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Published in:
Journal of natural products

DOI:
10.1021/np970271w

Citation for published version (APA):
Four New Bioactive Polybrominated Diphenyl Ethers of the Sponge Dysidea herbacea from West Sumatra, Indonesia

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Received June 2, 1997

The marine sponge Dysidea herbacea collected from Indonesia yielded four new polybrominated diphenyl ether congeners 2−5 and the known derivatives 1, 6, and 7. The structures of the new compounds were unambiguously established on the basis of NMR spectroscopic (1H, 13C, COSY, 1H-detected direct and long-range 13C−1H correlations) and mass spectrometric (EIMS) data. All of the compounds were active against the Gram-positive bacteria Bacillus subtilis and the phytopathogenic fungus Cladosporium cucumerinum. The isolated polybrominated compounds were also active in the brine shrimp lethality test. In the latter bioassay, compounds 1 and 6 were the most active with LC50's of 0.96 [SE ± 0.19] and 0.94 [SE ± 0.70] µg/mL, respectively.

The marine sponge Dysidea herbacea Keller (family Dysideidae, Order Dendroceratida) occurs in two chemotypes: one chemotype contains both polychlorinated amino acid derivatives and sesquiterpenes, while the second chemotype contains only polybrominated diphenyl ethers. Thus, it has been previously argued that this chemical variation in D. herbacea is due to different algal or bacterial symbionts associated with the sponge. Recently, it was suggested that the production of polybrominated diphenyl ethers of D. herbacea is due to the cyanobacterium (Oscillatoria spongelae) and not by the sponge or a symbiotic heterotrophic bacteria and that these compounds may play a role in the chemical defense of the sponge against potential predators and bacterial invasion. The polybrominated diphenyl ether derivatives have also been reported to inhibit enzymes implicated in tumor development and arteriosclerotic plaque, which indicates their potential as promising therapeutic agents. In this paper, we describe the isolation and structure elucidation of new polybrominated diphenyl ether derivatives obtained from the marine sponge D. herbacea, collected from West Sumatra, Indonesia, and report on their antibacterial, antifungal, and cytotoxic properties.

The marine sponge D. herbacea was collected off the shores of the Air island of West Sumatra, Indonesia. The ETOAc soluble material of a crude extract from the sponge was subjected to Sephadex (LH20) column chromatography using methanol as eluent, and 14 fractions were obtained. The polybrominated diphenyl ether congeners were isolated from fractions 9−14, and the last fraction 14 contained the major compound 1. The known compounds 1, 6, and 7 were readily identified from their spectroscopic data and by comparison with published data.

For this series of polybrominated diphenyl ether congeners, an inspection of the multiplicity patterns and the number of protons in the aromatic region indicated their relative distribution between the two ring systems. The occurrence of the 2-bromophenoxy ether ring (ring B, Chart 1) for compounds 2−5 was confirmed by direct comparison of the 1H and 13C NMR spectrum with a standard of 2-bromophenol (Fluka). Through-bond heteronuclear (1H-detected one-bond and multiple-bond 13C multiple coherence) correlations have been used to unambiguously establish the assignments and atom

![Chart 1](image-url)
of peaks at m/z 520, 518, 516, 514, 512 in the EIMS is compatible with the molecular composition of C_{53}H_{28}O_{29}Br_{14}. The methoxy signal was observed at δ 3.86 (s, 3H) in the 1H NMR spectrum, and the 1H NMR data are comparable to those of 4.

All isolated compounds were tested for their antibacterial and fungicidal activities and their response in the brine shrimp lethality test (Table 2). All compounds were found to be active against the Gram-positive bacteria B. subtilis. No inhibition was observed for the Gram-negative bacteria E. coli. As a guide to the sensitivity of B. subtilis to the isolated polybrominated diphenyl ether derivatives, the minimum inhibitory concentrations (MICs) for each of the compounds were determined. Compound 1 was found to be the most active with a MIC of 0.20 μg/mL (0.34 nmol), followed by the isomeric mixture of 3 and 4 then by compound 6, whereas compounds 2, 7, and 5 were less active with MICs of 6.25 μg/mL (12.45 nmol), 25 μg/mL (49.80 nmol), and 104 μg/mL (201.55 nmol), respectively. The activity of compound 1 toward B. subtilis was the same as that of the standard antibiotic gentamycin.

All compounds except 5 were active against the fungus C. cucumerinum. The isomeric mixture 3 and 4 was the most active at concentrations of 50 and 25 nmol, causing inhibition zones of 16 and 8 mm in diameter, respectively, followed by compounds 1, 2, and 6, and compound 7 with the weakest activity (Table 2).

For the brine shrimp lethality test, compounds 1 and 6 were found to be the most active with LC50's of 0.96 and 0.94 μg/mL, respectively, followed by the tribromophenol derivatives 2–4, while compounds 7 and 5 showed only very weak activities (Table 2).

From the results of these bioassays, preliminary statements can be made on the structure–activity relationship between the different polybrominated diphenyl ether derivatives. In general, the derivatives with the 2′-bromophenoxy ether ring system are more active than their 2,4′-dibromophenoxy ether congeners. In compound 2, the absence of a bromine substituent at C-6, which is ortho to the hydroxyl group of the phenolic ring system, decreases the bioactivity. In the case of the brine shrimp lethality test, the biological activity is directly proportional to the number of bromine substituents. Methylation of the hydroxyl group, as in 5, results in a weakening or in the loss of activity in all the bioassays performed.

### Experimental Section

**General Experimental Procedures.** 1H NMR and 13C NMR spectra (chemical shifts in ppm) were recorded on Bruker ARX 400 NMR and AVANCE DMX 600 NMR spectrometers, respectively. Mass spectra (EIMS) were measured on a Finnigan MAT 8430 mass spectrometer. UV spectra were recorded in MeOH. Percent purity of isolated compounds was analyzed by HPLC. For HPLC analysis, samples were injected into an HPLC system (Gynkotek, Germany) coupled to a photodiode-array detector. For each run, the separation column was equilibrated with 50% A (H2O adjusted to pH 2 in phosphoric acid) in B (MeOH) for 10 min, and then the separation was achieved by a linear gradient from 50% A to 100% B in 40 min, followed by an isocratic segment of 100% B for the last 5 min. Routine detection was at 254 nm. The separation column (125 × 4 mm, i.d.) was prefillled with Eurospher C-18 (Knauer, Germany).
Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements. TLC was performed on precoated TLC plates with Silica gel 60 F_254 (Merck, Darmstadt, Germany). The compounds were detected from their UV absorbance at 254 nm.

Animal Material. The gray-green sponge D. herbacea was collected by snorkelling off the shores of Air Island of West Sumatra, Indonesia. The sponge grows in shallow water on coral rubble and dead coral branches as a partly encrusting, partly thinly bladed erect form, with irregularly flattened digitate outgrowths. The surface is smooth and slippery. In places there are fine, faint conules. A convergent system of subdermal canals is visible. Consistency is cartilaginous, but rather fragile. The skeleton is a fairly meshed system of thin spongin fibers completely filled with sand grains and speculum debris. The size of the smallest meshes are about 250 × 400 µm, while many are larger. There is no clear distinction between primary and secondary fibers and their thickness varies between 30 and 50 µm. The ectosomes are covered by a thin coat of foreign material that is mostly spicule debris. The samples were immersed in methanol immediately after collection and transported to the University of Würzburg, Germany. A voucher fragment is kept in 70% methanol under the registration number ZMA POR.10983 in the Zoological Museum, Amsterdam.

Extraction and Isolation. The sample of D. herbacea (ca. 500 g, wet weight) was extracted successively with acetone and MeOH (500 mL × 3 for each). The total extract was evaporated under reduced pressure and was partitioned between EtOAc (200 mL × 5) and H_2O (100 mL). The organic fraction was taken to dryness (ca. 10 g) and chromatographed over a Sephadex LH-20 column using methanol as eluent, and 14 fractions were obtained. The polybrominated diphenyl ether congeners were isolated from fractions 9–14. Fraction 9 afforded the pure compounds 5 (2.3 mg, 0.0005%) and 7 (13.7 mg, 0.0027%) and a mixture (3:2, 17.6 mg, 0.0035%) of 3 and 4. Fraction 10 yielded 2 (5.4 mg, 0.0011%) and was purified by column chromatography on RP-18 Lobar (CH_3CN:H_2O, 70:30). Fraction 13 contained 6 (11.2 mg, 0.0024%) and was further purified on RP-18 Lobar (MeOH:H_2O, 70:30). The last fraction 14 yielded the major compound 1 (ca. 2.5 g, 0.5%). The identity of the fractions was confirmed by HPLC and UV spectra recorded online.

Bioautographic Detection of Fungicidal Activity. Spores of C. cucumerinum were cultivated on carrot-nutrient agar and were inoculated into a liquid yeast culture medium as previously described. Si gel TLC plates were spotted with the isolated compounds at concentrations of 50 nmol and 25 nmol, and the plates were sprayed with a suspension of spores of C. cucumerinum in liquid yeast culture medium. The fungitoxic compound was observed as a clear white spot of inhibition in a dark layer of the mycelia covering the TLC plate after the inoculated plates were incubated for 2 days at 25 °C.

<table>
<thead>
<tr>
<th>compd no.</th>
<th>serial dilution assay B. subtilis</th>
<th>zone of inhibition fungicidal activity against C. cucumerinum</th>
<th>brine shrimp lethality test A. salina</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (µg/mL (nmol))</td>
<td>dose = 50 nmol (mm diam)</td>
<td>dose = 25 nmol (mm diam)</td>
</tr>
<tr>
<td>1</td>
<td>0.20 (0.34)</td>
<td>14.0</td>
<td>7.0</td>
</tr>
<tr>
<td>2</td>
<td>6.25 (12.45)</td>
<td>13.0</td>
<td>8.0</td>
</tr>
<tr>
<td>3/4</td>
<td>1.56 (3.11)</td>
<td>16.0</td>
<td>8.0</td>
</tr>
<tr>
<td>5</td>
<td>104.00 (201.55)</td>
<td>8.0</td>
<td>5.0</td>
</tr>
<tr>
<td>6</td>
<td>3.13 (4.73)</td>
<td>3.5</td>
<td>1.5</td>
</tr>
<tr>
<td>7</td>
<td>25.00 (49.80)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Bioactivities of the Compounds Isolated from Dysidea herbacea.
Brine Shrimp Lethality Test. Eggs of Artemia salina (Dohse, Aquaristik GmbH, Bonn, Germany) were hatched in a small tank filled with artificial sea water, which was prepared with a commercial salt mixture (Sera Sea-Salt, Aquaristik GmbH, Bonn, Germany) and distilled water. After 48 h, the 20 phototropic nauplii were transferred to each sample vial using a pipet, and artificial sea water was added to make 5 mL. The percent deaths at each dose and control were determined. LC50s were calculated from the dose–response curve by probit analysis.

Acknowledgment. Financial support by grants of the DFG (Pr 229/7-1) and by the “Fonds der Chemischen Industrie” to P.P. is gratefully acknowledged. Furthermore, we would like to thank Dr. Dieter Gross and Ms. Monika Kummer (IPB, Halle, Germany) for giving us the opportunity to perform bioassays with C. cucumerimn, Ms. Reinhilde Merkert (Institut für Molekulare Infektionsbiologie, Würzburg, Germany) for the MIC analysis, C. Kakoschke and B. Jaschok-Kentner for recording NMR data (GBF, Braunschweig, Germany), and Ir. Yemfita Effendi and the students of Dr. Andreas Kunzmann from the Faculty of Fisheries, Bung Hatta University of Padang, Indonesia, for their help with the collection of the sponge specimens. D.H. and R.A.E. wish to thank the DAAD (Deutscher Akademischer Austauschdienst) for scholarships.

References and Notes