HYDROXYLATED AND METHOXYLATED POLYBROMINATED DIPHENYL ETHERS IN SALMON PLASMA: SYNTHESIS AND IDENTIFICATION

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Introduction

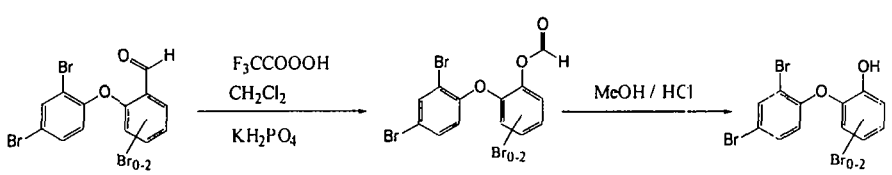
Hydroxylated and methoxylated polybrominated diphenyl ethers (HO- and MeO-PBDEs) are well known natural products most frequently found in marine sponges[1]. Moreover, HO-PBDEs have been determined as metabolites of polybrominated diphenyl ethers (PBDEs), e.g. of 2,2',4,4'-tetrabromodiphenyl ether (BDE-47)[2] in mice and rats[3] and of 2,2',4,4',5-pentabromodiphenyl ether (BDE-99) in rats[4]. PBDEs are a class of brominated flame retardant, which have become ubiquitous environmental pollutants[5, 6].

Recently, MeO-PBDEs have been reported to be present in pike[7], herring[8], salmon[8, 9], ringed seal[8], grey seal[8] and white-tailed sea eagle[10] in Scandinavia (northern Europe). From the same area several HO-PBDEs were found in salmon plasma[9] and one tetrabrominated HO-PBDE congener has also been observed in human plasma[11].

The aim of the present work was to synthesise HO- and MeO-PBDE congeners to determine the structural identity of these compounds in Baltic Sea salmon (*Salmo salar*) blood plasma.

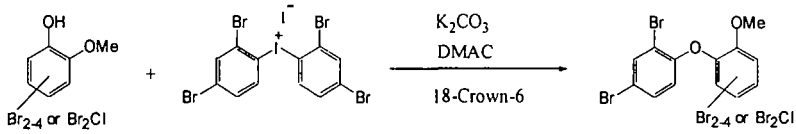
Methods and Materials

Synthesis of reference standards: Ten HO-PBDEs and the corresponding MeO-PBDEs (Figure 1) were synthesised using two major pathways. Seven of the HO-PBDEs and the corresponding MeO-PBDEs (**1-8** and **13-18**) were synthesised via brominated 2-phenoxybenzaldehydes (Scheme 1). The brominated 2-phenoxybenzaldehydes were either obtained by S_NAr reactions, coupling 2,4-dibromophenol with 2-flourobenzaldehyde and 5-bromo-2-fluorobenzaldehyde respectively or were prepared by the reaction of 2,2',4,4'-tetrabromodiphenyliodonium chloride with 3-bromosalicylaldehyde and 3,5-dibromosalicylaldehyde respectively. The aldehyde group was converted to a hydroxy group via a Baeyer-Villiger oxidation giving the formate ester, followed by an acid catalysed hydrolysis (Scheme 1). Dibromination (*ortho* and *para* relative to the hydroxy group) with benzyltrimethylammonium tribromide and monobromination (*ortho* relative to the hydroxy group) using a *tert*-butylamino/bromine complex led to the preparation of an additional number of HO-PBDE congeners.



Scheme 1

Via another pathway, HO- and MeO-PBDEs **1-2**, **9-12** and **19-20** were obtained by the reaction of bromo(chloro)guaiacol with 2,2',4,4'-tetrabromodiphenyliodonium iodide (Scheme 2). This pathway has previously been used by Utkina et. al.[12] All HO-PBDEs synthesised were methylated with iodomethane and all MeO-PBDEs were demethylated using borontribromine to give the corresponding MeO- and HO-PBDEs, respectively.



Scheme 2

Analysis: One pooled sample of blood plasma from 30 female sea-run Baltic Sea salmons (*Salmo salar*) from the Swedish River Dalälven sampled in 1995 was analysed. For more data and clean-up procedure see Asplund et.al.[9] The final GC-MS analysis was performed on a quadrupole SSQ 710 Finnigan MAT instrument equipped with a DB-5 column (30 m × 0.25 mm i.d. and 0.25 μm film thickness) using ECNI with methane as the reaction gas. The identification were done by comparison to the reference standards synthesised as described above using 2,2',3,4,4',5'-hexabromodiphenyl ether (BDE-138) as the internal standard as well as ECNI mass spectra.

Results and Discussion

Four tetrabromo-, one monochlorotetrabromo-, four pentabromo-, one hexabromo-HO-PBDEs and the corresponding ten MeO-PBDEs were synthesised (Figure 1).

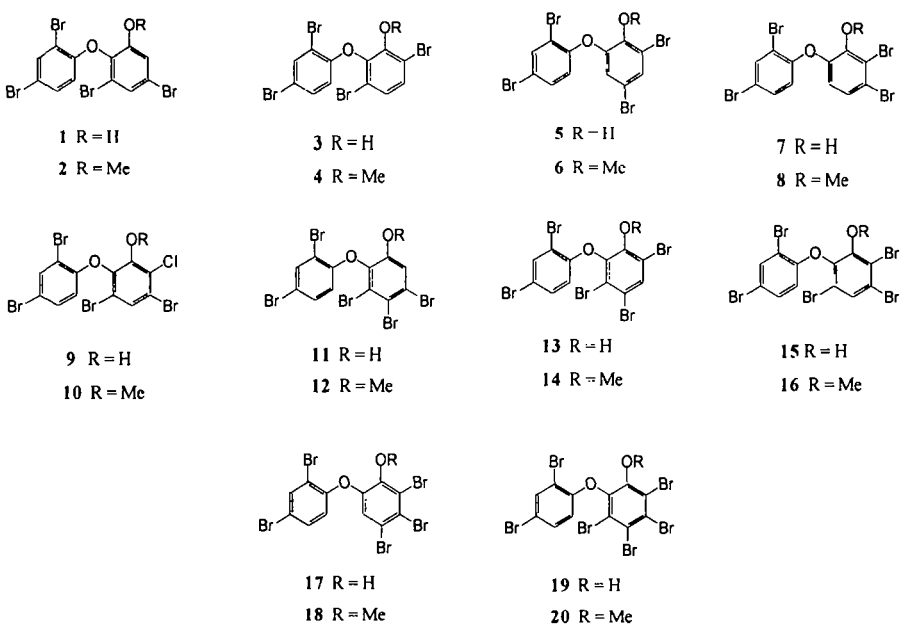


Figure 1. Structures of the synthesised HO-and MeO-PBDEs.

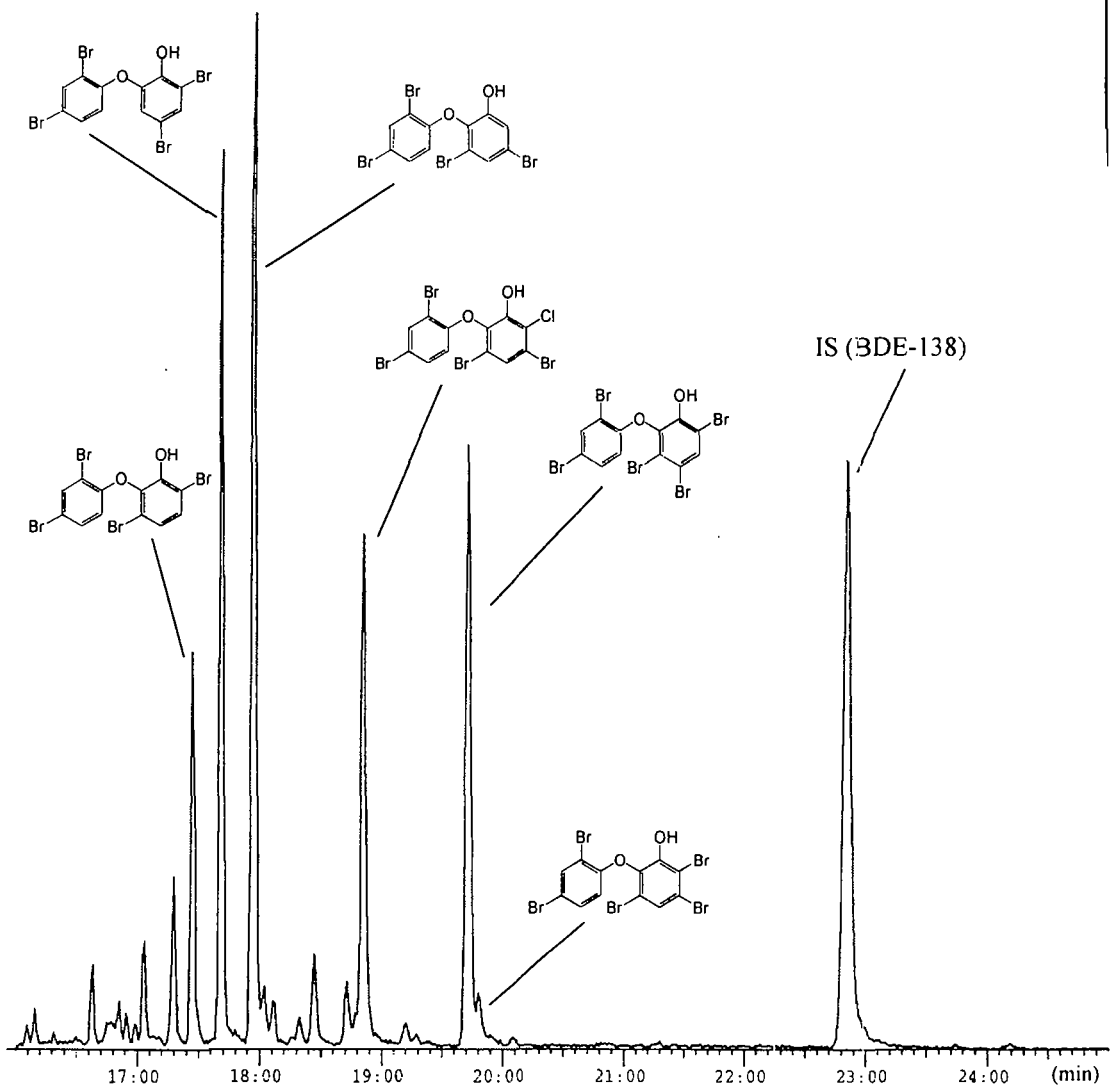


Figure 2. GC-MS chromatogram (ECNI, bromide ion *m/z* 79) of the methylated phenol fraction of the Baltic Sea salmon plasma.

The structures of six HO-PBDEs in the phenolic fraction (**1**, **3**, **5**, **9**, **13** and **15**) and five MeO-PBDEs (**2**, **4**, **6**, **10** and **14**) in the neutral fraction were identified in the salmon plasma (Figure 2). All five MeO-PBDEs identified in the neutral fraction corresponded to the derivatized methylated HO-PBDEs, in the phenolic fraction. The identification was made by comparison of retention times using BDE-138 as the internal standard. Previously, we reported the structures of two HO-PBDEs (**1** and **5**)[13]. To the best of our knowledge compounds **3**, **4**, **9**, **10**, **13** and **14** have not previously been structurally identified and reported. The compounds **1**[14], **2**[15], **5**[16], **6**[17] and **15**[18] have previously been reported as natural products.

No industrial production or use of HO- and MeO-PBDEs has been reported and neither have these compounds been reported as by-products in any industrial product. Most probably, the identified compounds are natural products and/or metabolites of PBDEs. The structures of the identified compounds **3-4**, **5-6**, **9-10** and **13-14** do not correspond to any expected metabolites of any major PBDE congeners. It is thus reasonable to believe that these compounds are of natural origin. Further, evidence of natural production of the HO-PBDE congeners discussed here are given by Asplund and coworkers at this symposium[19]. Compounds **1** and **2** cannot be excluded to be metabolites of BDE-47, but the high abundance of compounds **1** and **2** indicate that at least the major part most probably are natural products. In contrast it is difficult to say weather **15** is a natural compound or a metabolite of BDE-99, according to the relative small quantities determined of this compound. In the Baltic Sea salmon used in this work the concentration of the MeO-PBDEs were comparable with the concentration of PBDEs, i.e. 1-180 ng/g (lipid weight basis) and the levels of HO-PBDE was estimated to be 20-30% compared to those of the MeO-PBDEs[9].

A notable observation is that compound **8** (not found in this work) and **10** was found to co-elute with BDE-100 and BDE-99 respectively in GC on the DB-5 column used.

In conclusion, eleven compounds, HO- and MeO-PBDEs were identified in salmon plasma according to relative retention times with the comparison of synthesised reference standards. With great certainty, at least ten of the identified compounds are natural products. Compound **15** (or parts of it) and parts of **1** and **2** might be metabolites of PBDEs.

Additional work will be done to further confirm the identification of the found HO- and MeO-PBDEs and to further characterise the compounds, i.e. masspectrometry (EI and PICI).

Acknowledgements

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