

Synthesis and identification of polybrominated *ortho*-phenoxyanisoles and phenols in Baltic salmon (*Salmo salar*)

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Introduction

Polybrominated phenoxyanisoles (methoxylated polybrominated diphenyl ethers (MeO-PBDEs)) have recently been found in herring, ringed seal, grey seal (1) and salmon (1, 2) from the Baltic Sea and also in commercial fish oils (1). According to gas chromatography-mass spectrometry (GC-MS) data, three tetra- and one pentabrominated MeO-PBDE were found. At least one tetrabrominated MeO-PBDE seemed to have the methoxy-group in the *ortho*-position relative to the diphenyl ether oxygen (1). The concentration of the MeO-PBDEs were comparable with the concentration of polybrominated diphenyl ethers (PBDEs), i.e. 1-180 ng/g (lipid weight basis) in Baltic salmon (2).

In addition to MeO-PBDEs, also polybrominated phenoxyphenols (hydroxylated PBDEs (OH-PBDEs)) have been detected in blood from Baltic salmon at levels estimated to be 20-30% compared to those of the MeO-PBDEs (2).

The origin of these compounds is unknown, but the chromatographic profiles of the MeO-PBDEs and OH-PBDEs are similar, suggesting that the sources to these compounds in some way are connected (2).

The aim of the present study is to synthesize tetra- and pentabrominated MeO- and OH-PBDEs with the methoxy-/hydroxy-substituent in the *ortho*-position relative to the diphenyl ether oxygen in order to identify and quantify these substances in biota.

Material and Methods

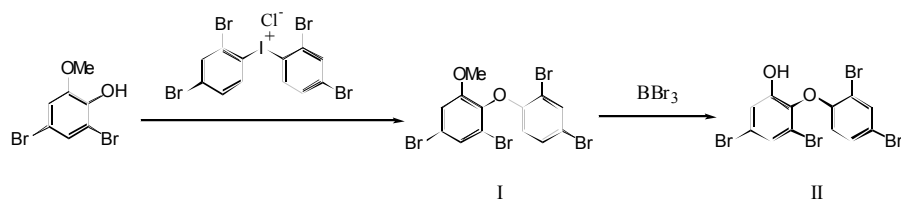
Chemicals: 2-Fluorobenzaldehyde, 2,4-dibromophenol, benzyltrimethylammonium tribromide (BTMA Br₃), borontribromide (BBr₃) and *m*-chloroperoxybenzoic acid (*m*-CPBA) were obtained from Aldrich. Iodomethane (CH₃I) was purchased from Merck. 2,2',4,4'-Tetrabromodiphenyliodonium chloride was synthesized according to Marsh et al. (3). 4,6-Dibromo-2-methoxyphenol was synthesized as previously described (4).

Instruments: Gas chromatography-mass spectrometry (GC-MS) was performed on an ion trap GCQ Finnigan MAT instrument. The GCQ Finnigan MAT gas chromatograph was equipped with a DB-5MS fused silica capillary column, 30 m × 0.25 mm, 0.025 mm film thickness (J&W Scientific). The temperature program was 80 °C, 1 min.; 20 °C min.⁻¹, 230 °C; 5 °C min.⁻¹, 300 °C, 5 min. Hydrogen was used as the carrier gas and the temperature of the injector was 260 °C. Electron ionization (EI) was used at an ion source temperature of 180 °C and an electron energy of 70 eV. Full scan mass spectra were recorded for *m/z* 250-700. A reconstructed ion chromatogram based on the sum of the most intense ions of MeO-tetraBDE (*m/z* 516) and MeO-pentaBDE (*m/z* 436) is shown in Figure 1.

Analysis: One pooled sample of blood plasma from fifteen female sea-run Baltic Sea salmon from the Swedish River Dalälven was analyzed. The salmon were sampled in 1995. The plasma was denaturated, extracted and cleaned-up as previously described (2). Thus, neutral and phenolic compounds were separated by partitioning between hexane and potassium hydroxide (0.5 M). The phenolic compounds were derivatized with diazomethane and the sample was further purified by high-resolution gel permeation chromatography (HR-GPC) and fractionation on silica gel.

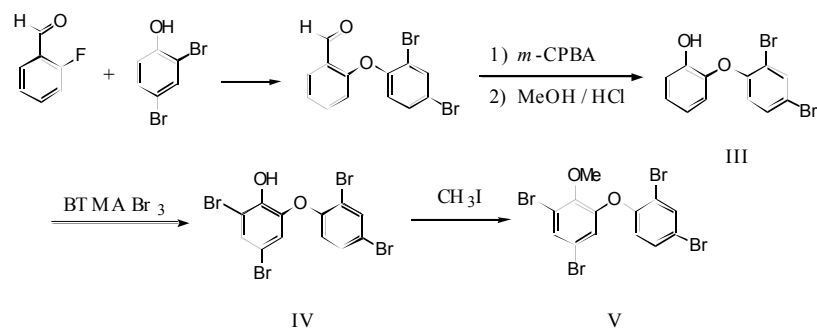
Synthesis: 3,5-Dibromo-2-(2,4-dibromophenoxy)anisole (I) was prepared by the coupling of 4,6-dibromo-2-methoxyphenol and 2,2',4,4'-tetrabromodiphenyliodonium chloride by a previously described method (3). The phenol derivative, 3,5-dibromo-2-(2,4-dibromophenoxy)anisole (II) was obtained by demethylation of 3,5-dibromo-2-(2,4-dibromophenoxy)anisole (I) with borontribromide, cf. Scheme 1.

Scheme 1



2-(2,4-Dibromophenoxy)phenol (III) was synthesized by the method of Yeager and Schissel (5). Thus, the coupling of 2-fluorobenzaldehyde and 2,4-dibromophenol resulted in 2-(2,4-dibromophenoxy)benzaldehyde which subsequently was converted into the corresponding formate ester in a Baeyer-Villiger oxidation using *m*-CPBA. The formate ester was hydrolyzed with methanol/HCl to 2-(2,4-dibromophenoxy)phenol (III). The latter was dibrominated with BTMA Br_3 (4) to 4,6-dibromo-2-(2,4-dibromophenoxy)phenol (IV). 4,6-Dibromo-2-(2,4-dibromophenoxy)anisole (V) was obtained by methylation of IV with iodomethane, cf. Scheme 2.

Scheme 2



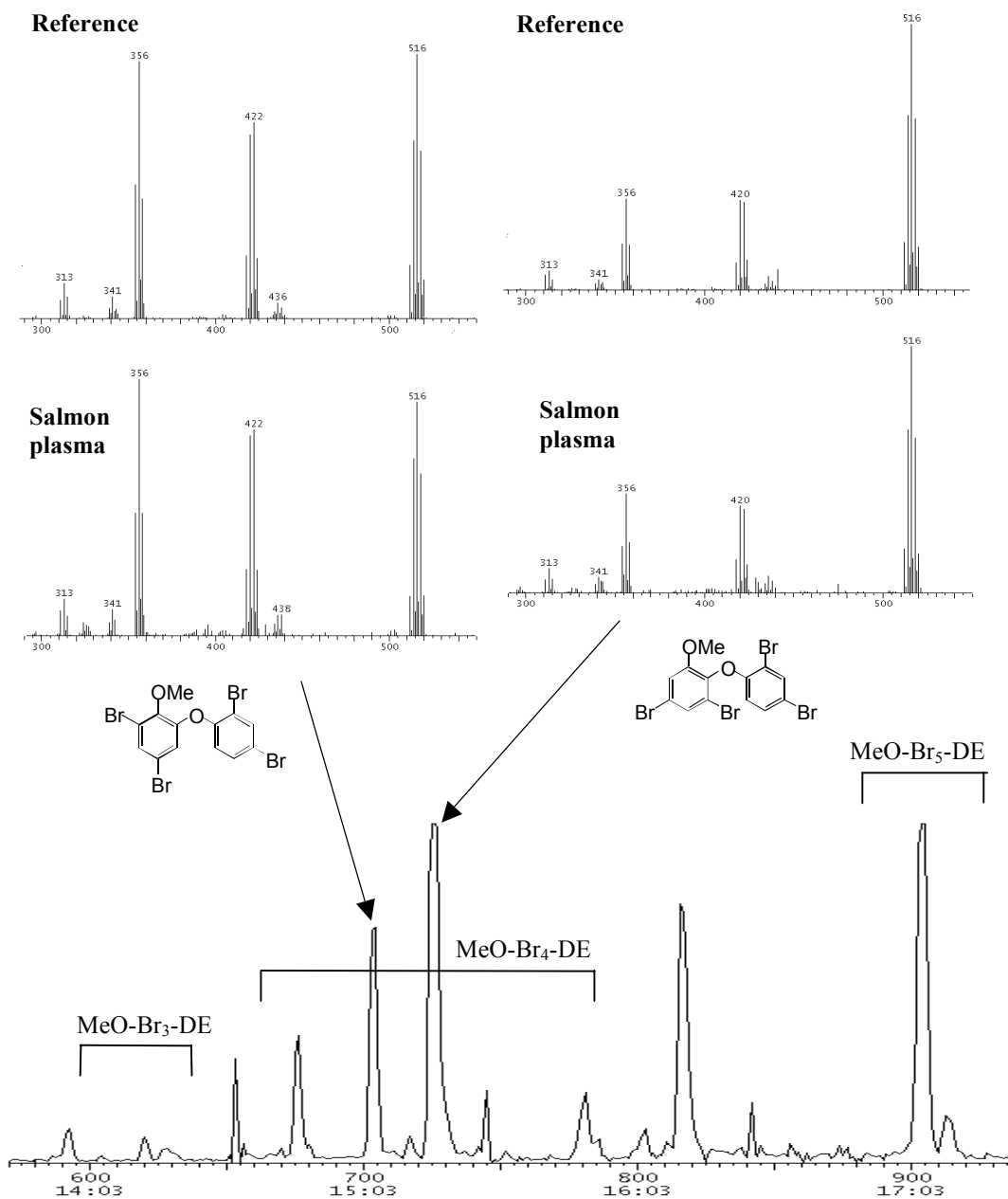


Figure 1. Reconstructed ion chromatogram based on the sum of the most intense ions of MeO-tetraBDE (m/z 516) and MeO-pentaBDE (m/z 436).

Results and Discussion

3,5-Dibromo-2-(2,4-dibromophenoxy)anisole (I), 3,5-dibromo-2-(2,4-dibromophenoxy)phenol (II), 4,6-dibromo-2-(2,4-dibromophenoxy)phenol (IV) and 4,6-dibromo-2-(2,4-dibromophenoxy)anisole (V) have been prepared by two different pathways, cf. Scheme 1 and 2. The preparations of these compounds have previously been described, but via longer pathways (6). Melting points and ^1H NMR spectra were recorded and they were found to be similar to data previously reported (6).

The presence of three tetra- and one pentabrominated MeO-PBDEs and also the corresponding OH-PBDEs have previously been reported in blood plasma from the Baltic salmon (2). 3,5-Dibromo-2-(2,4-dibromophenoxy)anisole (I) and 3,5-dibromo-2-(2,4-dibromophenoxy)phenol (II) were identified by the authors by comparison to synthesized reference compounds.

In the present study, at least two tribromo-, five tetrabromo- and two pentabromophenoxyphenols were found in the pooled sample of blood plasma from fifteen Baltic salmon, cf. Figure 1. In addition to the previously identified 3,5-dibromo-2-(2,4-dibromophenoxy)phenol (II), also 4,6-dibromo-2-(2,4-dibromophenoxy)phenol (IV) was identified in the present work. Mass spectra of the methyl derivatives of the identified compounds and the corresponding reference compound are shown in Figure 1.

Additional MeO-PBDE and OH-PBDE congeners will be synthesized, in order to identify and quantify these compounds in biota.

Acknowledgements

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References

1. Haglund P.S, Zook D.R, Buser H-R and Hu J; *Environ. Sci. Technol.* **1997**, 31, 3281
2. Asplund L, Athanasiadou M, Sjödin A, Bergman Å and Börjesson H; *Ambio* **1999**, 28, 67
3. Marsh G, Hu J, Jakobsson E, Rahm S and Bergman Å; *Environ. Sci. Technol.* In press
4. Kajigaeshi S, Kakinami T, Tokiyama H, Hirakawa T and Okamoto T; *Chem. Lett.* **1987**, 627
5. Yeager G.W and Schissel D.N; *Syntesis* **1995**, 28
6. Francesconi K.A and Ghisalberti E.L; *Aust. J. Chem.* **1985**, 38, 1271