IDENTIFICATION AND QUANTIFICATION OF NEW POLYBROMINATED DIMETHOXYBIPHENYLS (PBDMBS) IN MARINE MAMMALS FROM AUSTRALIA

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Introduction

Halogenated natural products (HNPs) have been identified with a variety of over 4,500 structures, most of which are produced by marine organisms[1]. During the last years, a few of these HNPs have also been detected in marine mammals. These predatory animals bioaccumulate HNPs in a similar way as it is known for anthropogenic persistent organic pollutants (POPs)[2]. Marsh *et al*. resolved the identity of an organobromine compound frequently found in marine mammal samples from the Pacific as 2,2´-dimethoxy-3,3´,5,5´-tetrabromobiphenyl (2,2´-diMeO-BB 80, BC-1)[3]. This compound was initially described as one of three major abundant brominated compounds (BCs) in marine mammals from Queensland (Australia)[4]. In addition to 2,2´-diMeO-BB 80, Australian cetaceans contained the tetrabromo-phenoxyanisoles 2´-MeO-BDE 68 (BC-2) and 6-MeO-BDE 47 (BC-3) along with relatively low concentrations of six earlier-eluting organobromine compounds (subsequently referred to as "minor compounds")[4,5]. Some of the minor compounds were identified as tribromo-phenoxyanisoles[6]. Since other minor products did not form *m/z* 159, it was hypothesised that these represented tribromo-2,2´-dimethoxybiphenyls. The goal of this study was to verify this hypothesis by the photolytical debromination 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.

Material and methods

Standards and chemicals. 2,2´-DiMeO-BB 80 (BC-1), 6-MeO-BDE 47 (BC-3), 2´-MeO-BDE 68 (BC-2), and 2,3-dibromopropyl-2,4,6-tribromophenyl ether (DPTE; used as IS) were previously prepared[3,7-9]. 2,2'-Biphenyldiol was purchased from Acros Organics (Geel, Belgium).

Photochemical treatment of 2,2´-diMeO-BB 80. 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[10]. 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, subsamples of 500 µL, respectively, were collected from the solution and analyzed by GC/ECD or GC/MS. The subsample collected after 5 min irradiation from the 2-propanol solution was condensed to 80 µL. An aliquot of 70 µL was further condensed to 20 µL for detailed analysis by GC/MS.

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. Purification included liquid-liquid extraction and chromatography on a silica gel column. From the batch with two equivalents 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, whereas the batch with three equivalents of BTMA Br_3 resulted in 144 mg (37%) of 2,6´-diMeO-BB 36 and 111 mg, (23%) of 2,2'-diMeO-BB80.

NMR data of the novel BDMBs. NMR measurements were recorded with a Varian Inova 300 MHz instrument. **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, 1.5Hz), 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). **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). **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).

Origin and processing of marine biota samples. Blubber was available from a female calf of common dolphin (*Delphinus delphis*), an adult male pygmy sperm whale (*Kogia breviceps*), an adult female melon-headed whale (*Peponocephala electra*) and a female adult bottlenose dolphin (*Tursiops truncates*) from SE Queensland. Details of the sample clean-up (accelerated solvent extraction, gel-permeation chromatography, and adsorption chromatography on silica) are described elsewhere[6]. One mL of the purified sample extract (corresponding with 0.25 g blubber) was evaporated to dryness and taken up with 25 µL of a 1 ng/µL solution of DPTE in isooctane.

GC/MS. Analyses were performed with a CP-3800/1200 system (Varian, Darmstadt, Germany). Helium (purity 99.9990%, Sauerstoffwerke, Friedrichshafen, Germany) was used as carrier gas at a constant flow of 1.2 mL/min. A DB5-like column (Factor Four CP-Sil 8MS, 30 m length, 0.25 mm I.D., 0.25 µm film thickness; Varian, Darmstadt, Germany) was installed in the GC oven. The oven temperature was initially kept at 70 °C for 1.5 min and then raised at 30 °C/min to 140 °C, at 3 °C/min to 230 °C, and at 4 °C/min to 270 °C (hold time 6.17 min. The total run time was 50 min. Injections were performed in splitless mode (split opened after 2 min). The electron energy was set at 70 eV, and the ion source temperature at 150 °C. In the electron ionisation mode (GC/EI-MS), full scan spectra (*m/z* 50-620) were recorded at a scan time of 2 cycles per second. In the SIM mode, the following ions were measured in four time windows: 10-26.5 min: *m/z* 292, *m/z* 294, *m/z* 296, *m/z* 297, *m/z* 330, *m/z* 332; 26.5 – 33.5 min: *m/z* 330, *m/z* 332, *m/z* 369, *m/z* 370, *m/z* 372, *m/z* 374; 33.5 – 39.7 min: *m/z* 330, *m/z* 332, *m/z* 447, *m/z* 448, *m/z* 450, *m/z* 452; 39.7 – 50 min: *m/z* 330, *m/z* 332, *m/z* 525, *m/z* 528, *m/z* 530, *m/z* 532. The presence of polybrominated dimethoxybiphenyls (PBDMBs) was verified by the retention time compared to the standards produced in the irradiation experiment as well as by the correct ratio of isotopic peaks. Deviations from the correct isotopic ratios in samples were <10% except where noted. Additional peaks in samples which gave response to the SIM masses monitored were detected as well, but these did not show the correct ratio of isotopic peaks for BDMBs. Quantitative determinations were carried out using an external standard of 2,2´-diMeO-BB 80. DPTE was added to both external standard and samples for compensation of instrumental instability. Mono- to triBDMBs were determined on the basis of GC/EI-MS full scan measurements of the irradiation solution (5 min, 2-propanol). The response of the compounds was adjusted to the carbon content relative to 2,2´-diMeO-BB 80.

Results and discussion

UV treatment of BC-1. Using *n*-hexane as the solvent led to a very fast breakdown of 2,2´-diMeO-BB 80. After fifteen minutes, neither 2,2´-diMeO-BB 80 nor any transformation product could be detected by GC/ECNI-MS. After five minutes, we found traces of 2,2´-diMeO-BB 80 and one minor diBDMB congener. Thus, this treatment was not suited for the production of debromination products of 2,2´-diMeO-BB 80. However, switching from *n*-hexane to 2-propanol as the solvent delayed the speed of transformation. Whereas traces of 2,2´-diMeO-BB 80 and products were detected after 15 minutes, solutions taken after five minutes contained eight PBDMBs including 2,2´-diMeO-BB 80 as was verified by GC/MS (**Figure 1**). At longer irradiation times, no BDMBs were detected in the solution.

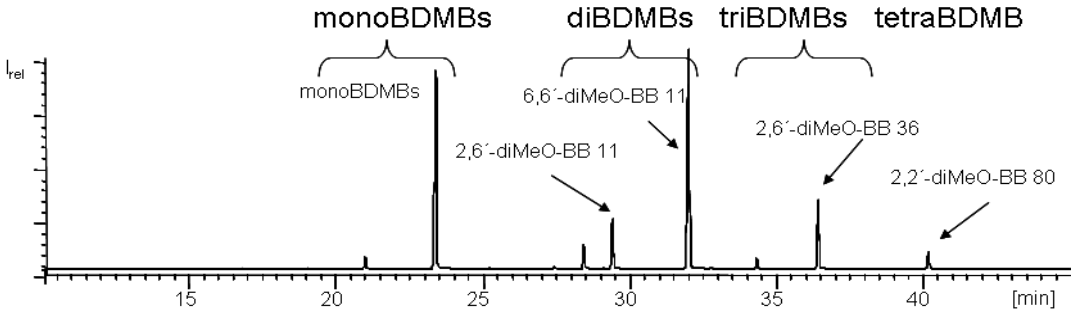

Figure 1: GC/EI-MS-SIM chromatogram of the UV transformation of 2,2´-diMeO-BB 80 (5 min in 2-propanol)

Isomer patterns and elution order of BDMBs after photochemical treatment of 2,2´-diMeO-BB 80. All but one debromination product that can be formed from 2,2´-diMeO-BB 80 (**Figure 2**) were observed (**Figure 1**).

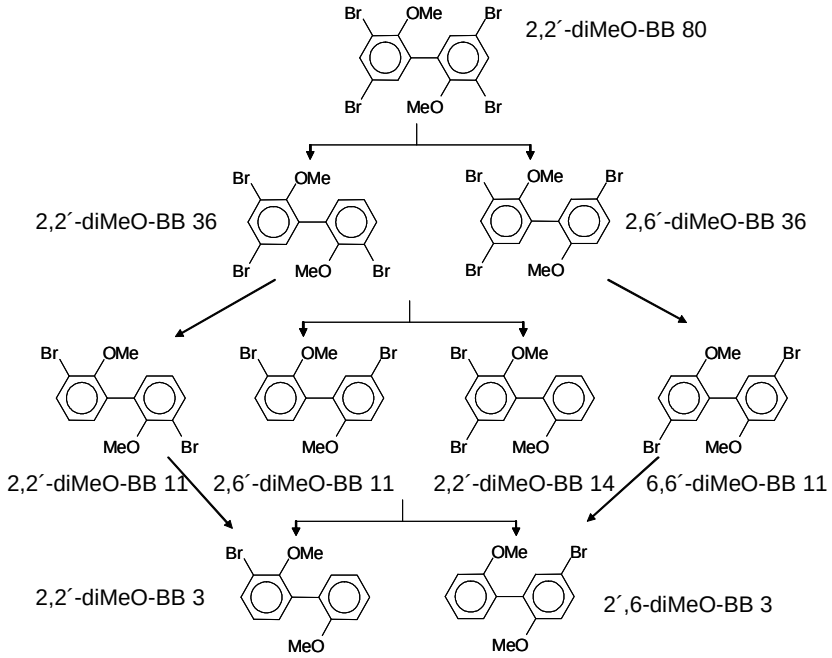

Figure 2: **Structures of 2,2´-diMeO-BB 80 and its possible debromination products**

The production rates and the retention times of individual tribromo- to monoBDMBs differed considerably from each other (**Figure 1**). The first eluting tribromo congener was identified as 2,2´-diMeO-BB 36 based on comparison with the (later eluting) synthesis product 2,6´-diMeO BB 36, which represents the only other possible tribromo congener from debromination of 2,2'-diMeO-BB 80 (**Figures 1 and 2**). With respect to diBDMBs, the second and third eluting congeners were also identified by subsequent synthesis. Since PBBs congeners with an excess of two bromines on one ring compared to the other ring are usually not formed[11], it is unlikely that 2,2´-diMeO-BB 14 (**Figure 2)** was produced from debromination of 2,2'-diMeO-BB 80. Thus, the structure of the remaining, first eluting diBDMB must be 2,2´-diMeO-BB 11 (**Figures 1 and 2**). The structures of the two monoBDMB that can be formed from 2,2'-diMeO-BB 80 could be assigned based on the relative abundance of isomers and their respective formation pathways during the UV experiment: the first eluting mono-, di-, and triBDMB was produced at significantly lower abundance compared to the last eluting isomer (**Figure 1**). Since the first (last) eluting monoBDMB can only be formed from the first (last) eluting triBDMB, the two monoBDMBs must elute in the order as shown in **Figures 1 and 2**.

Screening environmental samples for BDMBs. Using the authentic standard of 2,2´-diMeO-BB 80, it was for the first time possible to quantify residues of 2,2´-diMeO-BB 80 in blubber of dolphins and cetaceans from Australia. Moreover, the calibrated solution of lower BDMBs offered the opportunity to determine these debromination products of 2,2´-diMeO-BB 80 as well. 2,2´-diMeO-BB 80 was present at 200 – 1,800 ng/g lw in the marine mammals analysed for the present study (**Table 1**) which is higher than 2,2´-diMeO-BB 80 residues reported in marine mammals from Japan (12-800 ng/g lw)[3]. The present study also confirmed the presence of 6,6´-diMeO-BB-11, 2,2´-diMeO-BB 36, and 2,6´-diMeO-BB 36 in these samples. Furthermore, it was verified that all BDMBs detected in the biota samples are structurally related to 2,2´-diMeO-BB 80. The highest concentration was found for 2,6´-diMeO-BB 36 at ~ 6 ng/g lw, respectively, whereas 2,2´-diMeO-BB 36 and 6,6´-diMeO-BB 11 were ~ one order of magnitude lower concentrated (**Table 1**). The concentrations of the 2,2´-diMeO-BB 80-related BDMBs were 0.43 – 1.5 % of 2,2´-diMeO-BB 80. In two samples, only 2,2´-diMeO-BB 80 was detected. Since these samples contained rather low amounts of 2,2´-diMeO-BB 80 (**Table 1**) it can be assumed that di- and triBDMBs were below the detection limit of 0.05 – 0.10 ng/g lw for tri- and diBDMBs.

Table 1: Concentrations (ng/g) of BDMBs in blubber of marine mammals from Australia

Compound	6,6´-diMeO BB 11	2,2´-diMeO BB 36	2,6´-diMeO BB 36	2,2´-diMeO BB 80
t_R [min] (RRI)	32.02 (0.7973)	34.33 (0.8548)	36.42 (0.9069)	40.16 (1.000)
common dolphin	1.1	0.8	6.0	510
bottlenose dolphin	0.5	2.2	5.3	1840
pygmy sperm whale	< 0.1	< 0.1	< 0.1	205±15
melon-headed whale	< 0.1	< 0.1	< 0.1	530±20

Although the concentrations of the dominating 2,6´-diMeO-BB 36 was low compared to 2,2´-diMeO-BB 80 in the samples from Australia, its concentration was only half compared to 2,2´-diMeO-BB 80 reported in some marine mammals from Japan[3]. Moreover, the strong structural relationship of 2,2´-diMeO-BB 80 and the detected BDMBs is worth discussing. It is widely accepted that 2,2´-diMeO-BB 80 is a naturally produced organohalogen compound because its precursor 3,3´,5,5´-tetrabromobiphenyl-2,2´-diol (2,2´-diOH-BB 80) has been identified[12]. In addition, 2,2´-diMeO-BB 80 was tentatively identified in archived whale oil from 1921 which predates the industrial production of organobromine compounds[13]. However, to our knowledge, tribromobiphenyldiols have not been reported in the literature. It may be possible that the these BDMBs represent metabolites of 2,2´-diMeO-BB 80 and are not directly produced in nature. Similarly, it was recently suggested that at least some of the tribromophenoxyanisoles detected in related samples are metabolites of the known tetrabrominated phenoxyanisoles 6-MeO-BDE 47 and 2´-MeO-BDE 68[6]. However, the ratio of triBDMBs compared to the 2,2´-diMeO-BB 80 was ~one order lower than found for the brominated phenoxyanisoles.

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