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ARTICLE

Monitoring of Naturally Produced Brominated Phenoxyphenols and Phenoxyanisoles in Aquatic Plants from the Philippines

KOICHI HARAGUCHI,*,[†] YUICHI KOTAKI,[‡] JUAN R. RELOX JR.,[§]
MARC LAWRENCE J. ROMERO,[§] AND RYUTA TERADA[#]

[†]Daiichi College of Pharmaceutical Sciences, Fukuoka 815-8511, Japan, [‡]School of Marine Biosciences, Kitasato University, Ofunato, Iwate 022-0101, Japan, [§]Bureau of Fisheries and Aquatic Resources, 860 Arcadia Building, Quezon Avenue, Quezon City 1100, the Philippines, and [#]Faculty of Fisheries, Kagoshima University, Kagoshima 890-0056, Japan

Naturally produced brominated phenoxyphenols (OH-PBDEs) and phenoxyanisoles (MeO-PBDEs) were analyzed in aquatic plants (16 genera of green, brown, and red algae and angiosperms) collected from Luzon Island, the Philippines. Two brominated phenoxyphenols, 2′-hydroxy-2,3′,4,5′-tetrabromodiphenyl ether (2′-OH-BDE68) and 6-hydroxy-2,2′,4,4′-tetrabromodiphenyl ether (6-OH-BDE47), were detected in the phenolic fraction of extracts from most of the specimens; *Sargassum oligosystum* had the highest concentrations (101 ng/g fresh weight (fw)). The corresponding phenoxyanisole, 2′-methoxy-2,3′,4,5′-tetrabromodiphenyl ether (2′-MeO-BDE68), was most abundant in *Sargassum* aff. *bataanense* (229 ng/g fw), followed by *Padina* sp., and 6-methoxy-2,2′,4,4′-tetrabromodiphenyl ether (6-MeO-BDE47) was predominant in *Jania adhaerens* (29 ng/g fw). Hydroxy-pentaBDEs, hydroxy-methoxy-tetraBDEs, dihydroxy-tetraBDEs, dihydroxy-tetrabromobiphenyl, and hydroxy-tetrabromodibenzo-*p*-dioxins were also detected. The present study demonstrates that these aquatic plant species could be an abundant source of OH-PBDEs and MeO-PBDEs found in higher trophic organisms in the Asia−Pacific region.

KEYWORDS: Bromophenol; brominated phenoxyanisole; brominated phenoxyphenol; marine algae; *Sargassum*; *Jania*; the Philippines

INTRODUCTION

Benthic marine macroalgae in the Philippine archipelago include many endemic species and genera of red, brown, and green algae that are a major dietary source for many marine organisms (*1*). Bromophenols synthesized by the algae are important flavor components (*2*, *3*); 2,4,6-tribromophenol (2,4,6-TBP) is widely distributed in various seafood species, such as prawns, salmon, and fish dwelling in local waters (*4*). 2,4,6-TBP is the precursor of 2,4,6-tribromoanisole (2,4,6-TBA), which has been identified as a trace environmental contaminant in marine fish (*5*). It is believed that bacteria in algae could be responsible for the O-methylation of 2,4,6-TBP (*6*).

In recent years, lipophilic and bioaccumulative brominated compounds that are proposed to be of natural origin have been detected in marine biota from different sites throughout the world (*7*). One of the major congener groups is methoxylated polybrominated diphenyl ethers (MeO-PBDEs), which have been found in algae (*8*, *9*), sponges (*10*, *11*), mussels (*9*), fish (*12−14*), and marine mammals (*15−19*). Another group is dimethoxylated tetrabromobiphenyl (diMeO-BB), which has also been accumulated in fish and mammals (*14*, *17*). These compounds are likely to biomagnify within higher trophic organisms via the food chain due to their high lipophilicity (log K_{ow} > 5) (*20*). On the other hand, hydroxylated polybrominated diphenyl ethers (OH-PBDEs) such as 2′-hydroxy-2,3′,4,5′-tetrabromodiphenyl ether (2′-OH-BDE68) are produced by marine algae (*9*) and marine sponges (*21*, *22*) or their associated bacteria (symbiotic cyanobacteria) (*23*). Although OH-PBDEs have been detected in the marine food web (for example, in salmon blood) (*12*) and even in human blood (*24*), it is not clear whether they are derived from naturally produced secondary products or from metabolites of anthropogenic PBDEs.

The objective of this study was to investigate whether OH-PBDEs and their methoxylated analogues (MeO-PBDEs) are found in marine algae from shallow waters of the Philippines. In 2008, 31 specimens (16 genera) of green, brown, and red algae and angiosperms were collected from the littoral zones (four locations) and freshwaters (Taal Lake) of Luzon Island (**Figure 1**). The current survey was also undertaken to assess the source, distribution, and interspecific variation in aquatic plant brominated compounds that are found in fish and mammals from the Asia−Pacific region.

EXPERIMENTAL PROCEDURES

Sampling. In December 2008, a total of 23 marine algae (12 genera of green, red, and brown algae), 2 freshwater green algae (2 genera), and

*Author to whom correspondence should be addressed (phone 092-541-0161; fax 092-553-5698; e-mail k-haraguti@daiichi-cps.ac.jp).
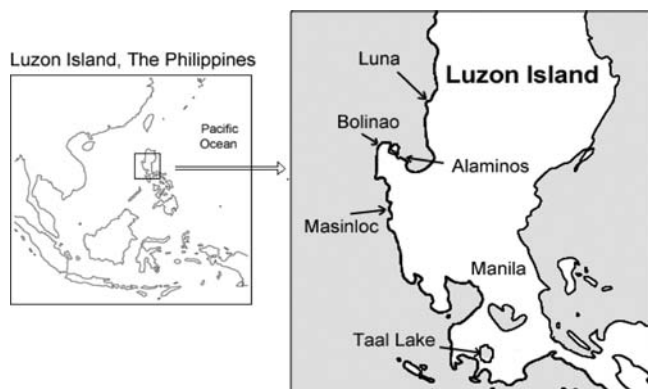
12386 *J. Agric. Food Chem.,* Vol. 58, No. 23, 2010

Haraguchi et al.

Figure 1. Sampling locations of macroalgae in the Philippines.

2 angiosperms were collected from four littoral zones (Masinloc, Bolinao, Alaminos, and Luna) and Taal Lake on Luzon Island, the Philippines (**Figure 1**). Each sample was frozen at −20 °C prior to chemical analysis.

Species Identification. Almost all of the algae were identified to genus/ species level (**Table 1**) using guidebooks and some literature (*1*, *25*−*32*). The identities of critical species monitored for OH-PBDEs and MeO-PBDEs were identified as *Sargassum oligocystum* Montagne (*25*, *26*), *Sargassum* aff. *bataanense* G.C. Trono (*27*), *Halymenia durvillei* Bory de Saint-Vincent (*28*, *29*), and *Jania adhaerens* Lamouroux (*30*). None of the algae investigated have been directly used as food source.

Chemicals and Reagents. Standards of 2,4,6-TBA, 2,4,6-TBP, 2′-methoxy-2,3′,4,5′-tetrabromdiphenyl ether (2′-MeO-BDE68), 2′-hydro-xy-2,3′,4,5′-tetrabromodiphenyl ether (2′-OH-BDE68), 6-methoxy-2,2′,4,4′-tetrabromodiphenyl ether (6-MeO-BDE47), and 6-hydroxy-2,2′,4,4′-tetra-bromodiphenyl ether (6-OH-BDE47) were purchased from AccuStandard Inc. (USA). Dr. G. Marsh (Stockholm University) kindly provided 4′-methoxy-2,3′,4,5′,6-pentabromodiphenyl ether (4′-MeO-BDE121), 2′,6-dimethoxy-2,3′,4,5′-tetrabromodiphenyl ether (2′,6-diMeO-BDE68), and 2,2′-dimethoxy-3,3′,4,4′-tetrabromobiphenyl (2,2′-diMeO-BB80). Their corresponding dihydroxylated analogues were prepared by demethy-lating the dimethoxylated analogues by boron tribromide (2 M) in dichloro-methane. We used 4′-MeO-BDE121 and 6-OH-[^{13}C]BDE47 (Cambridge Isotope Laboratories, Andover, MA) as internal standards (i.s.) for the determination of neutral and phenolic compounds, respectively. The chemical structures of the target analytes are shown in **Figure 2**.

Sample Cleanup. A sample (10 g of fresh weight (fw)) was cut into pieces, and the homogenate was extracted with MeOH (50 mL) for 1 week at room temperature. The extract was concentrated, and the residue was partitioned between 0.2 M HCl and ethyl acetate (EtOAc). The EtOAc-extractable organic matter (EOM) was determined gravimetrically. A portion (20 mg) of the EOM was spiked with two internal standards, 4′-MeO-BDE121 and 6-OH-[^{13}C]BDE47 (20 ng each). We then removed the EOM by gel permeation chromatography (Bio-Beads S-X3, Bio-Rad Laboratories), eluting with dichloromethane/*n*-hexane (1:1). Brominated products in the eluate were partitioned between 1 M KOH/ethanol (7:3, v/v) and *n*-hexane. The organic phase was concentrated and purified by silica gel column chromatography (0.5 g, Wako gel S-1, Wako Pure Industries, Osaka, Japan), eluting with 12% dichloromethane in *n*-hexane (15 mL) (neutral fraction). The aqueous phase was acidified by HCl and back-extracted with *n*-hexane/diethyl ether (8:2, v/v) (phenolic fraction). A portion (5 mL) of the phenolic fraction (15 mL) was reacted with diazomethane in diethyl ether (methylated phenolic fraction). The con-centrated fractions (500 μL) were subjected to gas chromatography−mass spectrometry.

Identification and Quantification. Analyses of natural organohalo-gens were performed using a gas chromatograph (GC, Agilent 6980N) equipped with a mass selective detector (5973i) in electron ionization and selected ion monitoring mode (EI-SIM). The GC was equipped with an HP-5MS column (30 m × 0.25 mm, 0.25 μm film thickness, J&W Scientific Inc.), and scan data were recorded in the range of m/z 200−800. Helium was used as a carrier gas at a constant flow rate of 1.0 mL/min. A 2 μL aliquot of each extract was injected in splitless mode with injector and transfer line temperatures of 250 and 280 °C, respectively. The ion source

temperature was 230 °C. The GC oven program was as follows: after injec-tion at 70 °C (1.5 min), the temperature was increased at a rate of 20 °C/ min to 230 °C (2 min) and then at a rate of 4 °C/min to 280 °C (20 min). The total run time was 40 min. The ions monitored were at m/z 342 and 344 for 2,4,6-TBA, at m/z 516 and 518 for two MeO-tetraBDEs, at m/z 530 and 532 for 2,2′-diMeO-BB80 and 6-OH-[^{13}C]BDE47 (i.s.), at m/z 546 and 548 for 2,2′-diMeO-BDE68, and at m/z 594 for 4′-MeO-BDE121 (i.s.). The identification of OH- and MeO-PBDEs in samples was made by compar-ing the relative GC retention times (RRTs) of samples and standard references versus those of 6-OH-[^{13}C]BDE47 for the phenolic fraction and 4′-MeO-BDE121 for the neutral fraction, respectively, on an HP-5MS column.

Quality Control. Five-point calibration curves (3−500 ng/mL) were linear ($r = 0.998$) for each compound. A standard solution containing all of the target analytes was analyzed every day to ensure that the calibration curves remained valid (variability, relative standard deviation = 12% ($n = 5$)) before sample analysis. The recoveries of i.s. and target analytes were assessed by spiking with 1.0 ng of each standard through the entire extraction procedure, and the results ranged from 85 to 102% for phenolic compounds and from 88 to 97% for methoxylated compounds. The limit of quantification (LOQ) using the EI-SIM mode, which was determined using a signal-to-noise ratio of 10, ranged from 5 to 15 pg on the GC column (2.5−7.5 ng/mL) for all analytes. Solvent blanks did not contain any of the analytes under investigation, indicating no carry-over effect between GC-MS runs.

RESULTS

Table 1 shows the sampling locations of each species and the concentrations (on a fresh-weight (fw) basis) of the eight target compounds. The concentrations of 2,4,6-TBP ranged from 0.3 ng/g fw in *Hydroclathrus clathratus* to 107 ng/g fw in *Jania adhaerens*, whereas the levels of 2,4,6-TBA ranged from < 0.02 ng/g fw in *Kappaphycus alvarezii* to 2.2 ng/g fw in *Caulerpa lenthillifera*. Among green algae, 2,4,6-TBP levels were lower in freshwater plants from Taal Lake than in marine algae from the littoral zones. The concentration ratios of 2,4,6-TBA/2,4,6-TBP were 0.2−1.44 for the aquatic plants and < 0.01−0.28 for the marine algae.

Figure 3 shows total ion chromatograms (TIC) of the bromi-nated compounds in the neutral and phenolic fractions of a brown alga (*Sargassum* aff. *bataanense*) and a red alga (*Jania adhaerens*) collected in Masinloc (**Figure 1**). The MeO-tetraBDEs (peaks a − d) detected in the neutral fractions of both genera were identified as 2′-MeO-BDE68, 2,2′-diMeO-BB80, 6-MeO-BDE47, and 2′,6-diMeO-BDE68, respectively (**Figure 3**). The correspond-ing OH-tetraBDEs in the methylated phenolic fraction (peaks a′ and c′) were identified as 2′-OH-BDE68 and 6-OH-BDE47, respectively. The mass spectrum of peak e′ in the phenolic fraction of *J. adhaerens* corresponded to dimethoxylated pentaBDE (M^{+}, m/z 620) (**Figure 4**), indicating the presence of OH-MeO-pentaBDE or diOH-pentaBDE (unknown structures). One of the major environmentally relevant anthropogenic com-pounds, BDE-47, was not present in any species investigated (LOQ, < 0.02 ng/g fw).

The methylated phenolic fraction of *J. adhaerens* was mon-itored at m/z 516 (MeO-tetraBDE), m/z 546 (diMeO-tetraBDE), and m/z 530 (diMeO-BB80 and MeO-tetraBDD) (**Figure 5**). In the SIM profile at m/z 546, several peaks were identified as diMeO-tetraBDEs, indicating the presence of diOH- or OH-MeO-tetra-BDEs in the algae. In the profile monitored at m/z 530, the peak ($t_R = 17.6$ min) was identified as 2,2′-diMeO-BB80, whereas the peaks between 21 and 25 min were tentatively identified as methoxy-tetrabromo-*p*-dioxin (MeO-tetraBDD) because the mass spectrum exhibited M^{+} (m/z 526) and a characteristic fragment [M − COCH$_3$]$^{+}$ (m/z 473), indicating the presence of OH-tetraBDD (Supporting Information, Figure S1). The identity

Article

J. Agric. Food Chem., Vol. 58, No. 23, 2010   **12387**

Table 1. Sample Characterization and Levels of Brominated Compounds in Freshwater and Marine Plants Collected from Philippine Waters in 2008

genus (species)	source	EOM[a] (%)	2,4,6-TBA	neutral fraction				2,4,6-TBP	phenolic fraction			
				2'-MeO-BDE68	6-MeO-BDE47	2,2'-diMeO-BB80	2',6-diMeO-BDE68		2'-OH-BDE68	6-OH-BDE47	2,2'-diOH-BB80	2',6-diOH-BDE68
angiosperms												
Ceratophyllum sp.	Taal Lake, Tanauan Batangas	0.28	0.6	ND[b]	ND	ND	ND	0.5	ND	ND	0.1	ND
Hydrilla sp.	Taal Lake, Tanauan Batangas	0.30	0.4	ND	ND	ND	ND	1.6	0.7	0.6	0.2	0.1
green algae												
Chara sp.	Taal Lake, Tanauan Batangas	0.21	0.3	ND	0.1	ND	ND	1.5	ND	ND	0.1	ND
Cladophora sp.	Taal Lake, Tanauan Batangas	0.35	1.3	ND	ND	ND	ND	0.9	ND	ND	0.1	ND
Caulerpa lentillifera	Lucero, Bolinao, Pangasinan	0.37	2.2	1.2	1.0	0.5	0.2	30	0.7	1.2	0.6	0.4
Caulerpa taxifolia	Lucero, Bolinao, Pangasinan	0.40	0.5	2.9	1.4	1.8	0.4	20	0.8	1.5	0.2	0.3
Chaetomorpha crassa	Lucero, Bolinao, Pangasinan	0.32	0.1	0.4	0.2	0.5	0.1	7.7	0.2	0.3	0.3	0.1
Chlorodesmis sp.	Lucero, Bolinao, Pangasinan	0.73	0.1	5.9	1.7	6.4	0.8	11	1.5	0.3	0.1	0.1
brown algae												
Padina minor	Lucero, Bolinao, Pangasinan	0.45	0.2	0.7	0.2	0.9	0.1	1.1	0.3	0.2	0.2	0.1
Padina sp.	Masinloc, Zambales	0.31	0.7	44	1.4	2.4	0.5	2.5	0.9	0.5	ND	0.1
Hydroclathrus clathratus	Masinloc, Zambales	0.46	ND	2.0	0.2	0.8	0.1	0.3	0.3	0.4	0.1	0.2
Sargassum oligosystum[c]	Zacarias Island, Alaminos	0.30	ND	0.7	0.2	1.9	0.1	3.3	10.4	91	0.1	0.1
Sargassum aff. bataanense[d]	Masinloc, Zambales	0.28	0.4	229	3.6	2.6	30	13	3.3	10	0.2	0.9
Sargassum sp.	Masinloc, Zambales	0.38	0.1	6.1	4.2	1.4	0.4	1.7	0.2	0.3	0.1	0.3
Sargassum sp.	Masinloc, Zambales	0.15	0.3	11	0.6	1.3	1.0	3.8	0.3	0.2	0.1	0.1
Sargassum sp.	Masinloc, Zambales	0.36	ND	4.7	1.5	4.8	0.4	0.5	8.8	5.6	0.5	1.8
Sargassum sp.	Luna, La Union	0.34	0.6	0.9	0.2	0.4	0.7	6.9	0.7	4.7	5.1	4.2
Sargassum sp.	Luna, La Union	0.27	0.5	1.8	0.1	0.5	0.9	7.7	4.2	0.3	0.3	2.5
Sargassum sp.	Lucero, Bolinao, Pangasinan	0.45	ND	0.3	0.1	0.4	0.1	0.4	0.1	0.3	0.2	ND
red algae												
Gracilaria edulis	Luna, La Union	0.22	0.1	0.3	0.1	0.1	0.6	2.3	0.1	0.1	ND	1.4
Hydropuntia edulis	Lucero, Bolinao, Pangasinan	0.05	1.3	5.4	4.5	2.6	1.2	32	0.1	0.1	ND	0.1
Acanthophora specifera	Lucero, Bolinao, Pangasinan	0.22	0.4	0.5	0.2	0.7	0.2	1.9	0.3	0.3	2.2	0.2
Halymenia durvillei[e]	Lucero, Bolinao, Pangasinan	0.46	0.1	0.3	0.1	0.2	0.1	1.1	0.2	0.1	0.2	0.1
Halymenia sp.	Lucero, Bolinao, Pangasinan	0.40	0.1	0.1	0.05	ND	0.1	6.3	0.1	0.1	0.1	0.2
Ceratodictyon spongiosum	Alaminos, Pangasinan	0.27	0.7	0.9	1.2	3.7	0.3	9.3	1.9	2.3	0.2	0.1
Kappaphycus alvarezii	Alaminos, Pangasinan	0.09	ND	0.3	0.05	0.3	0.1	18	0.1	0.1	ND	ND
Kappaphycus alvarezii	Alaminos, Pangasinan	0.07	ND	0.1	0.05	0.4	ND	58	ND	ND	ND	0.1
Kappaphycus alvarezii	Masinloc, Zambales	0.10	0.1	1.3	0.1	1.5	0.2	45	0.1	0.1	0.1	0.1
Kappaphycus alvarezii	Masinloc, Zambales	0.39	ND	1.3	0.05	0.5	0.1	13	ND	0.1	ND	ND
Jania adhaerens[f]	Masinloc, Zambales	0.20	0.8	78	29	44	13	107	25	6.2	2.8	4.4
Jania sp.	Masinloc, Zambales	0.13	0.1	3.6	1.7	6.3	0.5	105	1.1	1.7	0.3	0.5

concentration (ng/g fw)

[a] EOM, extractable organic matter. [b] ND, not detected (<0.02 ng/g fw). [c] References 25 and 26. [d] Reference 27. [e] Reference 28 and 29. [f] Reference 30.

12388 *J. Agric. Food Chem.,* Vol. 58, No. 23, 2010

Haraguchi et al.

of these compounds remains to be confirmed because the appropriate standards were not available for this survey.
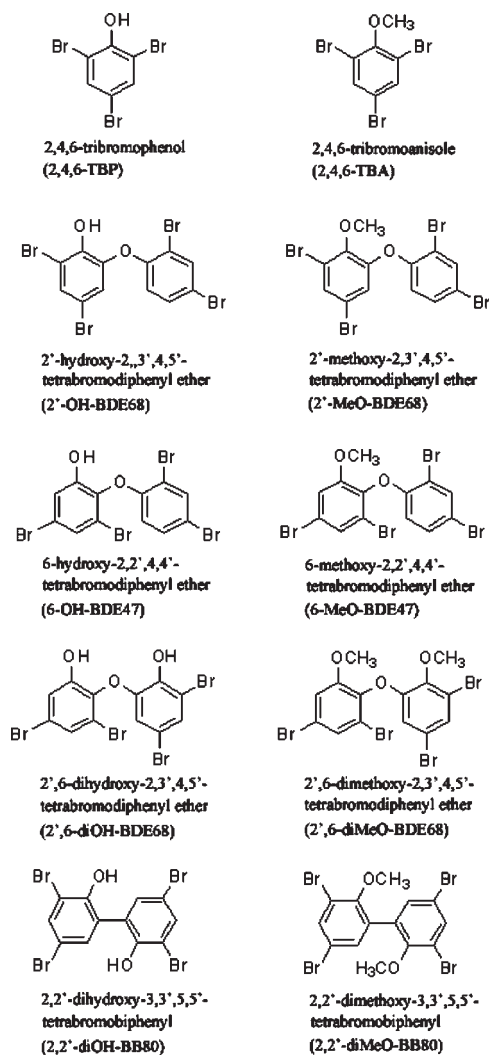

Figure 2. Chemical structures of target compounds monitored in this study.

In the neutral fraction, 2′-MeO-BDE68 and 6-MeO-BDE47 were detected in all of the samples except for the freshwater plants. The highest levels of 2′-MeO-BDE68 were detected in *Sargassum* aff. *bataanense* (229 ng/g fw), followed by *J. adhaerens* (78 ng/g fw) and *Padina* sp. (44 ng/g fw). In contrast, 6-MeO-BDE47 was predominant in *J. adhaerens* (29 ng/g fw), but its concentration was < 5 ng/g fw in the other species. The level of 2,2′-diMeO-BB80 was highest in *J. adhaerens* (44 ng/g fw), followed by *Chlorodesmis* sp. (6.4 ng/g fw), whereas 2′,6-diMeO-BDE68 was most abundant in *Sargassum* aff. *bataanense* (30 ng/g fw), followed by *J. adhaerens*.

In the phenolic fraction, 2′-OH-BDE68 and 6-OH-BDE47 were detected in all of the samples except for the freshwater algae and one red alga (*Kappaphycus alvarezii*). The highest concentrations of 2′-OH-BDE68 were observed in *J. adhaerens* (25 ng/g fw), followed by *Sargassum oligosystum* (10 ng/g fw). Concentrations of 6-OH-BDE47 were highest in *S. oligosystum* (91 ng/g fw), followed by *Sargassum* aff. *bataanense* (10 ng/g fw). The concentrations of the corresponding dihydroxylated analogues (2,2′-diOH-BB80 and 2′,6-diOH-BDE68) were < 5.1 ng/g fw in all samples. The concentrations of four analytes in *Sargassum* and *Jania* sp. are compared in Supporting Information, Figure S2.

DISCUSSION

To our knowledge, this is the first study to monitor OH- and MeO-PBDEs in aquatic plants from the Asia−Pacific region, although 2′-MeO-BDE68 has been isolated in green algae (*Cladophora fascicularis*) from Okinawa, Japan, by Kuniyoshi et al. (*8*). We found that the occurrence of OH- and MeO-PBDEs from the Philippines was species-dependent; the highest levels were observed in *Sargassum*, *Jania*, and *Padina* species from Masinloc, Zambales. The other algal species also contained smaller amounts of 2′-OH-/2′-MeO-BDE68 or 6-OH-/6-MeO-BDE47, indicating the wide distribution of these brominated products in shallow waters of the Philippines. The levels of OH- and MeO-tetraBDEs varied considerably within the genus *Sargassum*, implying that even in the same genus, particular species can produce preferably these brominated compounds. The variation may be attributed to biotic factors such as stress, grazing, water temperature, stage of the algae annual cycle. At present, it is not possible to tell whether the OH- and MeO-PBDEs
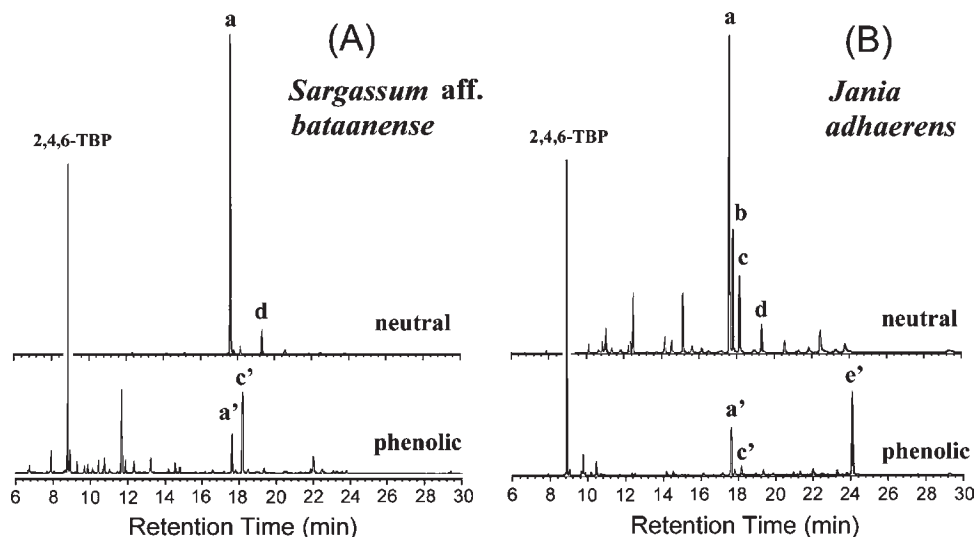

Figure 3. Total ion chromatograms of brominated compounds in neutral and methylated phenolic fractions from *Sargassum* aff. *bataanense* (**A**) and *Jania adhaerens* (**B**). In the neutral fraction: peak a, 2′-MeO-BDE68; peak b, 2,2′-diMeO-BB80; peak c, 6-MeO-BDE47; peak d, 2′,6-diMeO-BDE68. In the phenolic fraction: peak a′, 2′-OH-BDE68; peak c′, 6-OH-BDE68; peak e′, diOH-pentaBDE or OH-MeO-pentaBDE (as methylated derivatives).
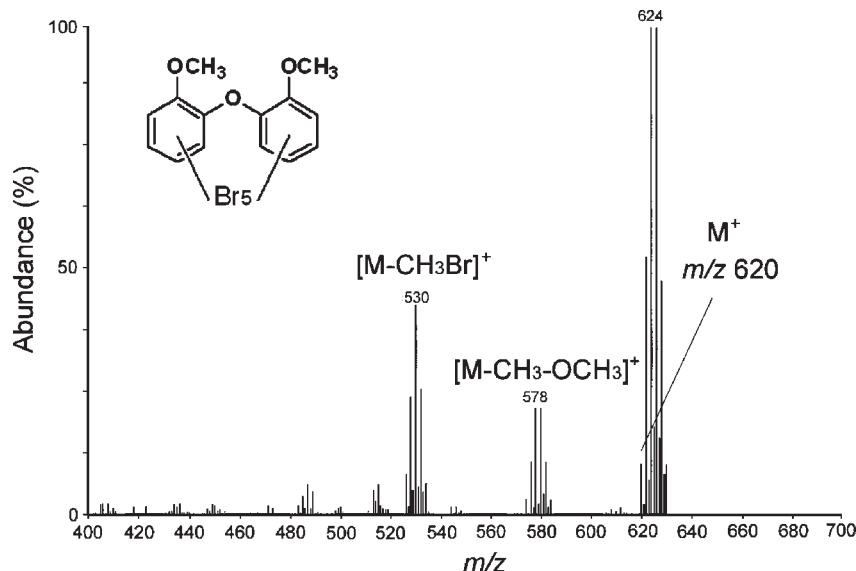
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J. Agric. Food Chem., Vol. 58, No. 23, 2010   **12389**

Figure 4. Mass spectrum of peak e′ in the methylated phenolic fraction of *Jania adhaerens* from **Figure 3**.
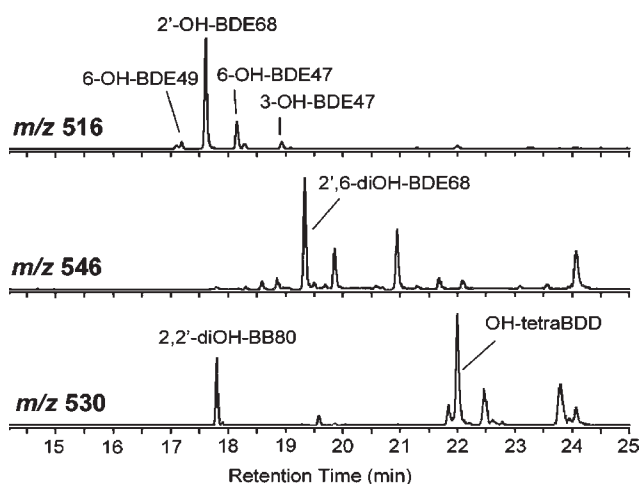

Figure 5. Selected ion monitoring of OH-tetraBDE (*m/z* 516), diOH-tetraBDE (*m/z* 546), and diOH-tetraBB/OH-tetraBDD (*m/z* 530) in the methylated phenolic fraction from *Jania adhaerens*. Some of the peaks were identified as 6-OH-BDE49 (t_R = 17.3 min), 3-OH-BDE47 (t_R = 18.8 min), 2′,6-diOH-BDE68 (t_R = 19.4 min), and OH-tetraBDD (t_R = 22.0 min).

are produced by the macroalgae themselves or by their associated symbionts (e.g., cyanobacteria) (*23*). It has been previously indicated that the producer of OH-PBDEs in the marine sponge *Dysidea herbacea* may be the symbiotic filamentous cyanobacterium *Oscillatoria spongeliae* (*23*).

The profiles of OH- and MeO-PBDEs in marine algae were similar among the five locations on Luzon Island, although the ratios and concentrations varied among species. However, the profiles of brominated compounds isolated from the Philippine algae seem to differ from those isolated in red algae from the Baltic Sea (*9, 33*). The Philippine algae produced higher concentrations of MeO-tetraBDEs (e.g., 229 ng/g fw in *Sargassum* aff. *bataanense*) than of the corresponding OH-tetraBDEs (e.g., 30 ng/g fw in *J. adhaerens*), whereas red algae (*Ceramium tenuicorne*) from the Baltic Sea contained primarily OH-pentaBDEs (10 ng/g fw for OH-PBDEs), with smaller amounts of MeO-tetraBDEs (0.4 ng/g fw for MeO-PBDEs) (*33*). Additionally, the profiles of the Philippine algae included 2,2′-diMeO-BB80 and 2′,6-diOH/diMeO-BDE68, whereas no information about these compounds

was reported for algae from the Baltic Sea. Finally, the Philippine algae contained OH-tetraBDDs and the corresponding MeO-tetraBDDs, whereas red algae from the Baltic Sea produced triBDDs and tetraBDDs (*33, 34*). Several OH-tetraBDDs, diOH-tetraBDEs and their methoxylated analogues have been isolated from Australian marine sponges (*35, 36*) and Palauan marine sponges (data not shown), suggesting that the profiles of hydroxylated, dihydroxylated, and methoxylated analogues are common in the Pacific.

In this study, the highest production of 2′-MeO-BDE68 reached 229 ng/g fw for *Sargassum* aff. *bataanense*, which corresponded to 62 µg/g when expressed per gram of extracted organic matter (EOM). For OH-PBDEs, 6-OH-BDE47 reached 91 ng/g fw in *Sargassum oligosystum*, corresponding to 30 µg/g EOM. 2′-MeO-BDE68 and 6-MeO-BDE47 have been isolated in the marine sponge *Dysidea* sp. from the Asia−Pacific region (*36, 37*). These products in sponge tissue (*Dysidea* sp.) may represent as much as 12% of the dry weight (*23*). Marine algae such as *Sargassum* and *Jania* sp. are more common in the Philippines' shallow waters than marine sponges (*Dysidea* sp.). As many invertebrates (e.g., thorny oyster) feed on these algae, naturally produced MeO-PBDEs could transfer and bioaccumulate in higher trophic levels via the food chain (*38, 39*). On the other hand, OH-PBDEs are expected to be more water-soluble than MeO-PBDEs and more mobile in the environment. In fact, they have been isolated in the blood of fish and animals (*12*).

The present survey further resulted in the isolation of 2,2′-diMeO-BB80 and 2′,6-diMeO-BDE68 from algae. These compounds have also been found to accumulate in bluefin tuna (*14*), tiger sharks (*38*), and killer whales (*19*) from Japanese coastal waters. For the corresponding hydroxylated analogues, 2,2′-diOH-BB80 and 2′,6-diOH-BDE68 were detected at levels of up to 5.1 ng/g fw in *Sargassum* or *Jania* sp. As we measured both dihydroxylated products as dimethoxy derivatives by GC-MS (**Figure 4**), the precursor may be either OH-MeO- or diOH-analogues. In fact, one product isolated in *J. adhaerens* was directly (without derivatization) identified as 2′-OH-6-MeO-BDE68 (data not shown). 2,2′-diOH-BB80 has been found to be produced by the marine bacterium, *Pseudoalteromonas phenolica* sp. (*40*), whereas both 2′,6-diOH-BDE68 and 2′-OH-6-MeO-BDE68 have been also isolated in marine sponges from Indonesia (*36, 37*).

Previous studies have discussed whether these OH-PBDEs in marine food webs originate from marine resources or from brominated flame retardants (e.g., PBDEs) through hepatic metabolism. This study strongly suggests that the OH-PBDEs identified are of natural origin because their possible precursors, such as BDE-47, were undetectable in any of the algae investigated. All of the OH- and MeO-PBDEs identified have the hydroxy/methoxy group in the ortho position(s) to the diphenyl ether oxygen and a 2-bromine or 2,4-dibromine substitution in the nonhydroxylated or nonmethoxylated phenyl ring, which is common for natural OH- and MeO-PBDEs (*37, 41*).

In addition to OH-PBDEs, we measured 2,4,6-TBP content in algae. Similar to OH-PBDE, 2,4,6-TBP varied considerably in concentration across species (from 0.5 to 107 ng/g fw). The concentration ranges of 2,4,6-TBP observed in this study appear to be low compared to those in marine algae from Australia (up to 1900 ng/g fw) (*2*). Seasonal changes in the concentrations of these compounds may occur (*3*). Marine algae are a major source of 2,4,6-TBP in herbivorous fish and a contributing source in fish that are diverse omnivores (*4*).

The levels of 2,4,6-TBA observed in this study ranged from < 0.02 to 2.2 ng/g fw, which is lower than 2,4,6-TBP levels in most cases. The 2,4,6-TBA is likely a natural product derived from bacterial O-methylation of bromophenols (*6*) rather than a contaminant derived from man-made flame retardants (*5*). Because the ratio of 2,4,6-TBA/2,4,6-TBP varies from < 0.01 to 1.44, depending on the species, the different concentration ratios of hydroxylated and methoxylated analogues in algae may be attributed to the O-methylation ability of the algae-associated bacteria.

Many species of algae have been shown to contain bromoperoxidases (BPO), capable of brominating organic substrates in the presence of bromide and hydrogen peroxide (*42, 43*). BPO isolated from red algae has been shown to catalyze the bromination of phenol and 2-hydroxybenzyl alcohol to 2,4,6-TBP (*44*). The condensation of 2,4,6-TBP to OH-PBDEs is presumably also catalyzed by BPO (*34*), as horseradish chloroperoxidase is known to catalyze the dimerization of chlorophenols to chlorinated dibenzo-*p*-dioxins (*45*). However, the levels of OH-PBDEs observed in this study were not correlated with those of 2,4,6-TBP (ANOVA, $p > 0.05$), indicating that the formation of OH-PBDE does not always depend on the bromophenol content as a substrate for BPO.

OH-PBDEs are bioactive against Gram-positive bacteria (*22*). Some authors have linked the survival of certain algae to the presence of volatile organohalogens in interalgal competition as a defense against bacterial and fungal infections (*2, 46, 47*). Thus, it is possible that OH-PBDE analogues may contribute to the chemical defenses of the algae. On the other hand, OH-PBDEs such as 6-OH-BDE47 have been shown to have higher transthyretin binding properties than PBDEs and to disrupt thyroid hormone homeostasis (*41*). Furthermore, OH-PBDEs possess neurotoxic properties of environmental significance (*48*). The OH-tetraBDDs isolated from *J. adhaerens* in this study may be the same as OH-tetraBDD congeners from Australian *Dysidea dendyi* that have been reported to be cytotoxic against mouse Ehrlich carcinoma cells (*35*). Because human populations have probably been exposed to these materials through seafood consumption over a long period of time, the exposure may have historical as well as current health implications.

In conclusion, the present study demonstrates the widespread occurrence of OH- and MeO-PBDE analogues in marine and freshwater algae and in angiosperms from the Philippines and suggests a possible source of such compounds accumulated in fish and higher trophic organisms in the Asia−Pacific region.

Our results show that it is important to characterize the origin and occurrence of OH-PBDEs as well as the details of their toxic properties.

Supporting Information Available: Mass spectrum of MeO-tetraBDD in the methylated phenolic fraction from *Jania adhaerens* (Figure S1) and comparison of the concentrations of four brominated compounds abundant in two algal species (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Trono, G. C., Jr. *Field Guide and Atlas of the Seaweed Resources of the Philippines*; Bookmark: Makati City, The Philippines, 1997; ISBN971-569-252-4.

(2) Whitfield, F. B.; Helidoniotis, F.; Shaw, K. J.; Svoronos, D. Distribution of bromophenols in species of marine algae from eastern Australia. *J. Agric. Food Chem.* **1999**, *47*, 2367−2373.

(3) Chung, H. Y.; Ma, W. C.; Ang, P. O.; Kim, J. S.; Chen, F. Seasonal variations of bromophenols in brown algae (*Padina arborescens*, *Sargassum siliquastrum*, and *Lobophora veriegata*) collected in Hong Kong. *J. Agric. Food Chem.* **2003**, *51*, 2619−2624.

(4) Whitfield, F. B.; Helidoniotis, F.; Shaw, K. J.; Svoronos, D. Distribution of bromophenols in species of ocean fish from eastern Australia. *J. Agric. Food Chem.* **1998**, *46*, 3750−3757.

(5) Watanabe, I.; Kashimoto, T.; Tatsukawa, R. Polybrominated anisoles in marine fish, shellfish, and sediments in Japan. *Arch. Environ. Contam. Toxicol.* **1983**, *12*, 615−620.

(6) Allard, A. S.; Remberger, M.; Neilson, A. H. Bacterial O-methylation of halogen-substituted phenols. *Appl. Environ. Microbiol.* **1987**, *53*, 839−845.

(7) Vetter, W. Marine halogenated natural products of environmental relevance. *Rev. Environ. Contam. Toxicol.* **2006**, *188*, 1−57.

(8) Kuniyoshi, M.; Yamada, K.; Higa, T. A biologically active diphenyl ether from the green alga *Cladophora fascicularis. Experientia* **1985**, *41*, 523−524.

(9) Malmvärn, A.; Marsh, G.; Kautsky, L.; Athanasiadou, M.; Bergman, Å.; Asplund, L. Hydroxylated and methoxylated brominated diphenyl ethers in the red algae *Ceramium tenuicorne* and blue mussels from the Baltic Sea. *Environ. Sci. Technol.* **2005**, *39*, 2990−2997.

(10) Anjaneyulu, V.; Nageswara, R. K.; Radhika, P.; Muralikrishna, M.; Connolly, J. D. A new tetrabromodiphenyl ether from the sponge *Dysidea herbacea* of the Indian Ocean. *Indian J. Chem.* **1996**, *35*, 89−90.

(11) Cameron, G. M.; Stapleton, B. L.; Simonsen, S. M.; Brecknell, D. J.; Garson, M. J. New sesquiterpene and brominated metabolites from the tropical marine sponge *Dysidea* sp. *Tetrahedron* **2000**, *56*, 5247−5252.

(12) Marsh, G.; Athanasiadou, M.; Bergman, Å.; Asplund, L. Identification of hydroxylated and methoxylated polybrominated diphenyl ethers in Baltic Sea salmon (*Salmo salar*) blood. *Environ. Sci. Technol.* **2004**, *38*, 10−18.

(13) Sinkkonen, S.; Ranthalainen, A. L.; Paasivirta, J.; Lahtiperä, M. Polybrominated methoxy diphenyl ethers (MeO-PBDEs) in fish and guillemot of Baltic, Atlantic and Arctic environments. *Chemosphere* **2004**, *56*, 767−775.

(14) Hisamichi, Y.; Endo, T.; Nishimura, E.; Haraguchi, K. Natural and anthropogenic POPs in bluefin tuna from the Japanese market. *Organohalogen Compd.* **2007**, *69*, 1709−1712.

Article

J. Agric. Food Chem., Vol. 58, No. 23, 2010   **12391**

(15) Vetter, W.; Stoll, E.; Garson, M. J.; Fahey, S. J.; Gaus, C.; Muller, J. F. Sponge halogenated natural products found at parts-per-million levels in marine mammals. *Environ. Toxicol. Chem.* **2002**, *21*, 2014–2019.

(16) Pettersson, A.; van Bavel, B.; Engwall, M.; Jimenez, B. Polybrominated diphenylethers and methoxylated tetrabromodiphenyl ethers in cetaceans from the Mediterranean Sea. *Arch. Environ. Contam. Toxicol.* **2004**, *47*, 542–550.

(17) Marsh, G.; Athanasiadou, M.; Athanassiadis, I.; Bergman, Å.; Endo, T.; Haraguchi, K. Identification, quantification, and synthesis of a novel dimethoxylated polybrominated biphenyl in marine mammals caught off the coast of Japan. *Environ. Sci. Technol.* **2005**, *39*, 8684–8690.

(18) Stapleton, H. M.; Dodder, N. G.; Kucklick, J. R.; Reddy, C. M.; Schantz, M. M.; Becker, P. R.; Gulland, F.; Porter, B. J.; Wise, S. A. Determination of HBCD, PBDEs and MeO-BDEs in California sea lions (*Zalophus californianus*) stranded between 1993 and 2003. *Mar. Pollut. Bull.* **2006**, *52*, 522–531.

(19) Haraguchi, K.; Hisamichi, Y.; Endo, T. Accumulation and mother-to-calf transfer of anthropogenic and natural organohalogens in killer whales (*Orcinus orca*) stranded on the Pacific coast of Japan. *Sci. Total Environ.* **2009**, *407*, 2853–2859.

(20) Vetter, W.; Haase-Aschoff, P.; Rosenfelder, N.; Komarova, T.; Mueller, J. F. Determination of halogenated natural products in passive samplers deployed along the Great Barrier Reef, Queensland/Australia. *Environ. Sci. Technol.* **2009**, *43*, 6131–6137.

(21) Fu, X.; Schmitz, F. J.; Govindan, M.; Abbas, S. A.; Hanson, K. M.; Horton, P. A.; Crews, P.; Laney, M.; Schantzman, 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.

(22) 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.

(23) Unson, M. D.; Holland, N. D.; Faulkner, D. J. A brominated secondary metabolite synthesized by the cyanobacterial symbiont of a marine sponge and accumulation of the crystalline metabolite in the sponge tissue. *Mar. Biol.* **1994**, *119*, 1–11.

(24) Qiu, X.; Bigsby, R. M.; Hites, R. A. Hydroxylated metabolites of polybrominated diphenyl ethers in human blood samples from the United States. *Environ. Health Perspect.* **2009**, *117*, 93–98.

(25) Trono, G. C., Jr. The genus *Sargassum* in the Philippines. In *Taxonomy of Economic Seaweeds with Reference to Some Pacific and Western Atlantic Species*; Abbott, I. A., Ed.; California Sea Grant College, University of California: La Jolla, CA, 1992; Vol. III, pp 43–94.

(26) Noro, T.; Ajisaka, T.; Yoshida, T. Species of *Sargassum* subgenus *Sargassum* (Fucales) with compressed primary branches. In *Taxonomy of Economic Seaweeds*; Abbott, I. A., Ed.; California Sea Grant College, University of California: La Jolla, CA, 1994; Vol. IV, pp 23–31.

(27) Trono, G. C., Jr. New species of *Sargassum* from the Philippines. In *Taxonomy of Economic Seaweeds with Reference to Some Pacific Species*; Abbott, I. A., Ed.; California Sea Grant College, University of California: La Jolla, CA, 1994; Vol. IV, pp 3–7.

(28) Kawaguchi, S.; Kato, A.; Masuda, M.; Kogame, K.; Phang, S. M. Taxonomic notes on marine algae from Malaysia. VII. Five species of Rhodophyceae, with the description of *Lomentaria gracillima* sp. nov. *Bot. Mar.* **2002**, *45*, 536–547.

(29) Kawaguchi, S.; Shimada, S.; Abe, T.; Terada, R. Morphological and molecular phylgenetic studies of a red alga, *Halymenia durvillei* (Halymniaceae, Halymeniales), from Indo-Pacific. *Coastal Mar. Sci.* **2006**, *30*, 201–208.

(30) Baba, M. An identification guide of coralline red algae in Japan. *Rep. Mar. Res. Inst.* **2000**, *1*, 1–68.

(31) Silva, P. C.; Meez, E. G.; Moe, R. L. Catalogue of the benthic marine algae of the Philippines. *Smithsonian Contribution to the Marine Sciences 27*; Smithsonian Institution Press: Washington, DC, 1987.

(32) Trono, G. C., Jr. *Field Guide and Atlas of the Seaweed Resources of the Philippines*; BAR, Marine Environment Resources Foundation: Quezon City, The Philippines, 2004; Vol. 2, ISBN 971-704-011-7.

(33) Malmvärn, A.; Zebuhr, Y.; Kautsky, L.; Bergman, Å.; Asplund, L. Hydroxylated and methoxylated polybrominated diphenyl ethers and polybrominated dibenzo-*p*-dioxins in red alga and cyanobacteria living in the Baltic Sea. *Chemosphere* **2008**, *72*, 910–916.

(34) Haglund, P.; Malmvärn, A.; Bergek, S.; Bignert, A.; Kautsky, L.; Nakano, T.; Wiberg, K.; Asplund, L. Brominated dibenzo-*p*-dioxins: a new class of marine toxins? *Environ. Sci. Technol.* **2007**, *41*, 3069–3074.

(35) Utkina, N. K.; Denisenko, V. A.; Scholokova, O. V.; Virovaya, M. V.; Gerasimenko, A. V.; Povov, D. Y.; Krasokhin, V. B.; Popov, A. M. Spongiadioxins A and B, two new polybrominated dibenzo-*p*-dioxins from an Australian marine sponge *Dysidea dendyi*. *J. Nat. Prod.* **2001**, *64*, 151–153.

(36) Utkina, N. K.; Denisenko, V. A.; Virovaya, M. V.; Scholokova, O. V.; Prokofeva, N. G. Two new minor polybrominated dibenzo-*p*-dioxins from the marine sponge *Dysidea dendyi*. *J. Nat. Prod.* **2002**, *65*, 1213–1215.

(37) Hanif, N.; Tanaka, J.; Setiawan, A.; Trianto, A.; de Voogd, N. J.; Murni, A.; Tanaka, C.; Higa, T. Polybrominated diphenyl ethers from the Indonesian sponge *Lamellodysidea herbacea*. *J. Nat. Prod.* **2007**, *70*, 432–435.

(38) Haraguchi, K.; Hisamichi, Y.; Kotaki, Y.; Kato, Y.; Endo, T. Halogenated bipyrroles and methoxylated tetrabromodiphenyl ethers in tiger shark (*Galeocerdo cuvier*) from the southern coast of Japan. *Environ. Sci. Technol.* **2009**, *43*, 2288–2294.

(39) Verreault, J.; Gabrielsen, G. W.; Chu, S.; Muir, D. C.; Andersen, M.; Hamaed, A.; Letcher, R. J. Flame retardants and methoxylated and hydroxylated polybrominated diphenyl ethers in two Norwegian Arctic top predators: glaucous gulls and polar bears. *Environ. Sci. Technol.* **2005**, *39*, 6021–6028.

(40) Isnansetyo, A.; Kamei, Y. MC21-A, a bactericidal antibiotic produced by a new marine bacterium, *Pseudoalteromonas phenolica* sp. nov. O-BC30^T, against methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* **2003**, *47*, 480–488.

(41) Hamers, T.; Kamstra, J. H.; Sonneveld, E.; Murk, A. J.; Visser, T. J.; van Veizen, J. M.; Brouwer, A.; Bergman, Å. Biotransformation of brominated flame retardants into potentially endocrine-disrupting metabolites, with special attention to 2,2′,4,4′-tetrabromodiphenyl ether (BDE-47). *Mol. Nutr. Food Res.* **2008**, *52*, 284–298.

(42) Flodin, C.; Whitfield, F. B. Biosynthesis of bromophenols in marine algae. *Water Sci. Technol.* **1999**, *40*, 53–58.

(43) Moore, C. A.; Okuda, R. K. Bromoperoxidase activity in 94 species of marine algae. *J. Nat. Toxins* **1996**, *5*, 295–305.

(44) Yamada, H.; Itoh, N.; Murakami, S.; Izumi, Y. New bromoperoxidase from coralline algae that brominates phenol compounds. *Agric. Biol. Chem.* **1985**, *49*, 2961–2967.

(45) Hoekstra, E. J.; de Weerd, H.; Leer, E. W. B.; Brinkman, U. A. T. Natural formation of chlorinated phenols, dibenzo-*p*-dioxins, and dibenzofurans in soil of a douglas fir forest. *Environ. Sci. Technol.* **1999**, *33*, 2543–2549.

(46) Mtolera, M. S. P.; Collén, J.; Pedersén, M.; Ekdahl, A.; Abrahamsson, K.; Semesi, A. K. Stress-induced production of volatile halogenated organic compounds in *Eucheuma denticulatum* (Rhodophyta) caused by elevated pH and high light intensities. *Eur. J. Phycol.* **1996**, *31*, 89–95.

(47) Kubanek, J.; Jensen, P. R.; Keifer, P. A.; Sullards, M. C.; Collins, D. O.; Fenical, W. Seaweed resistance to microbial attack: a targeted chemical defense against marine fungi. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 6916–6921.

(48) Suyama, T. L.; Cao, Z.; Murray, T. E.; Gerwick, W. H. Ichthyotoxic brominated diphenyl ethers from a mixed assemblage of a red alga and cyanobacterium: structure clarification and biological properties. *Toxicon* **2010**, *55*, 204–210.