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Four New Bioactive Polybrominated Diphenyl Ethers of the Sponge Dysidea herbacea from West Sumatra, Indonesia

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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;1 one chemotype contains both polychlorinated amino acid derivatives2,3 and sesquiterpenes,4,5 while the second chemotype contains only polybrominated diphenyl ethers.6,7 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.8 Recently, it was suggested that the production of polybrominated diphenyl ethers of D. herbacea is due to the cyanobacterium (Oscillatoria spongelaiae) 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.1,9,10 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.11 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.6,8,12–14

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

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connectivities for the protonated aromatic ring systems (Table 1). The magnitude of the long-range couplings, indicated by the intensity of the cross peaks in these spectra, to the carbons carrying oxygen substituents allowed unambiguous determination of the relative positions of the hydrogen atoms in each system. The relative assignments of the carbons carrying oxygen substituents was made by consideration of their substituent chemical shifts and indicated that C-2 is always to high field of C-1 and C-1′ in the compounds considered here.

Compound 2 was obtained as a viscous oil. A cluster of peaks at m/z 506, 504, 502, 500, 498 in the EIMS is compatible with the molecular composition of C_{12}H_{6}O_{2-Br}_{4} for compound 2. The 1H NMR spectrum of 2 showed a broad singlet at δ 7.34 (1H), which indicated the presence of a proton in the phenolic ring system A; subsequently, the broad form of the signal suggested a probable coupling with a hydroxyl group oriented ortho to the proton. In the HMBC spectrum, the presence of a strong correlation of H-6 to C-2 and a weak correlation of H-4 to C-2 and C-1′, respectively, while isomer 4 is 3,4,5-tribromo-2-(2′-bromophenoxy)phenol.

Compounds 3 and 4 are isomers of 2 and were obtained as an inseparable mixture in a ratio of 3:2, respectively, as observed from the 1H and 13C NMR spectrum. Overlapping signals appeared in the 1H NMR spectrum for the 2-bromophenoxy ring system B, but two distinct sets of signals were observed in the 13C NMR spectrum for both of the ring systems, and these allowed differentiation of the two isomers. Further experiments with HMBC established the assignments for both of the isomers. In the 1H NMR spectrum, the additional singlets at δ 7.55 and 7.75 for isomers 3 and 4, respectively, suggest the presence of a proton in the phenolic ring as in 2. Both of these protons are oriented para and meta to the hydroxyl groups as observed from the cross peaks in the HMBC spectrum, which consequently confirmed that H-5 of isomer 3 is meta- and para-oriented to the ether and hydroxyl substituents, respectively, while in isomer 4, the opposite is the case, H-4 is para- and meta-oriented to the ether and hydroxyl substituents, respectively. Therefore isomer 3 was determined to be 3,5,6-tribromo-2-(2′-bromophenoxy)-phenol, and isomer 4 was 3,4,6-tribromo-2-(2′-bromophenoxy)phenol. For isomers 2–4, the structure of the phenolic ring system A was also confirmed by comparison with published data on tribromophenolic compounds.12

Compound 5 is the methoxyl derivative of 4. A cluster of peaks at m/z 520, 518, 516, 514, 512 in the EIMS is compatible with the molecular composition of C_{12}H_{6}O_{2-Br}_{4}. The methoxyl 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 bromophenolic 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 x 4 mm, i.d.) was prefilled with Eurospher C-18 (Knauer, Germany).

**Table 1.** 13C NMR Data of Compounds 2–4 in CDCl3

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Notes
Solvents were distilled prior to use, and spectral grade solvents were used for spectroscopic measurements. TLC was performed on precoated TLC plates with Si gel 60 F254 (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, 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 fibers and the fibers are between 30 and 50 µm thick. 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) and H2O (100 mL). The organic fraction was taken to a TLC plate after the inoculated plates were incubated in a dark layer of the mycelial covering the plate was 125 µm (diam). The concentration of the test compounds in the initial hole of the microtiter plate was 125 µg/mL. Cultures of B. subtilis 168 were used in the assay. Growth inhibition was judged by comparison with growth in control holes prepared without test compound, which were set up at the same time as the test holes. The concentration of the tube at highest dilution, that was free from growth, was recorded as the minimum inhibitory concentration (MIC) value in µg/mL.

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.15,16 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.
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.

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References and Notes