POP-LIKE HALOGENATED NATURAL PRODUCTS IN ANTARCTIC SPONGES

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Introduction

Persistent organic pollutants (POPs) are major contaminants of our days. This group of chemicals comprises a number of halogenated compounds used as pesticides (DDT, lindane, chlordane, toxaphene and others) as well as industrial chemicals (PCBs, PCNs, CPs, and brominated flame-retardants). Although the list of known POPs including isomers and metabolites is long, there are frequent reports on the detection of unknown organohalogen compounds in the literature. Recent work demonstrated that some of these unknown peaks in gas chromatograms originate from halogenated natural products (HNPs) [1-5]. Sometimes, HNPs have been found at remarkably high concentrations in marine birds, mammals and fish [1,3,4,6]. Due to the structural similarities with anthropogenic POPs, these substances may possess a potential risk for wildlife and man. HNPs are known to be produced with an overwhelming variety by marine organisms such as algae, sponges, microorganisms and others [7-10].

In this study we have screened different species of Antarctic sponges on the occurrence of halogenated compounds which may be of environmental concern. Thus, we were only interested in lipophilic and persistent HNPs. Following that, we applied our standard sample clean-up procedure for the analysis of nonpolar POPs [11-12]. Two steps on deactivated and activated silica yielded compounds with similar polarity as PCBs, chloropesticides and brominated analogues in the sample extracts. Additionally, all samples were treated with concentrated sulphuric acid in order to eliminate labile (non-persistent) HNPs.

Material and Methods

Samples. Samples of sponges were collected in January 2001 on King George Island (62°14`S,58°40`W) at water depths of ~10 m. Voucher samples were used for taxonomic evaluation. The samples were assigned to *Kirkpatrickia variolosa*, *Artemisinaf. apollinis*, *Calcarea/Clathrinida* sp., *Phorbas glaberrima*, and one was tentatively identified as *Halichondridae* sp.

Sample clean-up. Samples were transported frozen to Germany. Portions of 2-8 g were air-dried for 72 h and then cleaned by means of combined open-vessel microwave assisted-extraction and gel permeation-chromatography using ethyl acetate/cyclohexane (1:1, v/v) as the solvent [11]. The system was equipped with a water trap[11] which allowed estimation of the water remaining in the samples after air-drying. The organohalogen fraction collected by GPC was concentrated and the

solvent was exchanged with isooctane (1-2 mL). The sample was placed on a 1 cm internal diameter glass column containing 3 g deactivated silica (30 weight-% of water added to activated silica). The halogenated compounds were eluted with 60 mL *n*-hexane. The extract was concentrated to ~10 mL, and the same volume of sulphuric acid was added. After >1 day, the acidic fraction was discarded, and the remainder washed with water. The n-hexane phase was concentrated (~ 1 mL) and then placed on a 1 cm internal diameter glass column partially filled with 8 g activated silica (>16 h at 130 °C) and topped with sodium sulphate. A first fraction was collected with 48 mL *n*-hexane and a second with 50 mL *n*-hexane/ethyl acetate (9:1, v/v). For some samples, a third fraction was collected with 50 mL ethyl acetate. The fractions were concentrated to 2 mL before GC/MS analysis.

Analyses. GC/ECD and GC/EI-MS, GC/PCI-MS, and GC/ECNI-MS investigations in the full scan mode were performed as recently described in detail [4,6,12]. In the GC/ECNI-MS mode the following SIM masses were recorded after a solvent delay of 6 or 8 min: m/z 35 and 37 ($[Cl]^-$), m/z 79 and 81 ($[Br]^-$), m/z 114 and 116 ($[BrCl]^-$), m/z 158 and 160 ($[Br_2]^-$), m/z 159 and 161 $[HBr_2]^-$), and m/z 386 and 388 ($[M]^-$ of Q1).

Results and Discussion

Samples were screened with the help of GC/ECNI-MS and halogen-specific m/z values (see above). Brominated components were identified by the bromide ion and the correct isotope ratio of m/z 79 and m/z 81. To receive information on chlorinated compounds, we monitored the chloride ion (m/z 35 and 37) which is common for organochlorines. Further m/z values were used for validation (see above). The concentrations and peak profiles in different species was subject to significant variations. Dependent on the compound and species, it was found that the area of m/z 79 in ECNI-MS varied by 4-5 orders in magnitude. Estimated highest concentrations were several ppm based on air-dried weight and lowest were in the sub-ppb range. Different species contained at least ten and up to 100 and more organobromine compounds. For instance the sample of *Halichondridae* sp. (**Figure 1**) contained 47 brominated compounds, according to the correct isotope ratio of m/z 79 and m/z 81.

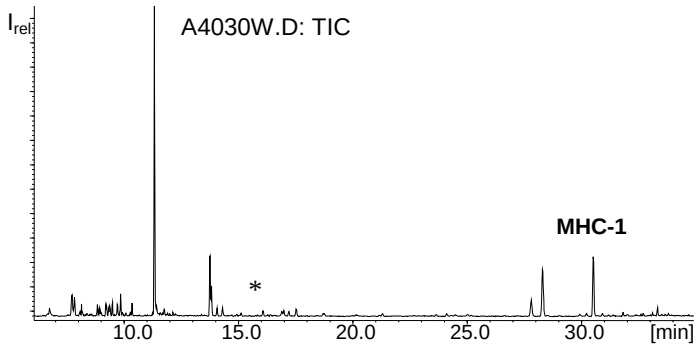

Figure 1: GC/ECNI-MS-SIM chromatograms (m/z 79) of samples of Halichondridae sp. *Asterisks denote retention time of α-HCH (15.7 min*

However, many brominated compounds were detected in different species, respectively. We also screened the samples for man-made POPs and found very low concentrations of α-HCH, lindane, and p,p´-DDE in the most species investigated as a result of long-range transport. Note that King George Island as an area with one of the lowest POP concentrations known to date [12]. Many of the brominated compounds eluted prior to α-HCH from the DB-5-like Ultra-2 column. This indicated relative low molecular weights of the compounds although we observed that non-polar brominated compounds that elute in the range of chlorinated compounds with similar backbone are roughly heavier at 25% by weight. E. g. 2,4,6-tribromoanisole (342 u) and HCH isomers (288 u) as well as BDE 47 (482 u) and PCB 180 (392 u) have similar retention times. Furthermore, brominated compounds possessed 2-3 halogens less than co-eluting organochlorines. Therefore, the brominated compounds eluting prior to α-HCH were expected to have two to four bromine substitutents. We also paid attention to the detection of mixed halogenated compounds although they were thought not to be present in sponges[8].

After a first screening by GC/ECNI-MS in the SIM mode, the retention times of brominated compounds were listed and full scan investigations using GC/ECNI-MS, GC/PCI-MS, and GC/EI-MS followed. In sponges, the lowest detected molecular masses were m/z 206 and m/z 210. The later was tentatively identified as a dibromobutadiene isomer. Furthermore, it was observed that different homolog groups were found. E. g. we detected the potential butadienes with two or three bromine substituents. In addition, 2,4,6-tribromoanisole (342 u) and dibromo homologues (264 u) were detected as well. Halogenated anisoles have been previously identified in Antarctic air [13,14].

In several sponge samples, an intense peak arose from a compound whose mass spectra are shown in **Figure 2**.

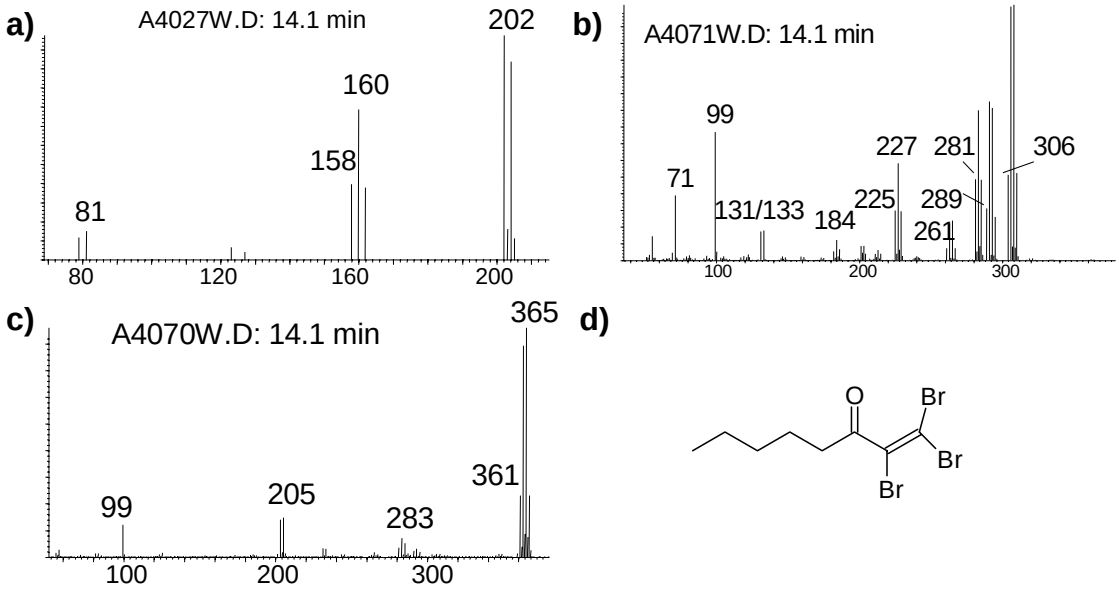

Figure 2: Mass spectra and structure of an important brominated compound in sponges. (a) GC/ECNI-MS (b) GC/MCI-MS, (c) GC/EI-MS, and (d) structure of the identified 1,1,2-tribromooct-1-en-3-one.

The major peak in the ECNI-MS (**Figure 2a**) was not the molecular ion since it only contained 1 Br whereas m/z 158 indicated the presence of at least 2 Br. For the same reason m/z 306 with in the EI-MS (**Figure 2b**) was not the molecular ion since m/z 281 contained one bromo substituent less. Finally, the molecular weight was identified by the quasi molecular ion $[M+H]^+$ in the PCI-MS (**Figure 2c**). Interpretation of the EI/MS (m/z 261: C_2Br_3; m/z 289: C_3Br_3O; m/z 71: C_5H_{11}; m/z 99: $C_7H_{11}O$) resulted in the identification of 1,1,2-tribromooct-1-en-3-one (**Figure 2d**) previously described by Cueto et al.[15].

A surprising result was found in the sample of *Halichondridae* sp. In addition to various brominated compounds at low retention time (i. e. shorter than α-HCH), this species did also contain several intense compounds in the retention range between oxychlordane and p,p´-DDE (**Figure 1**). The latest eluting major compound was identified as MHC-1, a dibromotrichloro-monoterpene recently identified in fish and seals (**Figure 3**) [3]. The producer of MHC-1 has not been identified yet, but isomers of MHC-1 were previously reported in seaweed. Usually, mixed halogenated compounds are not found in sponges[8].

Next to MHC-1 further mixed-halogenated compounds were detected in other sponge species. While it was not possible to identify all compounds, further distinguishing was obtained by fractionating on silica. Some very non-polar HNPs were found in the fraction that usually contains PCBs while others were found in the more polar fraction which usually contains chloropesticides and BDEs. Only very few compounds eluted in the more polar fraction 3 (see methods).

Figure 3a shows bromine-selective GC/ECNI-MS ion chromatogram of an Arctic hooded seal (*Cystophora cristata*). This sample contained three brominated compounds, whereof the most abundant was previously identified as MHC-1. In addition the second most abundant brominated compound in the sample was identical with a compound identified in Antarctic sponge (**Figure 3b and c**). The mass spectrum of the compound labelled SBC was dominated by a tribromo isotope pattern at m/z 328. Subtraction of the contribution of bromine (237 u) leaves 91 u for carbon, hydrogen and other hetero atoms. Therefore, SBC may have seven carbons or less. The detection of SBC in both Arctic and Antarctic samples underlines the global relevance of the compound. However, it is not clear whether or not SBC in the Arctic is also produced by *Phorbas glaberrima* or from another species.

Although the huge variety of the brominated compounds might be surprising, many brominated compounds have been detected previously in Antarctic air [13,14]. Due to the temperature gradient it is not likely that these compounds will be transported north of the Antarctic convergence. However, due to more and more intense fishing in this remote area these compounds possess a risk to reach the consumer if they are not metabolized by higher organisms. At present, we do not know anything about the persistence of these HNPs in higher organisms. The lack of any of the brominated compounds in Weddell seals from the same region[12] deserves to be noted. This indicates that there is no foodweb transport into marine mammals or that the pelagic compounds (~10 m) are not getting contact with food of the Weddell seal which is known to hunt commonly at depths as low as ~300 m. Furthermore, at least some of the lower concentrated compound could derive from uptake of the sponge by filtration of water. On the other hand, the residue pattern in the sponges was very different. Further studies are required to clarify these open questions. Irrespectively, our results demonstrate that at least some of the various brominated compounds detected in Antarctic sponges can be found in the blubber of marine mammals.

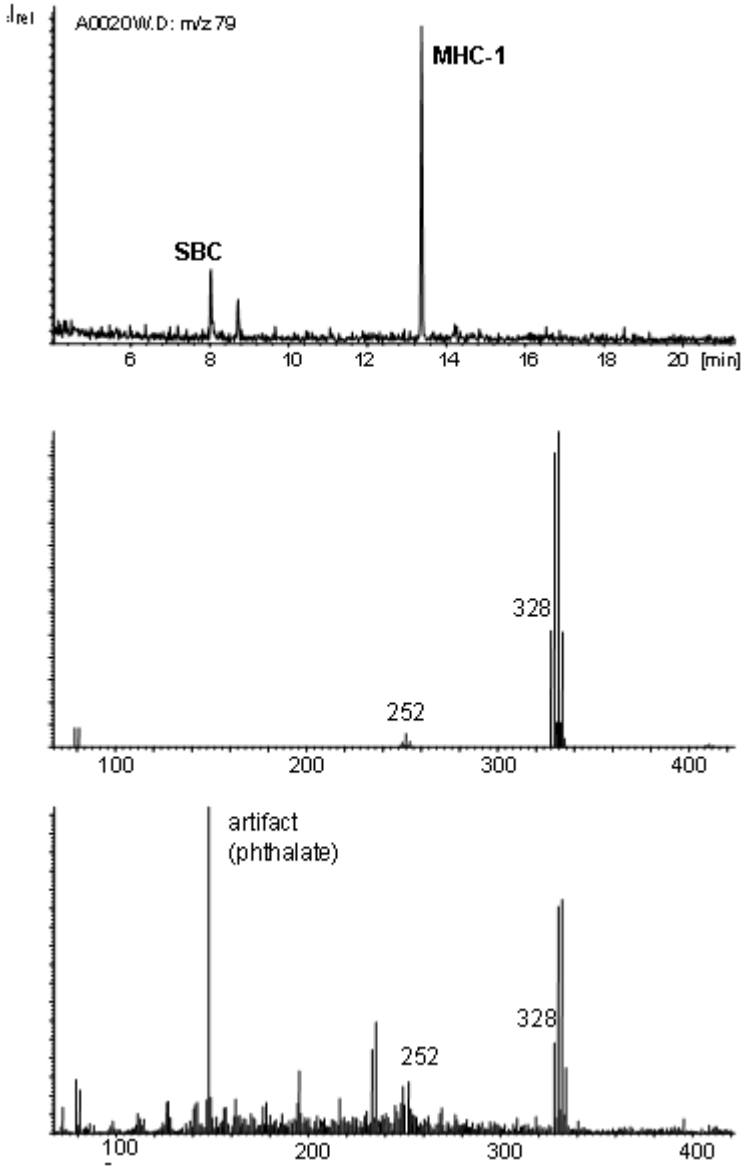

Figure 3: GC/ECNI-MS investigation of a sponge brominated compound (SBC) in Arctic hooded seal (Cystophory cristata) from Jan Mayen, and Antarctic sponge (Phorbas glaberrima)
a) bromine-selective m/z 79 extracted from the full scan chromatogram of the blubber extract of a hooded seal from the Arctic (SBC and the previously described MHC-1 are labelled). Note that different GC conditions were used for the analysis of the seal sample.
(b) mass spectrum of a brominated compound in the sponge
(c) mass spectrum of the same compound in the hooded seal

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