

Identification of a novel dimethoxylated polybrominated biphenyl bioaccumulating in marine mammals

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Introduction

The screening of persistent halogenated organic pollutants has in recent years ended up with findings of numerous compounds being natural products. These compounds have preferably been reported from the marine environment and comprise, several hydroxylated and methoxylated polybrominated diphenyl ethers (OH-PBDEs and MeO-PBDEs) including monochlorinated ditto¹, a dimethoxylated polybrominated diphenyl ether i.e. 2',6-dimethoxy-2,3',4,5'-tetrabromodiphenyl ether², four halogenated dimethylated bipyrrroles (HDBPs) with a 2,2'-bipyrrrole backbone³⁻⁵, a heptachlorinated 1,2'-bipyrrrole i.e. heptachloro-1'-methyl-1,2'-bipyrrrole (Q1)⁶ and a halogenated monoterpene holding two bromine and three chlorine atoms⁷. We have taken interest in an organobrominated compound with an unknown structure but with a molecule ion of $m/z = 526$ and a four bromine isotope pattern according to mass spectrometry (MS), detected in marine mammal samples of which the majority were collected from Japanese coastal waters. This compound has recently been reported from several marine mammals collected on the Southern hemisphere, but neither the chemical structure nor the type of substance group were established^{8,9}. We here report the structure of this organobrominated compound, a dimethoxylated polybrominated biphenyl, which likely is of natural origin. The identification was done by the comparison of relative retention time (RRT) on two GC columns with different polarities and by comparing mass spectra from three different ionization techniques i.e. PICI, ECNI and EI, of the unknown compound from the samples with an authentic reference compound. Levels of the novel dimethoxylated polybrominated biphenyl are reported as well as the levels (for comparison) of some other known polybrominated compounds, both brominated flame retardans (BFRs) and natural products i.e. BDE-47, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, 1,2,5,6,9,10-hexabromocyclododecane (HBCDD), 6-MeO-BDE-47, 2'-MeO-BDE-68 and 6,2'-diMeO-BDE-68.

Materials and Methods

Chemicals: The polybrominated diphenyl ethers BDE-47, BDE-99, BDE-100, BDE-138, BDE-153 and BDE-154^{10,11} and the methoxylated polybrominated diphenyl ethers 6-MeO-BDE-47 and 2'-MeO-BDE-68¹² were prepared as previously described. 2,2'-diMeO-BB-80 and 2',6-diMeO-BDE-68 were synthesized in house and their identity were in agreement with LRMS, ¹H NMR and ¹³C NMR (unpublished). All solvents used for analysis were of pesticide grade quality. Acids and other chemicals used in the analysis were of pro analysis quality. Silica gel (<0.063 mesh) was activated by heating over night, at 280°C, prior usage directly or impregnated with concentrated sulfuric acid.

Instruments: Gas chromatography-mass spectrometry (GC-MS) analysis of the samples and reference standards were performed on a Finnigan TSQ 700 (Thermoquest, Bremen, Germany) provided with a Varian 3400 gas chromatograph. Two GC columns were used *i*) a non-polar Equity column (30 m × 0.25 mm i.d. and 0.1 µm film thickness from Supelco, Bellefonte, USA) and *ii*) a polar column SPTM-2331 (30 m × 0,25 mm i.d. and 0,2 µm film thickness) also from Supelco. On-column injections were performed using a septum-equipped programmable injector fitted with a high performance insert. The injector temperature was 60°C and increased with 180°C /min up to 320°C for each injection. The GC temperature programs were: 80°C (1 min) - 10°C/min - 310°C (12 min) with helium as carrier gas (head pressure of 3 psi) for the nonpolar Equity column and 80°C (1 min), 20°C min-1 to 200°C (1 min), 3°C min-1 to 270°C (12 min) for the polar column SPTM-2331. The injector and transfer line temperatures were 260°C and 290°C, respectively, but the transfer line temperature was decreased to 260°C when the polar column was used. Methane (AGA, Stockholm, Sweden) of > 99,995 % purity and with < 5 ppm O₂ as the electron thermalization buffer gas at a pressure of 6.5 torr was used for electron capture negative ionization (ECNI) and positive ion chemical ionization (PICI). The electron energy was 70 eV and the temperature of the ion source was 150°C. Masses between 33-800 *m/z* were scanned on ECNI full scan runs and 200-800 for EI. The quantification of all bromine containing compounds was performed on ECNI measuring bromine ions *m/z* 79, 81.

Marine mammals' samples and clean-up: All 5 samples were collected in 1999 from retails for sale in Japan and DNA analyzed for Species ID¹³. The sources of 5 the samples are as follows; 1) fresh blubber from Striped dolphin (*Stenella coeruleoalba*) in Japanese coastal water (collected in Wakayama), 2) sliced bacon product from Bottlenose dolphin (*Tursiops truncatus*) in Japanese coastal water (collected in Wakayama), 3) cooked liver from Striped dolphin (*Stenella coeruleoalba*) in Japanese coastal water (collected in Wakayama), 4) fresh blubber from Minke whale (*Balaenoptera acutorostrata*) in North West Pacific Ocean (collected in Tokyo) and 5) shredded bacon product from Baird's beaked whale (*Berardius bairdii*) in Japanese coastal water (collected in Osaka). Ten to 20 g of the samples¹³ were homogenized and extracted with acetone:*n*-hexane (1:1). Extracts were dried over anhydrous sodium sulfate. The lipids were fortified with internal standard BDE-138 (5 ng per sample), dissolved in *n*-hexane (100 mg/ml) and partitioned with conc. sulfuric acid (1ml/100 mg of lipids). Samples of <0.500 mg where re-extracted twice with the same volume of *n*-hexane and samples with >0.500 mg lipids were re-extracted three times with equal volumes as used the first time. Subfractionated samples were pooled and further cleaned up on a silica column packed with conc. sulfuric acid impregnated silica (1g, 1:2 w/w) eluted with a mixture of dichloromethane and *n*-hexane (1:1, 8 ml). The bulk of PCBs was isolated in a separate fraction from a silica column packed with activated silica (0.9 g) and eluted with *n*-hexane (5 ml)

and dichloromethane (8 ml). This fraction was analyzed after solvent replacement with *n*-hexane. Quantification of the presenting compounds was performed relative to the corresponding individual authentic external standards.

Results and Discussion

The identity of the novel bioaccumulating compound, 2,2'-dimethoxy-3,3',5,5'-tetrabromobiphenyl (2,2'-diMeO-BB-80) in the marine mammal samples, was supported by similar RRT versus BDE-138 on two GC columns with different polarities compared to the authentic reference. The RRT were determined from full scan ECNI chromatograms by recording the ions m/z 79, 81. The difference in RRT between the reference standard and the corresponding compound in marine mammal samples were less than ± 0.003 on both GC columns used. A mass chromatogram from a striped dolphin (fresh blubber) on the non-polar GC column is shown in Figure 1, visualizing the elution order of 2,2'-diMeO-BB-80 in comparison to other brominated compounds detected. The identity of 2,2'-diMeO-BB-80 was supported by MS in ECNI, PICI and EI mode. An EI mass spectrum and the structure of 2,2'-diMeO-BB-80 are shown in Figure 2. The levels, ng/g lipids, of 2,2'-diMeO-BB-80 and other brominated compounds investigated are given in Table 1.

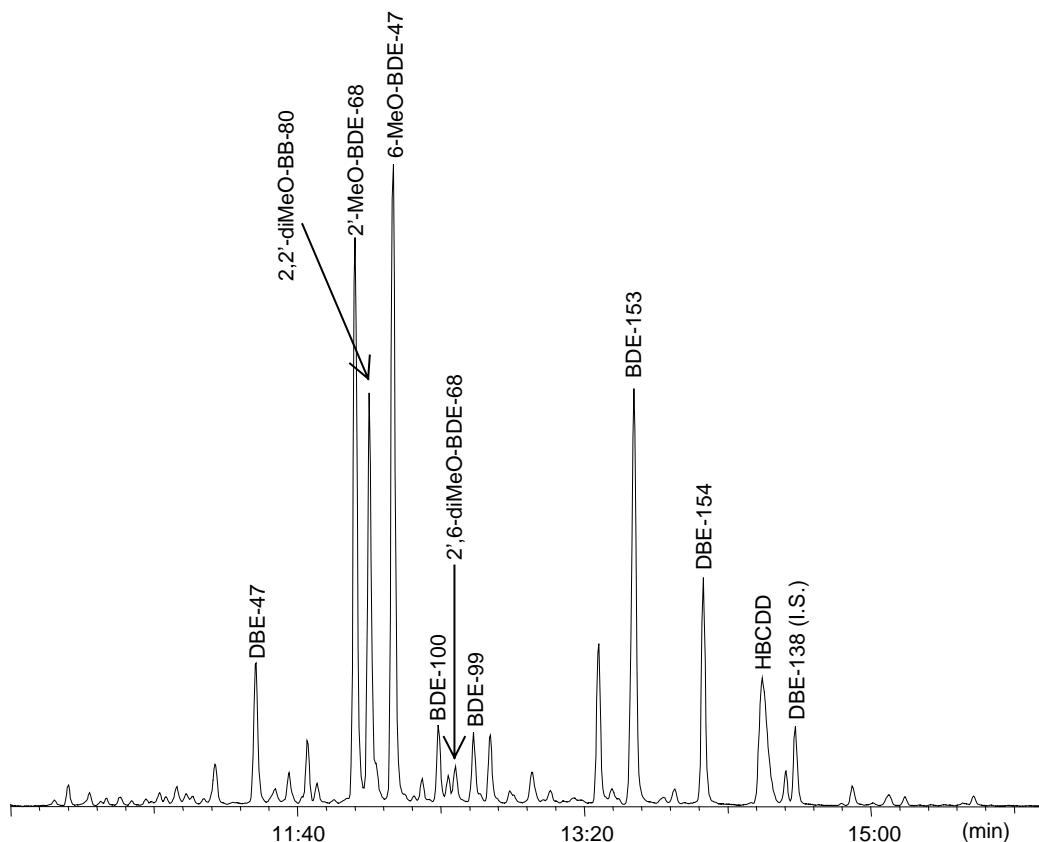


Figure 1. GC-MS (ECNI) chromatogram from a striped dolphin (fresh blubber) on the non-polar GC column recording bromide ions (m/z : 79, 81). BDE-138 has been used as internal standard (I.S.).

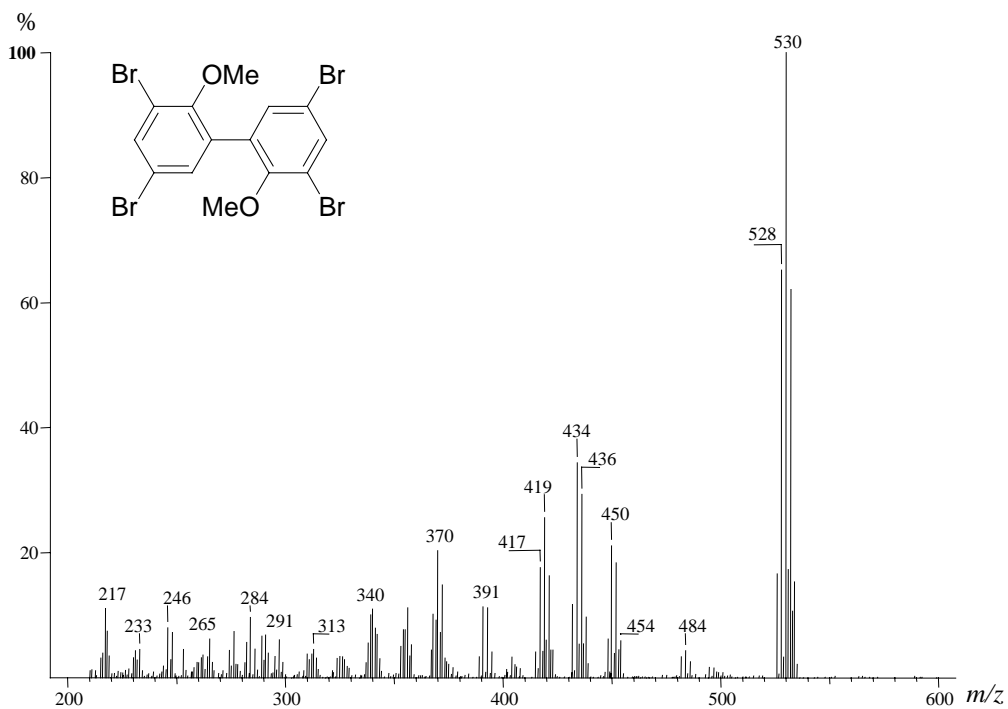


Figure 2. The structure and the fragmentation pattern on GC/MS in EI mode of the identified 2,2'-dimethoxy-3,3',5,5'-tetrabromobiphenyl (2,2'-diMeO-BB-80).

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Table 1. Concentrations (ng/g of lipid weight) of the novel 2,2'-diMeO-BB-80, some abundant PBDE congeners, a MeO-PBDE and hexabromocyclododecane (HBCDD) in marine mammals from the Pacific Ocean.

	Striped dolphin	Bottlenose dolphin	Striped dolphin	Minke whale	Baird's beaked whale
	fresh blubber	sliced bacon	cooked liver	fresh blubber	shredded bacon
BDE-47	4.4	272	73	26	3.0
BDE-99	3.0	87	29	2.7	0.8
BDE-100	3.4	47	22	5.7	1.9
BDE-153	25	180	76	7.3	1.0
BDE-154	15	50	33	5.6	0.8
6-MeO-BDE-47	25	708	252	32	54
2'-MeO-BDE-68	27	2180	294	16	4.1
2',6-diMeO-BDE-68	3.0	49	21	0.3	0.2
2,2'-diMeO-BB-80	12	800	410	12	49
HBCDD	90	256	134	57	25

The ECNI mass spectrum of 2,2'-diMeO-BB-80 has previously been published and the authors had the suggestions that this tetrabrominated compound had a backbone of either *i*) a methoxylated diphenyl ether, *ii*) a methoxylated and methylated diphenyl ether or *iii*) a dimethoxylated biphenyl⁸. The latter suggestion was thus shown to be correct as shown in the present work.

The levels of 2,2'-diMeO-BB-80 ranged from 12 to 800 ng/g of lipid weight in the five samples investigated (Table 1). Sliced bacon from the bottlenose dolphin showed generally the highest concentrations of all presenting compounds.

The origin of 2,2'-diMeO-BB-80 is not obvious since this compound has never been reported to be manufactured or used, nor reported as a natural product. However, our hypothesis is that this compound is a natural product, primary produced as the dihydroxylated derivative i.e. 3,3',5,5'-tetrabromo-2,2'-biphenyldiol (2,2'-diOH-BB-80) and consequently methylated by any kind of marine micro fauna. Just recently a novel marine bacterium (*Pseudoalteromonas phenolica* sp. nov.) was isolated from sea water¹⁴ and this marine bacterium was found to produce 2,2'-diOH-BB80¹⁵. Even though anthropogenic sources of 2,2'-diOH-BB-80 indeed exist, we believe that this contribution is negligible for the presence of 2,2'-diMeO-BB-80 in marine mammals. 2,2'-diOH-BB-80 has been reported to be a metabolite of bromofenofos, a mono dihydrogen phosphate derivative of 2,2'-diOH-BB-80, which is a drug given to sheep and cattle in order to combat distomatosis¹⁶. It can also be mentioned that the synthesis of bromofenofos goes via 2,2'-diOH-BB-80¹⁷. Methylated derivatives of polybrominated phenols existing as natural products is not unique. For example are the hydroxy derivatives of 6-MeO-BDE-47 and 2'-MeO-BDE-68 known natural products that have been isolated in e.g. marine sponges^{18,19}. However, in this case have also the methoxylated derivatives, 6-MeO-BDE-47²⁰ and 2'-MeO-BDE-68^{21,22} been isolated as natural

products. We do not believe that the identified 2,2'-diMeO-BB-80 is a transformation product of any congener of polybrominated biphenyls (PBBs). PBBs have been widely used as flame retardants and have become widespread environment pollutant, present also in marine mammals²³. However, there exist no relevant PBB congener precursor in the technical PBB products²⁴⁻²⁶, which likely can be a source for 2,2'-diMeO-BB-80. It could also here be mentioned that no peaks in the mass chromatograms had masses that matched with PBBs.

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