H-2), 10.64 (1H, bs, O*H*); ^{13}C NMR see Table 1; EIMS *m/z* 520 (16), 518 (68), 516 (100), 514 (68), 512 (18) [M$^+$], 439 (12), 437 (38), 435 (38), 433 (13) [M − Br]$^+$, 358 (10), 356 (20), 354 (10) [M − Br_2]$^+$; *anal.* C 27.89%, H 0.80%, Br 62.05%, calcd for $C_{12}H_4O_3Br_4$, C 27.94%, H 0.78%, Br 61.97%.

Spongiadioxin B (2): colorless needles ($CHCl_3$); mp 245−247 °C; UV (EtOH) λ_{max} (log ϵ) 242 (3.97), 295 (3.06) nm; IR ($CHCl_3$) ν_{max} 3540, 1567, 1467, 1415, 1320, 1255, 1070 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.47 (1H, d, J = 2.2 Hz, H-9), 7.14 (1H, d, J = 2.2 Hz, H-7), 6.94 (1H, s, H-4), 10.80 (1H, bs, O*H*); ^{13}C NMR see Table 1; EIMS *m/z* 520 (16), 518 (65), 516 (100), 514 (68), 512 (17) [M$^+$], 439 (12), 437 (37), 435 (38), 433 (13) [M − Br]$^+$, 358 (12), 356 (25), 354 (12) [M − Br_2]$^+$; *anal.* C 27.85%, H 0.79%, Br 61.95%, calcd for $C_{12}H_4O_3Br_4$, C 27.94%, H 0.78%, Br 61.97%.

Methylation of 1. A mixture of 16 mg of **1**, 3 mL of MeI, and 200 mg of K_2CO_3 in 5 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 **3** (15 mg).

Compound 3: colorless needles ($CHCl_3$); mp 208−209 °C; IR ($CHCl_3$) ν_{max} 2933, 1573, 1466, 1449, 1437, 1420, 1330, 1253, 1080 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.32 (1H, d, J = 2.2 Hz, H-9), 7.07 (1H, d, J = 2.2 Hz, H-7), 6.92 (1H, s, H-2), 3.88 (3H, s, OCH_3); EIMS m/z 534 (16), 532 (65), 530 (100), 528 (68), 526 (17) [M^+].

Methylation of 2. Methylation of **2** (10 mg) in a manner similar to that described for **1** gave compound **4** (9 mg).

Compound 4: colorless needles ($CHCl_3$); mp 204−206 °C; IR ($CHCl_3$) ν_{max} 2933, 1567, 1467, 1438, 1405, 1308, 1250, 1080 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.32 (1H, d, J = 2.2 Hz, H-9), 7.11 (1H, s, H-2), 7.07 (1H, d, J = 2.2 Hz, H-7), 3.90 (3H, s, OCH_3); EIMS m/z 534 (16), 532 (68), 530 (100), 528 (68), 526 (18) [M^+].

Acetylation of 1. A solution of **1** (16 mg), Ac_2O (0.5 mL) and pyridine (0.5 mL) in $CHCl_3$ (5 mL) was stirred at room temperature for 1 h. After evaporation of the solvents and excess of reactant the residue was dissolved in $CHCl_3$ (5 mL) and washed with water (2 × 10 mL). The organic layer was dried over Na_2SO_4 and evaporated to give **5** (17 mg).

Compound 5: colorless needles ($CHCl_3$); mp 212−213 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.33 (1H, d, J = 2.1 Hz, H-9), 7.10 (1H, s, H-2), 6.99 (1H, d, J = 2.1 Hz, H-7), 2.34 (3H, s, COCH_3); EIMS m/z 562 (2), 560 (7), 558 (10), 556 (7), 554 (2) [M^+].

Acetatylation of 2. Acetylation of **2** (3 mg) in a manner similar to that described for **1** gave acetate **6** (3 mg).

Compound 6: colorless needles ($CHCl_3$); mp 223−225 °C; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.33 (1H, d, J = 2.1 Hz, H-9), 7.24 (1H, s, H-2), 7.00 (1H, d, J = 2.1 Hz, H-7), 2.40 (3H, s, COCH_3); EIMS m/z 562 (2), 560 (7), 558 (10), 556 (7), 554 (2) [M^+].

Synthesis of 4 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 silica gel using hexane. The hexane fraction was repeatedly chromatographed on Sorbfil plates in $CHCl_3$−hexane (1:1) to yield pure dioxin **4** (3 mg, 69.7%), which exhibited mp, ^1H NMR, and EIMS similar to those for the product of methylation of **2** with MeI.

X-ray Crystallography. The methyl ether of spongiadioxin A (**3**) was crystallized from a $CHCl_3$ solution by slow solvent evaporation. A prismatic crystal, 0.3 × 0.1 × 0.1 mm, was selected for all crystallographic measurements. Cell parameters were obtained by least-squares fit of 960 reflections (2θ range 4.8−52.2°) measured at room temperature using Mo Kα radiation. All X-ray measurements were carried out on a Bruker SMART CCD area detector diffractometer with the use of the programs SMART and SAINT-Plus.[14]

Crystal data for 3: $C_{13}H_6Br_4O_3$, MW = 529.82, monoclinic, $P2_1/c$, a = 7.558(7), b = 20.479(2), c = 10.0518(9) Å, β = 111.978(2)°, V = 1442.3(2) Å^3, Z = 4, D_c = 2.44 g/cm^3, $F(000)$ = 992, λ(Mo Kα) = 0.71073 A, μ(Mo Kα) = 11.2 mm^{-1}. The intensity data of 4346 independent reflections (R_{int} = 0.047) within θ range 2.4−31.0° were collected at 295(2) K using Mo Kα radiation and employing ω-scan techniques. The crystal-to-detector distance was 4.5 cm. Data were collected from groups of 606, 435, and 230 frames at φ settings of 0°, 90°, and 180°, respectively. Each exposure covered −0.3° in ω for 10 s. The structure was solved by direct methods with the use of the program SHELXTL/PC[15] and refined by a full-matrix least-squares procedure of SHELXTL/PC,[15] in which $w = 1/[\sigma^2(F_o^2) + (0.0469P)^2]$, where $P = (F_o^2 + 2F_c^2)/3$. The hydrogen atoms were placed at calculated positions and refined isotropically. The U_{iso} value for each hydrogen atom was set at 1.2 times the equivalent isotropic displacement value of the carbon atom to which it is attached. The coordinates of nonhydrogen atoms and their equivalent isotropic thermal parameters are given in Table 2.The refinement converged to a final $R(F^2)$ = 0.0341, $wR(F^2)$ = 0.0762, S = 0.738 for 2338 reflections with $I > 2\sigma(I)$ and 182 parameters.[16]

Table 2. Atomic Coordinates (× 10^4) and Equivalent Isotropic Thermal Parameters (Å^2 × 10^3) for **3**

	x	y	z	U (eq)
Br(1)	4935(1)	2425(1)	5316(1)	58(1)
Br(2)	2281(1)	1418(1)	2652(1)	61(1)
Br(3)	−686(1)	−643(1)	1239(1)	55(1)
Br(4)	375(1)	−2619(1)	5418(1)	49(1)
O(5)	1865(4)	114(1)	3814(2)	46(1)
O(10)	3448(4)	−328(1)	6706(3)	50(1)
O(1A)	5492(4)	439(1)	8747(2)	46(1)
C(1)	4825(5)	712(2)	7419(4)	36(1)
C(2)	5156(5)	1345(2)	7091(4)	39(1)
C(3)	4399(5)	1553(2)	5681(4)	39(1)
C(4)	3333(5)	1151(2)	4584(4)	37(1)
C(4A)	2993(5)	512(2)	4914(4)	38(1)
C(5A)	1510(5)	−504(2)	4215(4)	36(1)
C(6)	353(5)	−920(2)	3162(4)	36(1)
C(7)	−31(5)	−1549(2)	3509(4)	38(1)
C(8)	789(5)	−1748(2)	4927(4)	37(1)
C(9)	1932(5)	−1344(2)	5970(4)	39(1)
C(9A)	2286(5)	−721(2)	5619(4)	37(1)
C(10A)	3749(5)	299(1)	6318(4)	35(1)
C(1B)	6259(6)	861(2)	9944(4)	54(1)

Acknowledgment. This research was supported in part by the Award No. REC-003 of the U.S. Civilian Research & Development Foundation for the Independent States of the Former Soviet Union (CRDF) and Ministry of Education of the Russian Federation and by Grant 99-07-90133 of the Russian Foundation for Basic Research (financial support of the license on use by the Cambridge Crystallographic Data Centre).

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(16) Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

NP0003544