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Bromination of 2-methoxydiphenyl ether to an average of tetrabrominated 2-methoxydiphenyl ethers

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ABSTRACT

Brominated 2-phenoxyanisoles (2-methoxydiphenyl ethers, 2-MeO-BDEs) are a class of halogenated natural products, produced by algae and sponges. Especially two tetrabrominated isomers, i.e. 2′-MeO-BDE 68 (BC-2) and 6-MeO-BDE 47 (BC-3), have also been frequently determined in environmental and food samples. In addition, 2-MeO-BDEs are under discussion as metabolites of polybrominated diphenyl ethers (PBDEs). In this study, we synthesized the backbone 2-methoxydiphenyl ether and brominated it to an average degree of four bromine substituents. The reaction mixture only contained one major product (~90%) along with three further MeO-BDEs and ~5% hydroxylated BDEs. In all likelihood, the HO-BDEs were formed in a side reaction by cleavage of the methoxy group. The major MeO-BDE was identified as 6′-methoxy-2,3′,4,4′-tetrabromodiphenyl ether (6′-MeO-BDE-66). The HO-BDEs were separated by KOH/*n*-hexane partitioning, and the resulting 2-MeO-BDEs were fractionated by means of high-speed counter-current chromatography (HSCCC). Due to the excellent enrichment facilities of HSCCC, some 15 MeO-BDEs, mainly present at traces only, could be detected in 26 fractions, and eight of them could be characterized by nuclear magnetic resonance spectroscopy (NMR). Only two of the compounds – 2′-MeO-BDE 68 and 6-MeO-BDE 123 – had been characterized as natural products while the prominent halogenated natural product 6-MeO-BDE 47 was not detected at all in the reaction product. The "non-natural" 2-MeO-BDEs may be useful internal standards in trace analysis.

1. Introduction

Polybrominated 2-phenoxyanisoles (a.k.a. polybrominated 2-methoxydiphenyl ethers, 2-MeO-BDEs) are a class of marine halogenated natural products (HNPs) biosynthesized by different algae and sponges (Faulkner, 1980; Gribble, 2000, 2010). Di- to hexabrominated MeO-BDEs have been isolated from different species (Sharma et al., 1970; Capon et al., 1981; Carté and Faulkner, 1981; Kuniyoshi et al., 1985; Salva and Faulkner, 1990; Unson et al., 1994; Handayani et al., 1997; Cameron et al., 2000; Utkina et al., 2002; Liu et al., 2004). Some of these HNPs have been identified in higher organisms which are not their natural producers (Vetter, 2006). In this point their environmental behavior was similar to anthropogenic pollutants. The predominant representatives of MeO-BDEs in the environment are the tetrabrominated 2′-MeO-BDE 68 (BC-2) and 6-MeO-BDE 47 (BC-3) (Haglund et al., 1997; Asplund et al., 1999; Vetter et al., 2001; Marsh et al., 2004a). Different MeO-triBDEs – structurally related to BC-2 and BC-3 – have also been detected, but these could as well be metabolites of the hydrodebrominaton of 2′-MeO-BDE 68 and 6-MeO-BDE 47 (Melcher et al., 2005). An additional, anthropogenic, source for MeO-BDEs in environmental samples has been suggested in different works (Haglund et al., 1997; Marsh et al., 2003; Stapleton et al., 2006; Feng et al., 2010). It has been mentioned that the structurally related polybrominated diphenyl ethers (PBDEs) may be oxidized to give HO-BDEs, and their subsequent *O*-methylation would then lead to MeO-BDEs (Hakk and Letcher, 2003; Feng et al., 2010). Recently, the photochemical formation of 2′-HO-BDE 68 from 2,4-dibromophenol was described (Liu et al., 2011). The HO-BDE 68 may also be a precursor of 2′-MeO-BDE 68. Another option could be the technical bromination of phenoxyanisole (2-MeO-BDE). However, in contrast to the synthesis of individual 2-MeO-BDEs (Francesconi and Ghisalberti, 1985; Marsh et al., 2003; Nikiforov et al., 2003; Vetter and Wu, 2003), the non-selective synthesis of MeO-BDEs has not been studied in detail.

In this study we aimed at synthesizing 2-MeO-BDE followed by its bromination to an average degree of bromination of four. The crude products should then be fractionated and analyzed for its constituents. Fractionation was attempted by means of high-speed counter-current chromatography (HSCCC). HSCCC is a separation technique based on the distribution of analytes between two immiscible liquid phases (Ito, 2005). While one phase is kept stationary, the second phase is moved through the system (Ito and Bowman, 1970). Under the impact of multiple phase equilibria, a compound

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mixture may elute separated from the column. This technique has recently been used in the separation of the polychlorinated multicomponent mixture toxaphene (Kapp and Vetter, 2009).

2. Materials and methods

2.1. Chemicals and solvents

Acetonitrile (HPLC gradient grade) and chloroform (reagent grade) were from Fisher Scientific (Leicestershire, UK). Cyclohexane (pure) was from VWR (Darmstadt, Germany). Ethyl acetate (purest), dibromomethane (puriss., ⩾98.5%), and $AlBr_3$ (98 + % extra pure, anhydrous) were from Acros Organics (Geel, Belgium). Br_2 (ACS reagent, ⩾99.5%), bromobenzene (99%, GC), guaiacol (purum, ⩾98.0%, GC), $MgSO_4$ (water-free, puriss., p.a.), Na_2SO_4 (water-free, ⩾99%, p.a.), silica gel 60 (for column chromatography), and $Na_2S_2O_3$ pentahydrate (puriss., p.a.) were from Sigma–Aldrich (Seelze, Germany). KOH (⩾85% Ph. Eur.) was from Carl Roth (Karlsruhe, Germany); methanol (puriss., p.a.) and *n*-hexane (HPLC grade) were from Th. Geyer (Renningen, Germany); iodomethane (for synthesis) was from Merck (Hohenbrunn, Germany).

2.2. Gas chromatography in combination with mass spectrometry (GC/MS)

Measurements in the electron ionization mode (GC/EI-MS) were performed with a 5890 series II plus/5972 GC/MS system fitted with a 7673 GC/SFC automatic injector (Hewlett–Packard/Agilent Technologies, Waldbronn, Germany). Samples were injected in splitless mode at 300 °C and were then transported at 1.2 mL/min by the carrier gas He (purity 5.0, Sauerstoffwerke, Friedrichshafen, Germany). An HP-5MS column (30 m, 0.25 mm internal diameter, 0.25 μm film thickness, J&W Scientific, Folsom, CA, USA) was installed in the GC oven. The oven temperature was programmed as follows: 50 °C (1 min), then at 10 °C/min to 300 °C (4 min); total run time 30 min. The transfer line temperature was set at 300 °C. After a solvent delay of 8 min, we recorded *m/z* 50 – *m/z* 700 in the full scan mode. The scan time was 1.19 scans s^{-1}.

2.3. High-speed counter-current chromatography

HSCCC analyses were run with a semi preparative PTR Model CCC-1000 counter-current chromatograph (Pharma-Tech Research, Baltimore, Maryland, USA) operated with a quaternary P580 HPLC pump (Dionex, Idstein, Germany) for solvent delivery and an ISCO Retriever 500 fraction collector (Teledyne Isco, Lincoln, Nebraska, USA) according to Kapp and Vetter (2009). The system consisted of three planetary rotating columns (column volume: 325 mL). The rotation speed was 1020 U/min. The separation was carried out in reversed phase mode (RP-HSCCC) by using the solvent system acetonitrile/*n*-hexane (1:1; *v/v*), elution mode tail to head. Extrusion of the stationary phase was 45 mL. The flow rate was set at 1 mL min^{-1}. The injection and fractionation scheme was as follows: the sample was loaded via a 10 mL sample loop 1 h after the mobile phase was added. Fractions of 15 min (~15 mL) were collected from the moment when mobile phase left the column system.

2.4. NMR analysis

One-dimensional ^1H and ^{13}C NMR spectra were recorded with a Varian Inova 300 MHz instrument fitted with a 5 mm ATB sample probe. Chemical shifts were reported relative to the solvent signal of $CDCl_3$ on the TMS scale. Gradient enhanced ^1H–^{13}C correlation measurements (HSQC) were performed according to Gaul et al. (2005).

2.5. Synthesis of MeO-BDEs

2.5.1. Synthesis of 2-phenoxyanisole (Ungnade and Orwoll, 1946)

Pulverized KOH (5.88 g, 0.086 mol) was placed in a reaction flask. Guaiacol **1** (13.27 mL, 0.12 mol) was added dropwise at room temperature. Once the exothermic reaction waned, the temperature was raised to 150 °C. A water stream pump was used to reduce the pressure in order to facilitate the separation of water formed under the reaction (and by this directing the equilibrium towards product). The resulting guaiacolate (2-methoxyphenolate) **2** was directly used in the following step.

The dry salt guaiacolate **2**, 0.06 g copper (catalyst), bromobenzene (10.8 mL, 0.102 mol) and further guaiacol (0.8 mL) was added stepwise. The temperature was gradually increased. At 180 °C the solid mixture became fluid. The temperature was set to 200 °C and the solution was stirred for 2 h. After cooling, product was extracted with ethyl acetate/cyclohexane (3 × 100 mL) and water (150 mL). The organic phase was washed with water (2 × 30 mL) and dried over Na_2SO_4. The solvent was evaporated and the product was refrigerated for crystallization. The crystalline product was washed with ice-cooled methanol, filtrated and 3× re-crystallized with hot methanol to give 4.52 g 2-methoxydiphenyl ether **3** (yield 26.3%).

NMR data of 2-methoxydiphenyl ether **3**: ^1H NMR (300 MHz, $CDCl_3$): δ [ppm] 7.33–6.90 (m, 9H, Ph*H*), 3.84 (s, 3H, OCH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] 157.9, 151.4, 145.0, 129.5, 124.8, 122.4, 121.1, 121.0, 117.2, 112.8, 55.9.

2.5.2. Bromination of 2-methoxydiphenyl ether 3 with five equivalents bromine

The synthesis followed the route described by Örn et al. (1996) for PBDEs. 2-Methoxydiphenyl ether **3** (0.879 g, 4.39 mmol) and 1 g $AlBr_3$ were placed in a double-necked flask. Then, 3.8 g bromine in 50 mL dibromomethane (DBM) was added dropwise at room temperature (Örn et al., 1996). The temperature was increased for 18 h to 100 °C. Another spatula tip of $AlBr_3$ was added and the temperature was raised to 130 °C. After cooling, 100 mL saturated $Na_2S_2O_3$ was added to reduce excessive Br_2 to bromide. The organic layer was extracted three times with 100 mL of dibromomethane (twice) and chloroform. The products were placed on 19 g silica 60 and all product was targeted in one fraction (50 mL) by means of *n*-hexane/ethyl acetate (1:0, then 9:1). Separation of MeO-BDEs from HO-BDEs was performed by adding 150 mL *n*-hexane and 200 mL 0.5 M KOH to the evaporated crude product (Athanasiadou et al., 2008). After shaking the organic phase was separated. The aqueous phase was re-extracted with 2 × 100 mL *n*-hexane. The crude product weighed 1.75 g.

The separated 2-HO-BDEs were acidified with 150 mL 1 M HCl and extracted with 3 × 100 mL *n*-hexane. After evaporation a total of 81 mg 2-HO-BDEs (~5% of the product) could be collected. Treatment of an aliquot of the HO-BDE fraction with CH_3I to give MeO-BDEs was performed according to Field and Monohan (1996). Initial bromination experiments of 2-methoxydiphenyl ether were also performed with seven equivalents of Br_2.

NMR data of the main product 6′-methoxy-2,3′,4,4′-tetrabromodiphenyl ether **4**: ^{13}C NMR (75 MHz, $CDCl_3$): δ [ppm] 152.8, 150.5, 144.3, 136.0, 131.5, 124.2, 119.8, 119.4, 117.7, 116.4, 114.9, 114.3, 56.5. The ^1H NMR data (Table 1) of **4** agreed with that of 6′-MeO-BDE-66 (P24-M45) (Nikiforov et al., 2003).

2.6. HSCCC fractionation

The partition coefficients *K* of four major compounds in the crude product were determined with acetonitrile/*n*-hexane (1:1; *v/v*) in shake-flask experiments. About 1 mg of the crude product (after separation of the phenols), 2 mL acetonitrile, and 2 mL