Identification of a C₉H₃N₂Br₆CI compound in dolphin: A novel bioaccumulated halogenated bipyrrole?

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Introduction

Over the past decade, two different types of halogenated bipyrroles have been found to bioaccumulate in marine food webs. A family of mixed halogenated 1,1'-dimethyl-2,2'-bipyrroles (DMBPs) have been observed by Tittlemier *et al.*, and a heptachlorinated 1'-methyl-1,2'-bipyrrole (MBP) referred to as Q1 has been reported by Vetter *et al.* (Figure 1).¹⁻³ Although there is no known natural source of these compounds, they have been assumed to be marine natural products. One DMBP has been shown to have at least in part a natural origin from analysis of its natural abundance radiocarbon content, and a structurally similar hexabrominated DMBP is a known marine bacterial metabolite.^{4,5} The heptachlorinated nature and absence of any bromines on Q1 makes it very unusual for a marine natural product. Even though chloride is 300 times more abundant in seawater than bromide, multi-halogenated marine natural products generally contain some bromine because the oxidation potential of bromide is 0.3 V lower than that of chloride.⁶ In the case of the DMBPs observed by Tittlemier, the most abundant congener contains two chlorine and four bromine atoms.¹ While surveying for halogenated organic compounds (HOCs) present in a variety of marine mammals that have stranded in the northeastern United States, we recently observed a number of unidentified halogenated compounds (UHCs) in several dolphin species. Here we identify one of the UHCs as a chloro hexabromo 1'-methyl 1,2'-bipyrrole (MBP-Br₆Cl; Figure 1), which has the same carbon skeleton as Q1.

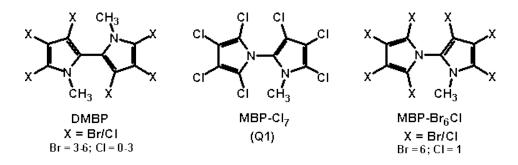


Figure 1. Bioaccumulated halogenated bipyrroles.

Materials and Methods

The *Delphinus delphis* blubber was provided by the Cape Cod Stranding Network, who found the animal fatally stranded in Westpoint, MA. The blubber was homogenized in a blender with a 1:1 solution of *n*-hexane: dichloromethane. The lipids were filtered and dried over anhydrous sodium sulfate. The HOCs were collected from 25 g of lipid by gel permeation chromatography (GPC), using Bio-beads SX-8 (BioRad Laboratories) as the stationary phase and 40% *n*-hexane in dichloromethane as the eluent. The HOC extract was separated using a column packed with silica gel (0.8 g) and aluminum oxide (0.5 g), eluting with *n*-hexane. Polychlorinated biphenyls (PCBs) were eluted in the first 5 mL. The second 5 mL fraction contained the UHCs and was analyzed by high resolution gas chromatography mass spectrometry (HR-GCMS). The HR-GCMS experiments were performed on a JEOL MSroute JMS-600H double focusing high resolution magnetic sector mass spectrometer, coupled with an Agilent 6890N network gas chromatograph, fitted with a Varian VF-5ms column (25m, 0.25 mm i.d., 0.25 µm film thickness). Instrument resolution was set to 3000 at 10% peak height. Electron impact ionization (EI) and electron capture negative ionization (ECNI) GCMS experiments were conducted on an Aglient 6890N series GC system installed with a J & W Scientific DB-XLB column (60 m, 0.25 mm i.d., 0.25 µm film thickness) connected to a 5973 network mass

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selective detector (70 eV ionization energy). ECNI experiments employed methane as a reagent gas. The concentration of MBP-Br₆Cl was estimated relative to a pure standard hexachloro 1,1'-dimethyl-2,2'-bipyrrole, by GC-FID, using HP 5890 Series II GC, with a CP-Sil 5CB column (60 m, 0.25 mm i.d., 0.25 μ m film thickness). Comparable relative response factors by FID have been observed for compounds with a similar number of carbons and a high degree of halogenation hence the standard used should adequately represent the response of MBP-Br₆Cl.^{7,8}

Results and Discussion

The HOC fraction isolated from *D. delphis* was analyzed by low resolution GCMS with both EI and ECNI. In addition to observing many PCBs and pesticides (and their metabolites), several halogenated compounds whose spectra did not match any reported bioaccumulated HOCs were observed (Figure 2). The most abundant of these UHCs observed by GCMS had an isotopic cluster resembling a compound containing one chlorine and six bromine atoms (Figure 3) at 647 Da. This was confirmed as the molecular ion by GCMS-ECNI, for which no ions heavier than those in this ion cluster were seen. The exact molecular formula was determined by HR-GCMS. The measured accurate mass of the molecular ion was compared to masses calculated for species with the following isotopic restrictions: H (0-20), C (7-14), N (0-3), Cl (0-5), Br (0-7), O (0-4), S (0-2). The elemental composition with the smallest difference between the measured and calculated masses, also containing an appropriate number of bromine and chlorine atoms to explain the observed isotopic pattern, was deemed the most probable molecular formula. According to these criteria the most abundant UHC was identified as $C_9H_3N_2Br_6CI$. Table 1 lists the measured and calculated masses for the molecular ion and the [M-Br] ion, which are within 2 ppm.

A literature search revealed no compounds with the elemental composition $C_9H_3N_2Br_6CI$. Clues about the molecular structure were revealed by low resolution MS, which shows fragmentation by loss of Br[•], Cl[•] and CH₃[•] (Figure 1). This information, in combination with the similarity of the elemental composition to that of Q1 and the propensity of both compounds to accumulate in marine mammal blubber, lead us to suspect that $C_9H_3N_2Br_6CI$ also contains a 1'-methyl 1,2'-bipyrrole (MBP) skeleton. There are five possible isomers of MBP-Br₆CI, but only one of these was observed in the *D. delphis* tissue examined. We do not currently have sufficient information to identify the location of the chlorine atom. A more thorough examination by HR-GCMS, isolation of sufficient quantities for single crystal X-ray analysis and/or synthesis of authentic standards should help in identification of the specific isomer. The concentration of MBP-Br₆CI in *D. delphis* was estimated to be 1.8 µg/ g of lipid, using GC-FID. A cursory investigation of a selection of marine mammals from the Northeastern United States indicated the concentration MBP-Br₆CI is almost an order of magnitude higher in the three species of dolphin species studied (*Lagenorhynchus actutus* and *Tursiops truncatus* in addition to *D. delphis*) than in the other five animals examined, including two

species of whale (*Balaenoptera physalus* and *Delphinapterus leucas*), two species of seal (*Phoca vitulina* and *Halichoerus grypus*) and one porpoise (*Phocoena phocoena*). In most cases the concentration of PCB-153 was 4 to 15 times that of MBP-Br₆Cl.

High resolution GCMS of another UHC observed in *D. delphis* is consistent with $C_9H_3N_2Br_7$ and is likely the fully brominated analogue of Q1. These results suggest there may be a family of MBPs, with identical carbon skeletons but differing in their halogenation patterns, akin to the DMBPs reported by Tittlemier *et al.*¹

Table 1. Measured and calculated masses for the molecular ion and [M-Br] ion of MBP-Br₆Cl, determined by HR-GCMS.

lon	Measured mass (Da)	Calculated mass (Da)	Error (ppm)
C ₉ H ₃ N ₂ Br ₆ Cl	647.5093	647.5085	1.3
C ₉ H ₃ N ₂ Br ₅ Cl	574.5747	574.5841	-1.7

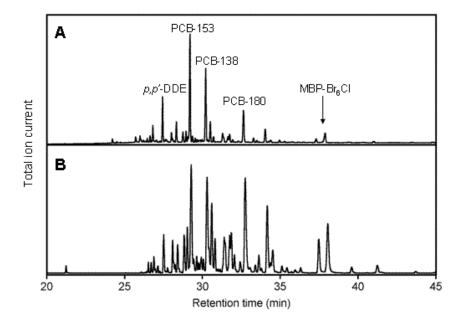


Figure 2. GCMS chromatograms of the HOCs extracted from *D. delphis*, using A) EI and B) ENCI as ionization sources.

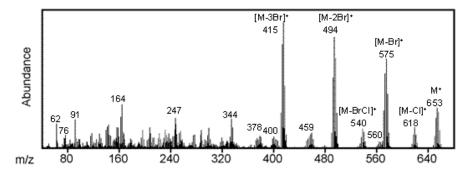


Figure 3. GCMS-EI spectrum for MBP-Br₆CI.

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