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Identification and quantification of new polybrominated dimethoxybiphenyls (PBDMBs) in marine mammals from Australia

Walter Vetter [a,*], Claudia Turek [a], Göran Marsh [b], Caroline Gaus [c]

[a] Institute of Food Chemistry, University of Hohenheim, Garbenstr. 28, D-70599 Stuttgart, Germany
[b] Department of Environmental Chemistry, Stockholm University, Svante Arrhenius väg 12, SE-10691 Stockholm, Sweden
[c] The University of Queensland, (EnTox), 39 Kessels Road, Coopers Plains 4108, Australia

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ABSTRACT

Marine mammals from Queensland, Australia, are bioaccumulating elevated concentrations of a range of polybrominated natural products. In this study, we detected three new polybrominated dimethoxybiphenyls (PBDMBs) in the blubber of selected marine mammal samples which were identified as 2,6′-dimethoxy-3,3′,5-tribromobiphenyl (2,6′-diMeO-BB 36), 2,2′-dimethoxy-3,3′-dibromobiphenyl (2,2′-diMeO-BB 36), and 6,6′-dimethoxy-3,3′-dibromobiphenyl (6,6′-diMeO-BB 11). These three PBDMBs are structurally related to the known natural product 2,2′-dimethoxy-3,3′,5,5′-tetrabromobiphenyl (2,2′-diMeO-BB 80). In the first part of this study, 2,2′-diMeO-BB 80 was photochemically debrominated under UV irradiation. This resulted in seven of eight possible mono- to triBDMBs as debromination products. In the second part of this study, the structure of all PBDMBs debromination products was investigated. This was supported by synthesis of two diBDMB and one triBDMB via bromination and subsequent methylation of 2,2′-biphenyldiol. Structures of the remaining PBDMBs were tentatively assigned by considering the retention times, mass spectra and amounts formed during UV irradiation of 2,2′-diMeO-BB 80 .

In the third part of this study, blubber of marine mammals from Australia was analysed for PBDMBs using gas chromatography in combination with electron ionization mass spectrometry (GC/EI-MS) in the selected ion monitoring mode. In these samples, 2,2′-diMeO-BB 80 was found at concentrations of 200–1800 ng g^{-1} lipid weight (lw). The latter represents the highest concentration reported for this compound in environmental samples. 6,6′-diMeO BB 11, 2,2′-diMeO BB 36, and 2,6′-diMeO BB 36 were present at ~7 ng g^{-1} lipids, or 0.43–1.5% of diMeO-BB 80. No further PBDMBs were detected in the samples. The di- and triBDMBs identified in marine mammal blubber have not been reported as natural products. They may represent either new natural products or transformation products of 2,2′-diMeO-BB 80.

1. Introduction

Halogenated natural products (HNPs) have been identified with a variety of over 4500 structures, most of which are produced by marine organisms (Gribble, 1999, 2000). During the last years, a number of these HNPs have also been detected in marine mammals. Predatory marine mammals have bioaccumulated the HNPs in a similar way as it is known for anthropogenic persistent organic pollutants (POPs) (Vetter, 2006). Not only the processes appeared to be the same, but some HNPs are in fact structural kins to certain POPs, including polybrominated diphenyl ethers (PBDEs) (Asplund et al., 1999; Petterson et al., 2004; Sinkkonen et al., 2004), polybrominated dibenzo-*p*-dioxins (PBDDs) (Malmvärn et al., 2005), and one compound even shared the backbone with polybrominated biphenyls (PBBs) (Marsh et al., 2005). Marsh et al. (2005) demonstrated that an abundant organobromine compound in Pacific samples was 2,2′-dimethoxy-3,3′,5,5′-tetrabromobiphenyl (2,2′-diMeO-BB 80, BC-1). 2,2′-DiMeO-BB 80 was initially described as one of three most abundant brominated compounds (BC's) in marine mammals from Queensland (Australia) (Vetter et al., 2001, 2002). In addition to 2,2′-diMeO-BB 80, the Australian cetaceans contained the tetrabromophenoxyanisoles 2′-MeO-BDE 68 (BC-2) and 6-MeO-BDE 47 (BC-3) and in some samples a dimethoxytetrabromodiphenyl ether at elevated concentrations, the structures (Fig. 1) of which were verified in subsequent experiments (Marsh et al., 2005; Melcher et al., 2005; Vetter et al., 2002). In addition to the most abundant brominated compounds, six earlier-eluting organobromine compounds were detected at lower levels in marine mammals (Vetter et al., 2001). Identification of the congeners with low abundances was more challenging; however, a refined method (Vetter, 2001) allowed detection of several tribromophenoxyanisoles structurally related to 2′-MeO-BDE 68 and 6-MeO-BDE 47 in samples from Australia (Melcher et al., 2005). It was assumed that tribromo-2,2′-dimethoxybiphenyls related to 2,2′-diMeO-BB 80 may also exist. The goal of this study was to verify this hypothesis

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* Corresponding author. Tel.: +49 711 45932 4016.
E-mail address: w-vetter@uni-hohenheim.de (W. Vetter) .

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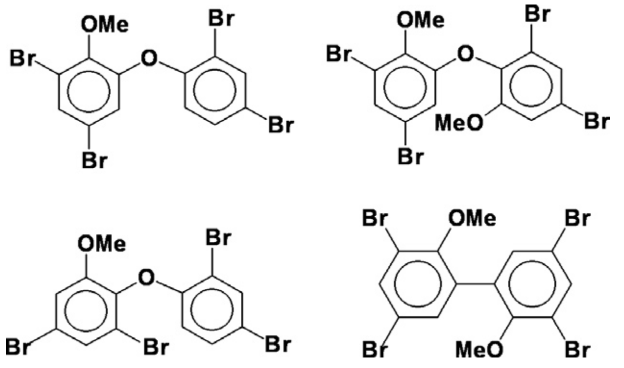

Fig. 1. Structures of prominent halogenated natural products in marine mammals. Clockwise from top left: 4,6-dibromo-2-(2′,4′-dibromo)phenoxyanisole (2-MeO-BDE 68, BC-2), 2′,6-dimethoxy-2,3′,4,5′-tetrabromodiphenyl ether (BC-11), 2,2′-dimethoxy-3,3′,5,5′-tetrabromobiphenyl (2,2′-diMeO-BB 80, BC-1), and 3,5-dibromo-2′-(2′,4′-dibromo)phenoxyanisole (6-MeO-BDE 47, BC-3).

by the photolytical production of mono- through tribromo as well as synthesis of dibromo and tribromo congeners related to 2,2′-diMeO-BB 80, followed by the screening of these products in environmental samples.

2. Materials and methods

2.1. Standards and chemicals

2,2′-DiMeO-BB 80 (BC-1) and 6-MeO-BDE 47 (BC-3) were prepared by Marsh et al. (2003, 2005) whereas 2′-MeO-BDE 68 (BC-2) was prepared by Vetter and Jun (2003). 2,3-Dibromopropyl-2,4,6-tribromophenyl ether (DPTE), which was used as internal standard, was synthesized as shown by von der Recke and Vetter (2007a). For the UV irradiation, *n*-hexane (UniSolv) was obtained from Merck (Darmstadt, Germany) and 2-propanol (99.95% purity) was sourced from Roth, Germany. Chemicals used for sample clean-up were described elsewhere (Melcher et al., 2005). For the synthesis of PBDMBs, MATREX silica (6 nm, 35–70 μm) was obtained from Millipore (Bedford, USA) and 2,2’-biphenyldiol was purchased from Acros Organics (Geel, Belgium). Solvents and other chemicals used for this study were of “for organic trace analysis” quality. All organic extracts were dried with anhydrous sodium sulfate before they were concentrated in a rotary evaporator under reduced pressure at temperatures not exceeding 35 °C. Solutions containing iodomethane were concentrated in a hood at 30 °C using a stream of nitrogen.

2.2. Photochemical treatment of 2,2′-diMeO-BB 80 (irradiation experiment)

2,2′-DiMeO-BB 80 (0.24 mg) was dissolved in *n*-hexane or 2-propanol (10 ml) and placed in cylindrical quartz vials (30 mm diameter, 60 mm height) coated with Teflon locks (Kapp et al., 2006). The system was cooled with a flow of cold water and the solutions were stirred. Irradiation was carried out with a 150 W medium pressure mercury vapor lamp (TQ150, Heraeus Noblelight, Hanau, Germany). After 5, 15, 30, 45, 60, and 90 min of irradiation, 0.5 ml was taken, respectively, from the solution and analyzed by gas chromatography with either electron capture detection (GC/ECD) or mass spectrometry (GC/MS). The sample solution (500 μl) obtained after five min irradiation in 2-propanol was condensed to 80 μl. An aliquot of 70 μl was further condensed to 20 μl for detailed analysis by GC/MS.

2.3. Synthesis of 3,5,5′-tribromo-, 3,5′-dibromo-, and 5,5′-dibromo-2,2′-dimethoxybiphenyl

Either 2.0 mmol (0.78 g) or 3.0 mmol (1.17 g) benzyltrimethylammonium tribromide (BTMA Br$_3$) was added at room temperature to a solution of 2,2’-biphenyldiol (0.19 g, 1.0 mmol) in dichloromethane (50 ml) and methanol (20 ml). The mixtures were stirred for 24 h and subsequently concentrated. Water (100 ml) was added to the crude solid which was extracted with diethyl ether (4×100 ml) and the combined diethyl ether extracts were evaporated to dryness. The crude solids were dissolved in 8 ml dry dimethylformamide (DMF) to which potassium carbonate (0.69 g, 5.0 mmol) was added and the mixtures were stirred for 1 h at 50 °C. A solution of iodomethane (0.50 ml, 8.0 mmol) in dry DMF (2 ml) was added dropwise at room temperature and the mixture was stirred at 50 °C for 12 h. The reaction mixtures were diluted with diethyl ether (30 ml) and washed with water (2×50 ml), aqueous NaOH (1 M, 30 ml), water (3×30 ml) and the organic layer was concentrated. The crude products were chromatographed on a silica gel column eluted with 2-methylpentane/ethyl acetate (14:1, v/v) for the dimolar batch, and with 2-methylpentane/ethyl acetate (20:1, v/v) for the trimolar batch. From the batch with 2 equiv. of BTMA Br$_3$, we obtained 40 mg (13%) of 2,6′-diMeO-BB 11 as an oil and 206 mg (66%) of 6,6′-diMeO-BB 11 (m.p. 129–130 °C; lit. 130–131 °C (Gilman et al., 1940), whereas the batch with 3 equiv. of BTMA Br$_3$ resulted in 144 mg (37%) of 2,6′-diMeO-BB 36 and 111 mg (23%) of 2,2’-diMeO-BB 80 (m.p. 84–85 °C; lit. 86–87 °C (Gilman et al., 1940). Melting points were determined on a Büchi 353 apparatus and were not corrected.

2.4. NMR data of the novel PBDMBs

NMR measurements were recorded with a Varian Inova 300 MHz instrument.

(a) 2,6′-diMeO-BB 11: ^{13}C (75 MHz, CDCl$_3$, ppm): 156.0, 154.9, 133.3, 133.0, 132.6, 131.8, 130.9, 128.8, 124.8, 117.5, 112.7, 112.5, 60.8, 55.9. ^{1}H (300 MHz, CDCl$_3$, ppm): 7.56 (dd, 7.8 Hz, 1.5 Hz), 7.45 (dd, 8.8 Hz, 2.5 Hz), 7.39 (d, 2.5 Hz), 7.18 (dd, 7.5 Hz, 1.5 Hz), 7.02 (t, 7.8/7.5 Hz), 6.86 (d, 8.8 Hz), 3.76 (s), 3.52 (s).

(b) 6,6′-diMeO BB 11: ^{13}C (75 MHz, CDCl$_3$, ppm): 156.1, 133.8, 131.6, 128.4, 112,7, 112.5, 55.9. ^{1}H (300 MHz, CDCl$_3$, ppm): 7.43 (dd, 8.8 Hz, 2.5 Hz), 7.3 (d, 2.5 Hz), and 6.8 (d, 8.8 Hz).

(c) 2,6′-diMeO-BB 36: ^{13}C (75 MHz, CDCl$_3$, ppm): 155.8, 154.3, 135.1, 133.9, 133.6, 133.5, 132.3, 127.4, 118.3, 116.5, 112.7, 112.5, 60.9, 55.9. ^{1}H (300 MHz, CDCl$_3$, ppm): 7.69 (d, 2.3 Hz), 7.47 (dd, 8.8 Hz, 2.5 Hz), 7.36 (d, 2.5 Hz), 7.32 (d, 2.3 Hz), 6.86 (d, 8.8 Hz), 3.77 (s), 3.50 (s).

2.5. Origin and processing of marine biota samples

Blubber was available from the following samples collected in Australia: a female calf of common dolphin (*Delphinus delphis*; P13b, lipid content in blubber 99.6%) from SE Queensland, an adult male pygmy sperm whale (*Kogia breviceps*; P14b, lipid content 93.4%) from Moreton Bay, Brisbane; an adult female melon-headed whale (*Peponocephala electra*; P15a, lipid content 100%) from SE Queensland; and a female adult bottlenose dolphin (*Tursiops truncates*; P17b, lipid content 99.3%) from Moreton Bay, Brisbane. The sample clean-up was described elsewhere and consisted of accelerated solvent extraction, gel-permeation chromatography, and adsorption chromatography on silica (Melcher et al., 2005). One milliliter of the purified sample extract (corresponding to 0.25 g blubber) was gently evaporated to dryness and taken up with 25 μl of a 1 ng μl^{-1} solution of DPTE in isooctane.

2.6. Gas chromatography coupled with mass spectrometry (GC/MS)

GC/MS analyses were performed with a CP-3800/1200 triple–quadrupole system (Varian, Darmstadt, Germany). Helium (purity