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Non-polar halogenated natural products bioaccumulated in marine samples. II. Brominated and mixed halogenated compounds

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Abstract

Several identified and potential natural brominated bioaccumulative compounds were studied in this work. 4,6-dibromo-2-(2′,4′-dibromo)phenoxyanisole (BC-2) previously detected in Australian marine mammals and isolated from sponges, was synthesized. Two byproducts (a tetrabromo isomer and a tribromo congener) were investigated as well. The byproducts of the synthesis were not identified in the environmental samples investigated. Previously described natural brominated compounds (BC-1, BC-2, BC-3, BC-10, BC-11, MHC-1) and anthropogenic brominated diphenyl ethers (BDE-47, BDE-99, BDE-100, BDE-154) were detected in a sample of human milk. The sample was from a woman from the Faeroe Islands who frequently consumed fish as well as whale blubber and meat. The most abundant compound originated from the natural tetrabromo phenoxyanisole BC-3 which may have a 3:1 distribution of bromine on the two phenyl units. This sample also accumulated a dibromochloroanisole, as well as a previously unknown mixed halogenated compound (MHC-X) and an unknown, most likely aromatic brominated compound. Co-elutions on a DB-5 column were found for BDE-99 and BC-11 as well as BDE-154 and the unknown brominated compound. This suggests that quantification of these two compounds has to be carried out carefully.

Two samples of lower trophic level, namely Baltic cod liver and Mexican mussel tissue, were investigated as well. The cod liver samples contained BDE congeners but also abundant signals for the natural 2,3,3′,4,4′,5,5′-heptachloro-1′-methyl-1,2′-bipyrrole Q1 and tribromoanisole (TBA). The mussel sample contained Q1, TBA, another halogenated anisole, BC-1, BC-2, and BC-3, as well as additional, potential natural brominated compounds in the elution range of tribromophenoxyanisoles.

Keywords: Naturally produced organohalogens; 4,6-dibromo-2-(2′,4′-dibromo)phenoxyanisole (BC-2); Synthesis; Brominated flame retardants; Marine samples; Human milk

1. Introduction

In 1774 Schiele discovered chlorine and Faraday synthesized hexachlorocyclohexane and thus the first chloropesticide—albeit this discovery was reserved to Bender (1933)—in 1825. Bromine, however, was only discovered the year after in 1826. This historic comparison appears to be typical of the scientific relevance of the two halogens. It seems that brominated compounds always lagged behind their chlorinated kins. In the middle of the 20th century, a wide range of chlorinated compounds were produced at million-ton-scales for industrial (e.g. PCBs and PCNs) and agricultural (e.g. toxaphene, DDT, chlordane, and lindane)

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applications. It took again some decades until the brominated compounds received industrial attention. These days, it almost appears that large amounts of brominated compounds were only produced after the ban of the lighter halogen.

Today's major industrial field of application of brominated compounds is for flame retardation. In past years the records on the identification of residues of brominated flame retardants (BFRs) in the environment increased (Marsh et al., 1999; de Boer et al., 2000). Consequently, it seemed that the brominated compounds that bioaccumulated in marine organisms are BFRs due to the lack of other plausible sources. From this point of view it was thought, that compounds appearing at non-typical retention times in gas chromatograms might be unknown yet unidentified congeners of BFRs. This is not true for a number of brominated compounds we have detected in various samples (e.g. BC-2, BC-11, MHC-1) (Vetter et al., 2001a,b). Two of them (BC-2 and BC-11) were recently identified as naturally produced organohalogens (Vetter et al., 2002b), and the same was expected for several other (previous) unknowns.

In order to receive more information on the structure of brominated compounds, we suggested to monitor additional low-mass fragment ions in GC/ECNI-MS-SIM studies (see Section 2) (Vetter, 2001; Vetter et al., 2002a, 2003). When methane is used as the CI gas, $[Br]^-$ and $[HBr_2]^-$ next to $[Br]^-$ qualifies aromatic organobromines, $[Br_2]^-$ next to $[Br]^-$ qualifies non-aromatic organobromines, and $[BrCl]^-$ next to $[Br]^-$ qualifies mixed halogenated non-aromatics (Fig. 1) (Vetter, 2001; Vetter et al., 2002a). In this presentation we add more knowledge to the current state of the art of potential natural brominated compounds bioaccumulated in the lipids of members of marine food webs. Chlorinated compounds are discussed elsewhere (Vetter et al., 2003).

2. Materials and methods

2.1. Synthesis of BC-2

2.1.1. Synthesis of 2-methoxy-3,5-dibromophenol (Green, 1991)

2,4,6-Tribromoanisole (10 g, 30 mmol) was suspended in 200 ml of dry *n*-pentane and cooled to −20 °C in an Ar atmosphere. Twenty milliliter of *n*-butyllithium (1.6 g, 32 mmol) in *n*-hexane was added over 10 min with vigorous mechanical stirring. This suspension was allowed to warm to −10 °C over 15 min. Upon cooling to −30 °C, neat trimethyl borate 3.2 g (32 mmol) was added all at once. The solution was warmed to 0 °C over 30 min and then cooled −10 °C. A solution of 40% peracetic acid/acetic acid (25 ml) was added over 30 min. Upon completion of the addition, the solution was warmed to 0 °C over 30 min and then cooled to −10 °C, and 25 ml of saturated aqueous $NaHSO_3$ was added dropwise over 30 min. Upon warming to room temperature equal volumes of water and diethylether were added. The organic layer was separated, dried over $MgSO_4$, and evaporated to provide a colorless oil. Yield 7.4 g (87%), b.p. 120 °C (0.24 Torr).

2.1.2. Synthesis of 2,2′,4,4′-tetrabromodiphenyliodonium chloride (III) (Marsh et al., 1999)

A mixture of concentrated sulfuric acid (2.4 ml) and 30% fumic sulfuric acid (4.8 ml) was added to iodine (2.03 g, 8 mmol) under stirring. Then a mixture of concentrated sulfuric acid (0.64 ml), 65% fumic sulfuric acid (0.32 ml), and 100% fumic nitric acid (1.04 ml) was slowly added. The reaction mixture was stirred at 70–80 °C for 1.5 h, at which time yellow crystals of iodyl sulfate precipitated. The mixture was then cooled and 1,3-bromobenzene (10 g, 42 mmol) was slowly added at 10–15 °C. The mixture was stirred at 45 °C for 2 h and then cooled to 0 °C. Water was carefully added in small

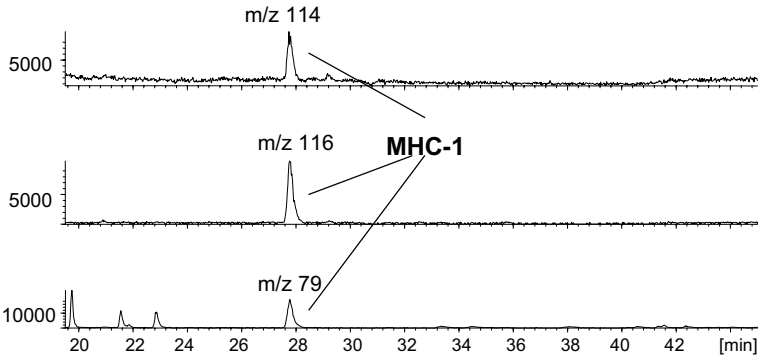

Fig. 1. Identification of MHC-1 in a blubber extract of a monk seal (*Monachus monachus*) from northwest Africa on the basis of fragment ions at *m/z* 114 and 116 (both corresponding with $[BrCl]^-$) and *m/z* 79.

portions at 5–10 °C. Nitrogen oxides were removed by introducing a gentle stream of nitrogen into the reaction solution. The product was collected, dissolved in methanol, and crystallized as the chloride salt by dropwise addition of concentrated hydrochloric acid. Yield 6.64 g (62%).

2.1.3. Synthesis of BC-2

2-Methoxy-3,5-dibromophenol 0.7 g (2.5 mmol) was dissolved in an aqueous solution (20 ml) of sodium hydroxide (0.1 g, 2.5 mmol). The diphenyliodonium salt (2.5 mmol) was added and the mixture refluxed for 1.5 h. The product was extracted from the crude mixture using diethylether (2×30 ml). The organic layer was washed with water and then dried over sodium sulfate. The solution was filtered, the solvent was evaporated, and the product was purified on a silica gel column with *n*-hexane as the mobile phase. Upon re-crystallisation from *n*-hexane and chromatography on 50 g silica gel, a colourless oil was obtained which proved to be neat BC-2.

2.2. GC/ECD and GC/MS parameters

GC/ECD analyses were performed with a Hewlett-Packard 5890 series II gas chromatograph modified by Gerstel (Mülheim, Germany). A T-piece behind the PAS split/splitless injector (splitless time 1.5 min) divided the samples equally onto two capillary columns that ended in ^{63}Ni electron capture detectors (ECDs). The samples were injected automatically (HP 7673 autosampler). The capillary columns CP-Sil 2 and CP-Sil 8/20% C_{18} (both: length 50 m, 0.25 mm i.d., 0.25 μm film thickness) were from Chrompack (Middelburg, The Netherlands). Helium (5.0 quality, Institute of Physics, University of Jena, Germany) was used as carrier gas at a constant flow of 1.3 ml/min, and nitrogen (5.0 quality, Linde, Germany) was used as the ECD make-up gas. Moisture and oxygen filters from Varian-Chrompack were used. The injector and detector temperatures were set at 250 and 300 °C, respectively. The GC oven program was the following: after injection at 60 °C (1.5 min) the temperature was ramped at 40 °C/min to 150 °C (5 min), then ramped at 2 °C/min to 230 °C (0 min), and finally ramped at 5 °C/min to 270 °C (15 min).

A Hewlett-Packard 5890 II plus gas chromatograph interfaced to a 5989B MS Engine was used for electron capture negative ion mass spectrometry (GC/ECNI-MS) and electron impact mass spectrometry (GC/EI-MS). Helium (see above) was used as the carrier gas. Injections into a split/splitless injector (maintained at 250 °C) were performed in the splitless mode (1.5 min). A HP-5 (manufacturer: Hewlett-Packard/Agilent; 95% dimethyl-, 5% diphenylpolysiloxane; equivalent with DB-5 and CP-Sil 8) capillary column (30 m, 0.25 mm i.d., 0.18 μm film thickness) was used for analyses. The GC oven was programmed from 80 °C (hold time 1 min) at 20 °C/min to 180 °C (hold time 2 min), at 2 °C/min to 200 °C (hold time 5 min), and at 20 °C/min to 280 °C (hold time 15 min).

The transfer line, ion source and quadrupole temperatures were set at 280, 150 and 100 °C, respectively. Methane (quality 5.0; Linde, Germany) was used as the CI moderating gas. The system was optimized by manual tuning with perfluorotributylamine (PFTBA). In the total ion current mode (TIC), m/z 50–650 were scanned. In the selected ion monitoring (SIM) mode, the following m/z values (corresponding compositions are given in parentheses) were recorded in parallel after a solvent delay of 20 min: m/z 79 ($[^{79}Br]^-$) and 81 ($[^{81}Br]^-$), m/z 158 ($[^{79}Br^{79}Br]^-$), m/z 160 ($[^{79}Br^{81}Br]^-$), m/z 159 ($[^{1}H^{79}Br^{79}Br]^-$), m/z 161 ($[^{1}H^{79}Br^{81}Br]^-$), m/z 114 ($[^{79}Br^{35}Cl]^-$), m/z 116 ($[^{79}Br^{37}Cl]^-$ and $[^{81}Br^{35}Cl]^-$), as well as m/z 115 $[^{1}H^{79}Br^{35}Cl]^-$ and m/z 117 ($[^{1}H^{79}Br^{37}Cl]^-$ and $[^{1}H^{81}Br^{35}Cl]^-$).

2.3. Sample clean-up

All samples shown in this presentation were cleaned using our validated method consisting of microwave-assisted extraction, gel permeation chromatography, adsorption chromatography on both deactivated and activated silica (Vetter et al., 1998; Weichbrodt et al., 1999, 2000). The internal standard was perdeuterated α-HCH (α-PDHCH).

Screening on planar molecules was performed with 1 g of a 1:1 mixture of Supelclean Envicarb (120–400 mesh) and Celite 545-AW (Supelco, Deisenhofen, Germany). The first fraction was eluted with 50 ml *n*-hexane, and four further fractions were collected with 50 ml toluene, respectively.

3. Results and discussion

The presence of halogenated compounds in top predators demands that the compounds are both lipophilic ($\log K_{ow} > 5$) and persistent. Both criteria are fulfilled by non-polar chlorinated xenobiotica such as PCBs, DDT and other chloropesticides. Phenolic compounds are considered as the most stable group of natural organohalogens (Faulkner, 1980). However, phenolic organohalogens have never been detected in adipose tissue of higher organisms (Letcher et al., 2000). This suggested that natural (halogenated) products are not bioaccumulative.

On the other hand, high application rates of non-polar BFRs (at $\sim 10^5$ tons per year) caused increasing pollution in the marine ecosystem upon release of the compounds into the environment. In particular, brominated diphenyl ethers (BDEs) were repeatedly detected

in wildlife (de Boer et al., 2000). Therefore, analysis of brominated compounds in the environment has become a subject for more and more scientists from all over the world.

The determination of non-polar brominated compounds in environmental samples—irrespective as to whether they are natural or anthropogenic in origin—is different to chlorinated compounds. Sample preparation (extraction and elimination of sample matrix) of both classes can be performed in the same way, but chlorinated and brominated compounds behave differently on silica. Activated silica (or deactivated with small amounts of water) is often used for the separation of aromatic organochlorines (PCBs) from alicyclic chloropesticides (DDT, chlordane, HCH, toxaphene). Usually, chlorinated aromatic compounds are targeted with *n*-hexane in the first fraction, and the brominated analogues have to be eluted with a more polar solvent. In that way they are found in a fraction which also contains the chloropesticides. It seems, however, that this is different in NP-HPLC where the brominated and chlorinated aromatics elute together. The combination of both techniques can thus be used for the selective enrichment of brominated aromatics. This can be necessary since the concentrations of brominated compounds in marine samples are often lower than those of the chlorinated compounds. Therefore, Br-selective GC/MS methods are required while many chlorinated compounds can be determined with GC/ECD. The most sensitive technique for detection of non-polar brominated compounds is GC/ECNI-MS (Crow et al., 1981; Buser, 1986). Chlorinated and brominated compounds alike form abundant fragment ions in the low mass range. However, intense fragment ions at high mass are often missing in the spectra of organobromines, so that the bromide ion is often used for quantification of BFRs (Sellström, 1999; de Boer et al., 2000). This method also allows detection of natural organobromines (Vetter, 2001).

A major drawback in the analysis of natural halogenated compounds is that most of them are not available as reference standards. This also impedes the determination of the environmental relevance of the compounds (e.g. ecotoxicity). For this reason we have started to synthesize some of the natural products. Recently, we have prepared the natural heptachloro compound Q1 (Wu et al., 2002), and in this study we present the synthesis of 4,6-dibromo-2-(2′,4′-dibromo)phenoxyanisole (BC-2) (Fig. 2). BC-2 and six further brominated phenoxyanisoles have been previously synthesized by Francesconi and Ghisalberti (1985) in a four step reaction as intermediate products on the way to achieve brominated phenoxyphenols. To simplify the method we have applied the iodonium reaction suggested by Marsh et al. (1999) for the preparation of individual brominated diphenyl ethers (BDEs). The reaction partner 2,4-dibromo-6-hydroxyanisole was prepared from 2,4,6-tribromoanisole (Fig. 2). The crude product was fractionated on 60 g silica (elution with *n*-hexane) which yielded neat BC-2. The NMR spectrum of the purified oil and the chromatographic and mass spectrometric properties were identical with previous BC-2 isolates (Francesconi and Ghisalberti, 1985; Cameron et al., 2000).

GC/MS analysis of later fractions of the silica fractionation resulted in the detection of a second tetrabromophenoxyanisole ($RRI_{BC\text{-}2}$ on DB-5 column = 0.9485) and a tribromophenoxyanisole ($RRI_{BC\text{-}2}$ = 0.8153). The tetrabromo isomer of BC-2 may be explained by formation of the *p*-hydroxyanisole instead of the *o*-hydroxyanisole shown in Fig. 2. Coupling of this dibromo-*p*-hydroxyanisole with the 2,2′,4′,4′-tetrabromodiphenyl iodonium chloride would result in 2,6-dibromo-4-(2′,4′-dibromo)phenoxyanisole. The by-product which eluted significantly earlier from DB-5 like columns (see RRI, above) has not been identified in environmental samples which would be plausible in the case of the structural suggestion given above since all natural brominated anisoles identified to date possess the anisole group adjacent to the phenoxy group. The EI mass spectra of BC-2 and its isomer (Fig. 3a) were almost identical but the ECNI-MS showed one remarkable difference. The molecular ion at m/z 512 was not

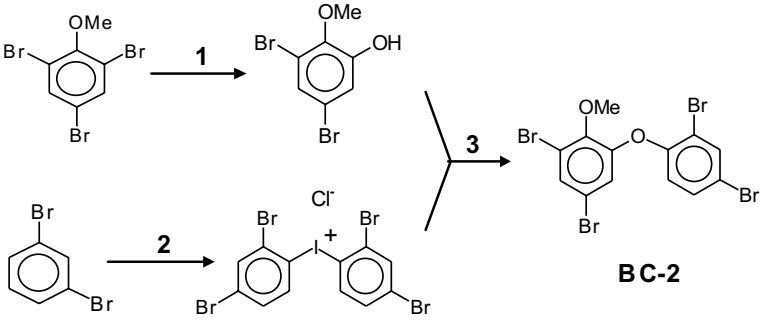

Fig. 2. Scheme of the synthesis of BC-2.

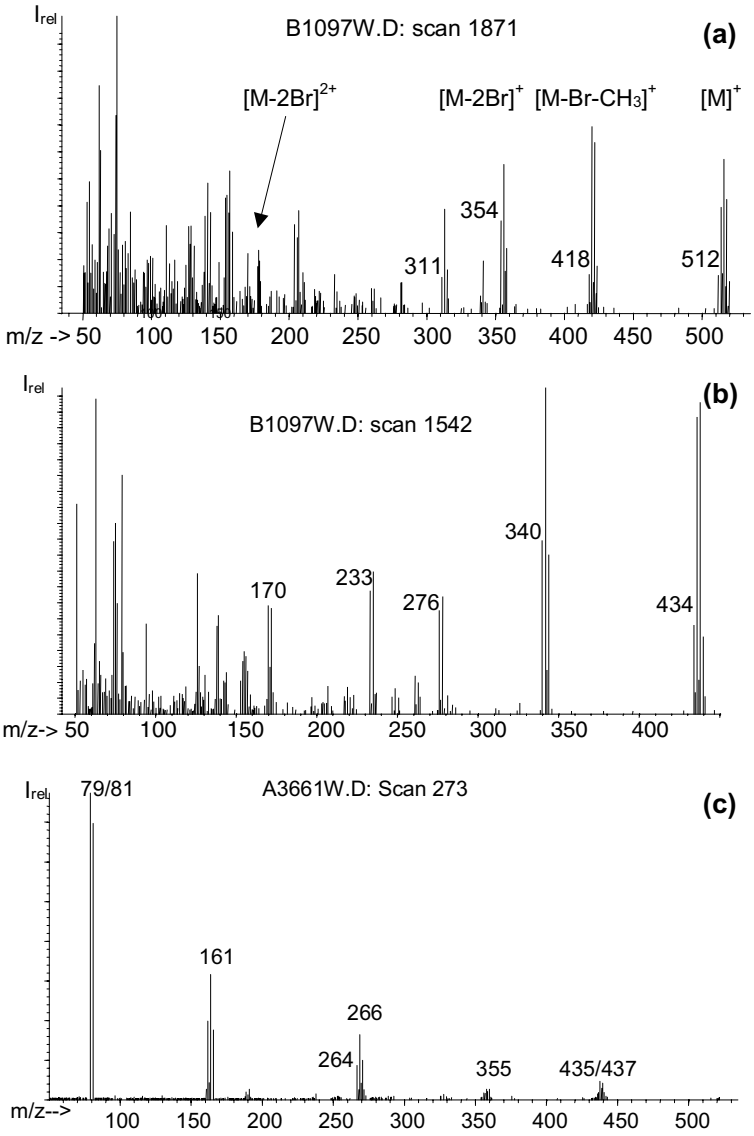

Fig. 3. GC/MS investigation of two byproducts of the BC-2 synthesis. EI-MS of (a) the tetrabromo isomer and (b) the tribromo congener as well as (c) ECNI-MS of the tetrabromoisomer.

observed in the ECNI-MS of the byproduct (Fig. 3c) which is different to BC-2 (Vetter et al., 2001b). The highest visible fragment ion in the ECNI-MS of the byproduct was found at m/z 433 (i.e. $[M–Br]^-$). Interestingly, such an ECNI-MS fragmentation pattern has been reported previously for BC-3 which is not identical with the byproduct and whose structure is still unknown (Vetter, 2001). However, the similarity of the ECNI mass spectra of BC-3 and the BC-2 byproduct supports the earlier prediction that BC-3 is a tetrabromophenoxyanisole as well (Vetter, 2001). BC-3 and the BC-2 isomer can be distinguished by the ECNI-MS fragment ion at m/z 264 (formally $[C_7H_6Br_2O]^-$ or [M-dibromophenoxy+H]$^-$) which was found for BC-2 and its isomer but not in the ECNI-MS of BC-3. This may serve as an indication that BC-3 has a 3:1 distribution of bromine on the phenyl rings. MeO-BDEs which fulfill this criterion were previously identified by natural products chemists (Handayani et al., 1997). A further tetrabromophenoxyanisole detected in the Baltic Sea (Haglund et al., 1997) was identified as 3,5-dibromo-2-(2′, 4′-dibromo)phenoxyanisole (Asplund et al., 1999).

BC-2 and BC-11 have been previously identified in Australian sponges (Cameron et al., 2000). It is curious

that sponges, i.e. species largely without any lipids, should be the producer of highly lipophilic compounds such as BC-2. There is some indication in the literature that it is not the sponges but cyanobacteria living on the sponge (symbiosis) that are responsible for the production of brominated secondary metabolites (Unson et al., 1994). It has been suggested that *Oscillatoria* may be the originate producer of BC-2 (Moore et al., 2002). *Oscillatoria* have reasonably high lipid content which would make this source of production plausible. However, another explanation is suggested here. We submit that sponges might be involved in the production of the brominated phenoxyphenols and that the possible symbiont (cyanobacteria) produces the methylether (anisole) from the phenoxyphenols. This would be a symbiotic synthesis by sponge and bacteria. However, distinguishing the role of the two organisms in the production of brominated phenoxyphenols and other natural products is not simple. For such studies it may be helpful to note that standards of brominated phenoxyphenols can be synthesized from the respective phenoxyanisoles by treatment with BBr_3 in 1,2-dichloroethane (Francesconi and Ghisalberti, 1985). Assuming that two organisms are involved we would expect seasonal variations in the ratio of brominated phenoxyanisoles and brominated phenoxyphenols. A closer study of these ratios may thus provide the key to the understanding of these natural processes. Furthermore, brominated phenoxyanisoles can be regarded as condensation products of brominated phenols and anisoles. In fact, brominated anisoles (and particularly tribromoanisole) have been previously detected in numerous samples such as fish and mussels from the North Sea (Rimkus and Wolf, 1991; Rimkus and Wolf, 2001), birds of prey (Herzke et al., 2001), as well as Antarctic (Wittlinger and Ballschmiter, 1990; Vetter et al., 2002a) and Arctic air (Vetter et al., 2002a). The recent identification of an organism that produces TBA (Flodin and Whitfield, 1999, 2000) supports the fact that some residues of TBA are natural although there are also anthropogenic sources.

Assuming that a two step reaction, i.e. (a) formation of phenols and (b) formation of anisoles is occurring, the precursor of BC-11 may be the brominated methoxyphenoxyphenol shown in Fig. 4a. This tetrabromomethoxyphenoxyphenol may then be transformed into 3,5-dibromo-2-(3′,5′-dibromo,2′-methoxy)phenoxyanisole (BC-11) (Fig. 4b). On the other hand, the tetrabromomethoxyphenoxyphenol may also result in a brominated methoxy dibenzo-*p*-dioxin (Fig. 4c). The molecular weight (m/z 526) of the brominated methoxy dibenzo-*p*-dioxin ($C_{13}H_6Br_4O_3$) is the same as that previously determined for BC-1 (Vetter et al., 2001b). Initially, BC-1 has been described as a brominated phenoxyanisole with an additional "CH_2" unit ($C_{14}H_{10}Br_4O_2$) (Vetter et al., 2001b). To distinguish between these two interpretations we have applied chromatography on charcoal (see Section 2). Using this method, it was shown that BC-1 eluted together with BC-2 and BC-3 into the *n*-hexane fraction. If BC-1 would have been the tetrabromomethoxydibenzo-*p*-dioxin (Fig. 4c), this planar molecule would have been retained on the column. Chromatography of planar molecules from charcoal is displacement, so that planar molecules such as dioxins will only be eluted with Π-electron containing solvents such as toluene (but not *n*-hexane). Another possibility to distinguish between the two compounds is high resolution mass spectrometry. The mass difference of 0.036 u (exact masses of $C_{13}H_6Br_4O_3$ and $C_{14}H_{10}Br_4O_2$ are 525.705087 and 525.741472, respectively) can be distinguished with this technique.

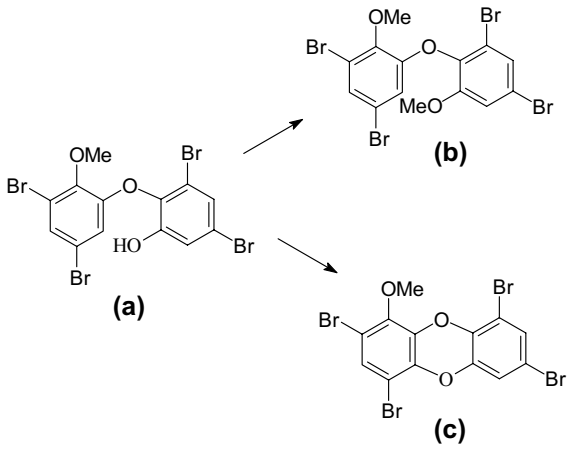

Fig. 4. Possible formation of BC-11 (b) from the tetrabromomethoxyphenoxyphenol (a) and reaction of this to the brominated methoxy dibenzo-*p*-dioxin (c) which has the same molecular mass as BC-1.

The identity of further BCs detected in Australian samples was also partly resolved. BC-10 was identified as the Br_4,Cl_2-dimethyl-2,2′-bipyrrole discovered by Tittlemier et al. (1999) and which was recently synthesized by Gribble et al. (1999). Along with these compounds in Australian samples which have also been detected in samples from other continents, further potential natural products are the unknown tetrabromo compound UBC-1 detected in samples from the Arctic (Vetter, 2001). Another group of bioaccumulative natural products are mixed halogenated compounds.

Recently, we have described a dibromotrichloro monoterpene (MHC-1) in samples from different locations in the world (Vetter et al., 2001a). Two compounds with the molecular formula of MHC-1 ($C_{10}H_{13}Br_2Cl_3$) were previously isolated from the red algae *Plocamium cartilagineum* (Stierle and Sims, 1979; Higgs et al., 1997). It was not possible to verify that MHC-1 is identical with one of the two monoterpenes produced by red algae

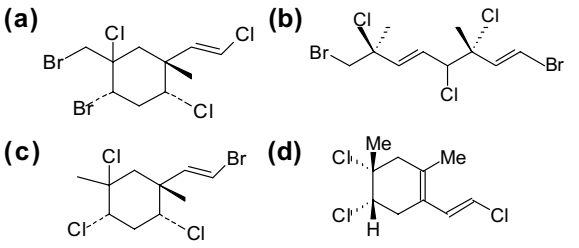

Fig. 5. Structures of MHC-1 related compounds. (a,b) Natural products of *P. cartilagineum* isomeric to MHC-1, (c) telfairine and (d) procomene.

(Fig. 5a,b). However, our identification of MHC-1 in the Antarctic, Europe, Northern Europe, Greenland Sea and other places agrees with the regions where *P. cartilagineum* is sedentary.

It is noteworthy, that one of the two structures are very similar with telfairine (Fig. 5c) and procomeme (Fig. 5d), two known monoterpenes which have been previously isolated and whose chemical properties have been studied (Stierle and Sims, 1979; Higgs et al., 1997). It was found that telfairine is 100% lethal to mosquito larvae at 10 ppm, and that procomene is a fourfold stronger insecticide than the known anthropogenic pesticide lindane (Gribble, 1998). The monoterpene shown in Fig. 5b and four further structurally similar monoterpenes from *P. cartilagineum* were tested with the Ames test for mutagenicity since structurally similar anthropogenic substances such as 1,2-dibromo-3-chloropropane (DBCP) induced stomach cancer in test rats and mice (Leary et al., 1979). The compound shown in Fig. 5b was mutagenic in the Ames test as were the other halogenated monoterpenes. Using the S9-mix, the compound depicted in Fig. 5b was active with strain TA100 but not TA98. Further compounds with structures similar to the aliphatic monoterpene (Fig. 5b) were also reported in the literature (Gribble, 1998). A dibromotrichloro monoterpene isolated from *Portieria hornemannii* has a very pronounced anticancer activity against human cancer cell lines (Fuller et al., 1992; Gribble, 1998). In view of these reports, it seems to be likely that MHC-1 possesses similar biological activity. However, a disadvantage of the mixed halogenated monoterpene products in general and particularly of the compounds with aliphatic backbone is that their synthesis is difficult. Therefore, toxic evaluation of MHC-1 and further mixed halogenated compounds detected in samples (Vetter, 2001; Vetter et al., 2002a) (see also below) without identification of the natural producer and isolation of the compounds from natural organisms, is not possible at this time.

The brominated natural products have properties similar to brominated diphenyl ethers. Therefore, it is an important task to elucidate if the natural and the anthropogenic brominated aromatics may co-elute. Fig. 6 shows the GC/ECNI-MS chromatogram of a sample extract of human milk from a Faeroese woman who frequently consumed whale blubber and meat as well as fish (Abraham et al., 1995). Interestingly, this samples contained a variety of new and known brominated compounds. At short retention time a dibromochloroanisole was identified on the basis of the full scan mass spectrum. Note that the retention time was very similar to TBA, which was not detected in the sample. Next to MHC-1, another mixed halogenated compound (MHC-X) was identified on the basis of the $[BrCl]^-$ fragment ion (Vetter, 2001). At higher retention times, several polybrominated diphenyl ethers were determined (BDE-47, 99, 100, and 154). The most abundant signal originated from the natural product BC-3. The analysis of this sample also demonstrates the interference of BDEs with halogenated natural products on the most

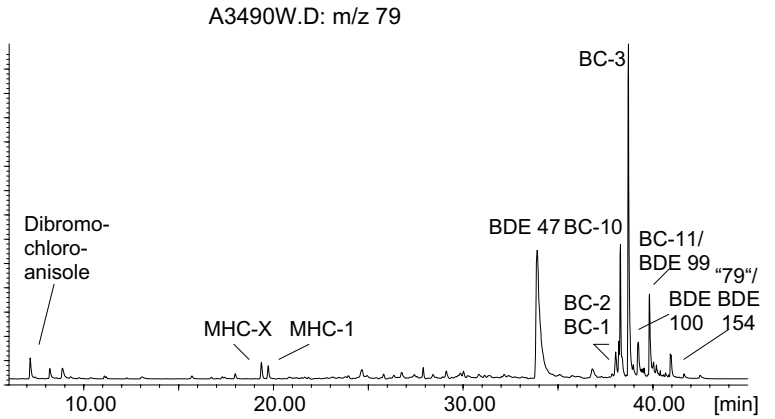

Fig. 6. GC/ECNI-MS SIM chromatogram (m/z 79) showing natural and xenobiotic brominated compounds detected in a sample of human milk from the Faeroe Islands.

frequently used GC stationary phase for the determination of organohalogen compounds. BDE–99 co-eluted with BC-11 and BDE-154 co-eluted with an unknown brominated that did not form low-mass fragment ions other than $[Br]^-$ (“type 79”, Vetter, 2001). Such coelutions may cause false-positive identification of BFRs in wildlife.

Our previous work was mainly restricted to top predators of marine food webs. We have also started screening of samples from lower tropic levels (Fig. 7). The cod sample from the Baltic Sea showed an intense signal for TBA. Next to the natural chlorinated compound Q1 (identified by Q1-selective m/z 386), we also identified BDE-47 and several other compounds (Fig. 7a). The mussel sample from Mexico also showed TBA and Q1 as well as a number of unknown brominated compounds some of which may be tribromo compounds (Fig. 7b).

Different biological activities have been ascribed to natural halogenated products (Faulkner, 1980; Gribble, 1998). However, most of these effects were found for more polar compounds, which are the majority of natural organohalogens found to date. Nevertheless, the bioaccumulative natural organohalogens investigated so far are active against bacteria, viruses and fungi. This partly explains the insecticidal activity of procomeme and telfairine. These chemicals may protect their producers against predators and enemies. Some of the compounds may cause autotoxicity and thus, a natural regulation in population dynamics may be obtained. This distinguishes them from the halogenated xenobiotica which can be produced at any amount and concentration. However, the mode of bioaccumulation seems to be the same for anthropogenic and natural organohalogens. Finally, we need to state that there is a lack of research on the relevance of the bioaccumulation of naturally produced organohalogens.

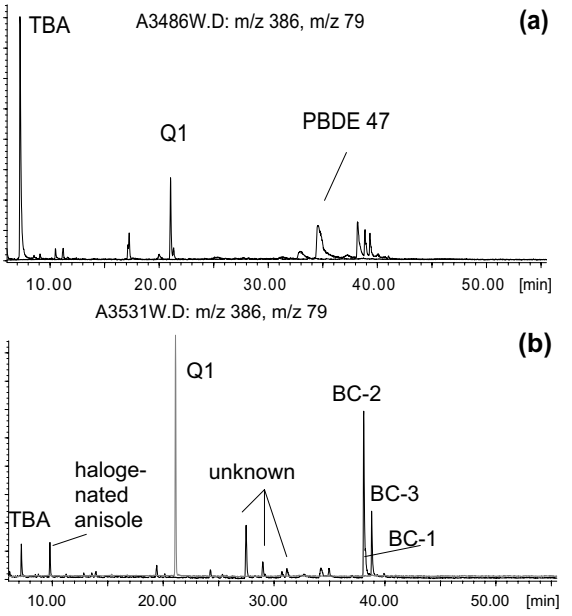

Fig. 7. GC/ECNI-MS SIM chromatogram (m/z 79, 386) of sample extracts of (a) Baltic cod liver and (b) Mexican mussel tissue.

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