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Identification of a Novel $C_{10}H_6N_2Br_4Cl_2$ Heterocyclic Compound in Seabird Eggs. A Bioaccumulating Marine Natural Product?

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A novel brominated and chlorinated compound, $C_{10}H_6N_2Br_4Cl_2$, bioaccumulating in seabird eggs was identified and characterized by low- and high-resolution electron impact ionization (EI), electron capture negative ionization (ECNI), and ammonia positive chemical ionization (PCI) mass spectrometry. This compound is the major congener of a series of four hexahalogenated species. The major congener was determined in egg samples from Leach's storm-petrel, rhinoceros auklet, glaucous-winged gull, and black-footed albatross from the Pacific coast area; Leach's storm-petrel, Atlantic puffin, and herring gull from the Atlantic coast; and herring gull from the Great Lakes using GC-ECNI-MS. The concentrations of $C_{10}H_6N_2Br_4Cl_2$ in the Pacific Ocean samples ranged from 1.8 to 140 ng/g (wet weight), and were significantly higher than the Atlantic Ocean samples ($p = 0.037$). The Pacific Ocean samples contained levels of $C_{10}H_6N_2Br_4Cl_2$ approximately 1.5−2.5 times higher than in the Atlantic Ocean samples of the same or ecologically similar species. The compound was not detected in any of the samples from the Great Lakes. The Pacific Ocean offshore surface feeders had the highest concentrations (34−140 ng/g) when compared to the other samples (0.61−5.6 ng/g). Its strictly marine occurrence and relatively high nitrogen content indicate that $C_{10}H_6N_2Br_4Cl_2$ probably is a marine natural product, found at highest concentrations in the Pacific Ocean surface feeding birds. A possible structure of $C_{10}H_6N_2Br_4Cl_2$ is 1,1′-dimethyl-tetrabromodichloro-2,2′-bipyrrole.

Introduction

The known occurrences of mixed brominated and chlorinated compounds fall into two general areas: incineration byproducts and marine natural products. Mixed halogenated dibenzodioxins/furans (*1*) and phenols (*2*) have already been detected and identified as incineration byproducts. These compounds are formed in high-temperature environments, such as combustion engines and municipal waste incinerators, that contain traces of aromatic and halogenated species (*1*). Incineration byproducts are presumed to be formed via free radical halogenation of the aromatic compounds.

Marine natural products are the second source of mixed brominated and chlorinated organic species. There is a wide variety of such compounds, including polyhalogenated monoterpenes (*3*, *4*), phenols (*5*), diphenyl ethers (*6*), pyrroles, and indoles (*7*). Sources of these compounds appear to be limited to sessile or slow-moving organisms with limited physical defenses. The principal producers are certain types of red alga, but sponges, tunicates, marine worms, and some microbes have also been shown to manufacture mixed brominated and chlorinated compounds (*6*). It is widely believed that the organohalogens act as a chemical defense. The suggested mechanism of production involves haloperoxidases, enzymes which catalyze the oxidation of halide ions by hydrogen peroxide (*8*).

Even though nearly 2400 naturally produced organohalogens have already been identified (*6*), natural sources are often ignored. A 1992 survey on naturally produced organohalogens (*7*) drew on two examples to illustrate this observation. The first involves a report from the Science Advisory Board to the International Joint Commission on the Great Lakes which stated: "There is something non-biological about halogenated organics (excluding iodinated compounds)..." (*9*). The other example is taken from an article in *Science* which contained the quote "...some types of synthetic compounds, including halogenated hydrocarbons such as PCB, are not found in nature." (*10*).

Knowledge of natural sources of organohalogens is essential when regulations concerning anthropogenic organohalogens are being drafted since many organohalogens have both natural and anthropogenic sources. For example, methyl bromide has a large number of natural sources including giant kelp (*11*), Antarctic ice algae (*12*), and marine phytoplankton (*13*). It is also industrially produced for use as a soil fumigant.

In 1988, relatively high levels of an unknown halogenated compound (UHC) were detected in Leach's storm-petrel eggs from both the Pacific and Atlantic coasts (*14*). It appeared to be pentabrominated, but the exact halogen content was not determined. In this study, the novel UHC was characterized using various GC-MS techniques, including low and high-resolution electron impact ionization (EI), electron capture negative ionization (ECNI), and positive chemical ionization (PCI). The more energetic ionization technique of EI provides information on the fragmentation of the compound, while the less energetic ionization techniques of ECNI and PCI provide information on the identity of the molecular ion. The high-resolution mass spectrometry determines the specific elemental composition of the ions. The distribution of the novel compound was determined in bird eggs from the Great Lakes, Atlantic, and Pacific areas and compared to that of a major bioaccumulating PCB, CB-153.

Experimental Section

Isolation of UHC. Bald eagle (*Haliaeetus leucocephalus*) liver samples from Port Hardy, BC were used as a source because of their relatively high levels of UHC. Two 12 g samples of homogenized liver were each combined with 145 g of activated Na_2SO_4 and mixed until free-flowing mixtures were

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obtained. The mixtures were extracted with dichloromethane/hexane (1:1) for 17 h on a Soxhlet extraction apparatus. The extracts were reduced to approximately 5 mL and filtered using precleaned 0.2 μm PTFE disposable filters and plastic syringes.

Lipids were removed from both samples according to the method described by Letcher et al. (*15*). After lipid removal, the samples were reduced to approximately 1 mL and separated on a Florisil column into three fractions. The procedure outlined by Norstrom et al. (*16*) was used, with the following changes: F1 36 mL hexane, F2 36 mL dichloromethane/hexane (85%/15%), and F3 56 mL dichloromethane/hexane (1:1). The F2 fractions from both samples were combined and reduced to a final volume of 390 μL.

Identification of UHC. The molecular formula of UHC was determined using both low- and high-resolution mass spectrometry. The initial mass spectrometry experiments were performed at unit resolution on a Hewlett-Packard 5987B mass spectrometer in the electron impact ionization mode (electron energy 70 eV). The mass spectrometer was linked via a 5988 GC/MS direct interface to a 5890 Series II GC equipped with a 30 m × 0.25 mm i.d. DB-5MS column with a film thickness of 0.25 μm (J&W Scientific). The initial temperature and hold time was 85 °C for 3.00 min, first ramp 10 °C/min to 180 °C, second ramp 5 °C/min to 300 °C, injector temperature 250 °C, interface temperature 280 °C, and source temperature 190 °C. Splitless injection mode was used, with volumes of 2.0 μL injected by a Hewlett-Packard 7673 automatic injector.

Low-resolution ECNI and PCI mass spectra were also obtained on the Hewlett-Packard 5987B mass spectrometer to help in the identification of the unknown compound. These spectra were run under the same GC and interface temperature conditions used above. The ECNI spectrum was obtained using CH_4 (99.97%) at a pressure of 1.0 Torr measured at the source inlet and a source temperature of 175 °C. The PCI spectrum was obtained at a source temperature of 150 °C and electron energy of 100 eV using NH_3 (99.999%, 0.8 Torr) as a reagent gas. The ECNI and PCI spectra were full scans, from 75 to 650 and 200 to 750 Da, respectively.

The high-resolution mass spectrometry was done at resolutions of 10 000 and 20 000 using a VG AutoSpec double-focusing mass spectrometer in full scan electron impact ionization mode (electron energy 70 eV). The mass spectrometer was linked to a Hewlett-Packard 5890 Series II GC fitted with 30 m × 0.25 mm i.d. DB5-MS column (J & W Scientific). The GC conditions used were as follows: initial temperature 100 °C held for 3 min, initial ramp 20 °C/min to 180 °C, and final ramp 5 °C/min to 300 °C. The injector, interface, and source were all kept at a temperature of 280 °C. Volumes of 1.0 μL were injected in the splitless mode by a CTC Analytics A200S automatic injector.

Quantification of UHC in Eagle Liver Sample. The concentration of UHC in the combined F2 and F3 from the bald eagle liver was determined using gas chromatography with flame ionization detection (FID). Determination of the molecular formula by mass spectrometry indicated that the compound contained 10 carbon atoms. Yieru et al. (*17*) demonstrated that carbon weight response factors for a variety of compounds are constant even though FID responses are affected by compound structures (*18*). As a precaution, a variety of compounds was used to ensure adequate representation of all of the structural elements that could be present in the unknown compound. The following chemicals were used as standards in the quantification: nicotine, hexachlorobenzene, heptachlor epoxide, pyrene, 2,2′,4,4′,5,5′-hexachlorobiphenyl, 2,3,3,4′,5,5′,6-heptachlorobiphenyl, mirex, t-chlordane, 2-(*p*-tolyl)-pyridine, α,α,α-tribromoquinaldine, 1,3-dibromoadamantane, and 2,3,3′,4,4′,5,6-heptabromodiphenyl ether.

The compounds were analyzed with a Hewlett-Packard 5890 GC with FID detection under the following conditions: detector temperature 270 °C, initial oven temperature 85 °C held for 3.00 min, initial ramp of 20 °C/min to 200 °C, final ramp of 5 °C/min to 300 °C, and held for 3.00 min. A volume of 0.5 μL of each solution was injected onto a 30 m × 0.25 mm i.d. DB-5MS column (0.25 μm film thickness, J&W Scientific) using automated cool on-column injection to avoid discrimination against the higher molecular weight compounds. All solutions were injected in triplicate. Average carbon weight response factors (*19*) were calculated for each of the 12 compounds. After determination of its carbon content by the mass spectrometry experiments, the carbon concentration of the UHC in the eagle liver extract was determined using the mean of the average carbon weight response factor for each of the 12 compounds. The UHC concentration was then determined from a knowledge of its molecular formula.

Sample Analysis. Seabird and gull egg samples were obtained from the National Wildlife Research Centre Specimen Bank. The samples (Figure 1) represent offshore surface feeders [Leach's storm petrel (*Oceanodroma leucorhoa*) and Black-footed albatross (*Diomeda nigripes*)], offshore subsurface feeders [Rhinoceros auklet (*Cerorhinca monocerata*) from the Pacific and Atlantic puffin (*Fratercula arctica*) from the Atlantic], and inshore omnivores [Glaucous-winged gulls (*Larus glaucescens*) from the Pacific and Herring gulls (*Larus argentatus*) from the Atlantic and Great Lakes]. The Leach's storm petrel was classified as a surface feeder even though it feeds on vertically migrant fish and crustaceans because it also consumes fish, crustaceans, and soft-bodied animals that inhabit the surface layer (*20*). Accurately weighed amounts of homogenized egg contents (around 5 g) were mixed with a 10-fold amount of activated Na_2SO_4. The mixtures were then spiked with an internal standard (^{13}C-labeled heptachlor epoxide, 20 μL, 100 ng/μL). After 45 min the mixtures were wet-packed with dichloromethane/hexane (1:1) into a glass column (2 cm i.d.) and extracted with 220 mL of dichloromethane/hexane (1:1). After extraction, the lipid content of the samples was determined, and the lipid was then removed by GPC (*15*). The GPC fractions were reduced to approximately 1 mL, and the organohalogens were fractionated on a Florisil column following the same procedure as for the bald eagle liver. Fractions F2 and F3 were combined and reduced to exactly 2.00 mL. Fraction F1 was also reduced to a final volume of 2.00 mL. Four method blanks containing no sample were used. One blank was carried through all of the steps of the sample preparation and analysis with each set of samples run.

The F1 fractions were analyzed for CB-153 using a method similar to the one outlined by Norstrom et al. (*16*). The combined F2 and F3 fractions were analyzed for UHC using gas chromatography electron capture negative ionization mass spectrometry (GC-ECNI/MS) in the single ion monitoring mode. The analyses were performed on a Hewlett-Packard 5987B mass spectrometer connected to a 5890 Series II GC. The GC was equipped with a 30 m × 0.25 mm i.d. DB-5MS column with a film thickness of 0.25 μm (J&W Scientific). The temperature program was as follows: injector temperature 250 °C, initial temperature and hold time 85 °C for 3.00 min, first ramp 10 °C/min to 180 °C, second ramp 5 °C/min to 300 °C. Splitless injection mode was used, with an injection volume of 2.0 μL. The mass spectrometer was operated in the negative chemical ionization mode with CH_4 (99.97%, 1.0 Torr) as the moderating gas, an interface temperature of 280 °C, and a source temperature of 190 °C. Previous to injection, 50.0 μL of each sample and standard were spiked with 1.0 μL of a performance standard of

FIGURE 1. Seabird and gull egg sampling sites. A, Storm Islands, BC; B, Cleland Island, BC; C, Five Finger Rocks, BC; D, Silver Islet, Lake Superior; E, Big Sister Island, Lake Michigan; F, Chantry Island, Lake Huron; G, Middle Island, Lake Erie; H, Snake Island, Lake Ontario; I, Machias Seal Island, NB; J, Kent Island, NB; K, Sable Island, NS; L, Gull Island, NF. The Sand Island, Pacific Ocean site is part of the Midway Island group and is not shown on this map.

decachlorodiphenyl ether (50 ng/μL). Quantitation was performed using the bald eagle liver extract UHC solution.

Results and Discussion

Identification of UHC. The UHC was first detected in 1988 during a screening program to identify contaminants in Atlantic and Pacific coast seabirds using low-resolution EI-MS. At the time, the compound was believed to be a pentabrominated species (*14*). Further low-resolution EI-MS work on a highly contaminated bald eagle liver sample indicated that the unknown compound contained not only bromine but also chlorine. The observed isotopic patterns of the unknown compound most closely matched four bromine and two chlorine atoms. A pentabromo isotopic cluster looks very similar to a tetrabromodichloro cluster, except the latter contains six ions.

High-resolution mass spectra of the major congener were obtained to determine its molecular formula. Software provided as part of the OPUS operating system of the VG AutoSpec was used to determine the possible elemental compositions of the ions studied by comparing the experimentally determined mass to theoretical masses of species provided as possibilities by the software. Limits were set on the possible elements, and their amounts, present in the suggested fragments. The possibilities given by the software were restricted to those that contained C (0−15), H (0−15), N (0−4), O (0−4), S (0−4), P (0−4), Br (0−4), and Cl (0−4). Seven different ions from the most abundant clusters in the EI mass spectrum of the major congener were analyzed under the high resolution conditions. The most probable elemental composition of each ion was the composition which gave a theoretical mass with the smallest difference from the experimentally determined mass (Table 1) and contained the correct proportion of bromine and chlorine to produce the isotopic pattern observed for UHC. The experimental masses listed in Table 1 were obtained at a resolution of 10 000.

TABLE 1. Elemental Composition of Ions from the Major UHC Congener Found in Bald Eagle Liver Extract, Analyzed at a Resolution of 10 000

experimental mass	theoretical mass	difference	most probable elemental composition
139.031 067	139.029 623	0.001 444	$C_9H_3N_2$
190.913 635	190.913 738	−0.000 103	C_5H_3NBrCl
366.802 704	366.804 001	−0.001 297	$C_9H_3N_2Br_2Cl_2$
381.828 430	381.827 476	0.000 954	$C_{10}H_6N_2Br_2Cl_2$
419.719 086	419.719 263	−0.000 177	$C_8H_3NBr_2Cl_2$
460.748 535	460.745 812	0.002 723	$C_{10}H_6N_2Br_3Cl_2$
539.664 063	539.664 148	−0.000 085	$C_{10}H_6N_2Br_4Cl_2$

According to the results in Table 1, the parent ion at 540 Da has a molecular formula of $C_{10}H_6N_2Br_4Cl_2$. There were other possible elemental compositions provided by the program, but they did not contain both bromine and chlorine; or if they did, it was not in the right proportion that would produce isotopic patterns that resembled those of the major congener. There were some suggested compositions which did contain the right proportion of bromine and chlorine; however, their theoretical masses ranged from 1 to 27 mDa higher than the experimental masses listed in Table 1.

The ion of largest mass in the EI spectrum (540 Da) was assumed to be the molecular ion. This assignment was confirmed in the PCI experiments. The even mass is

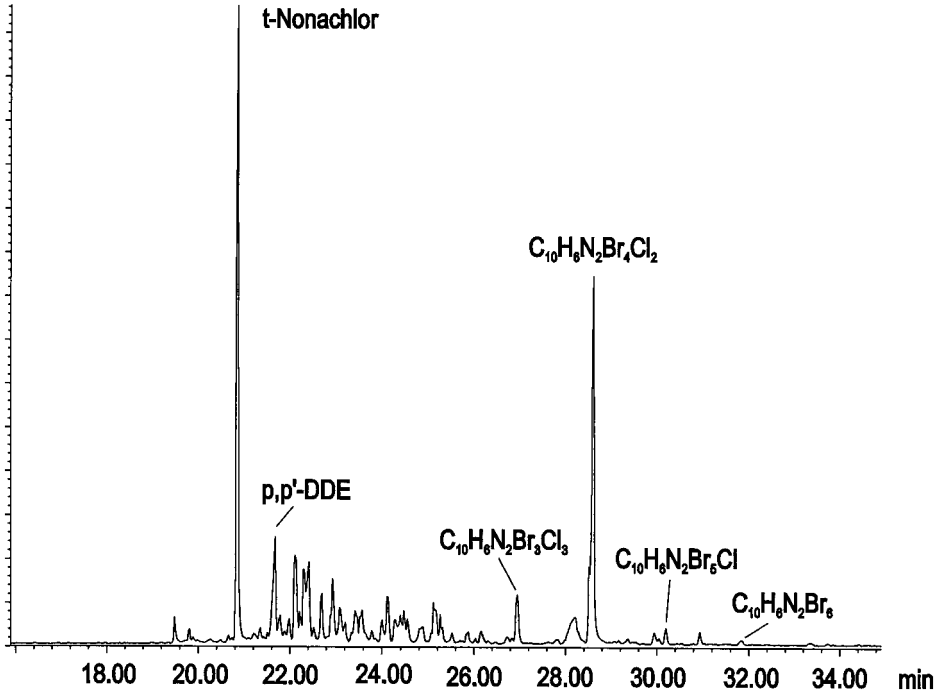


FIGURE 2. ECNI total ion chromatogram of bald eagle liver extract.

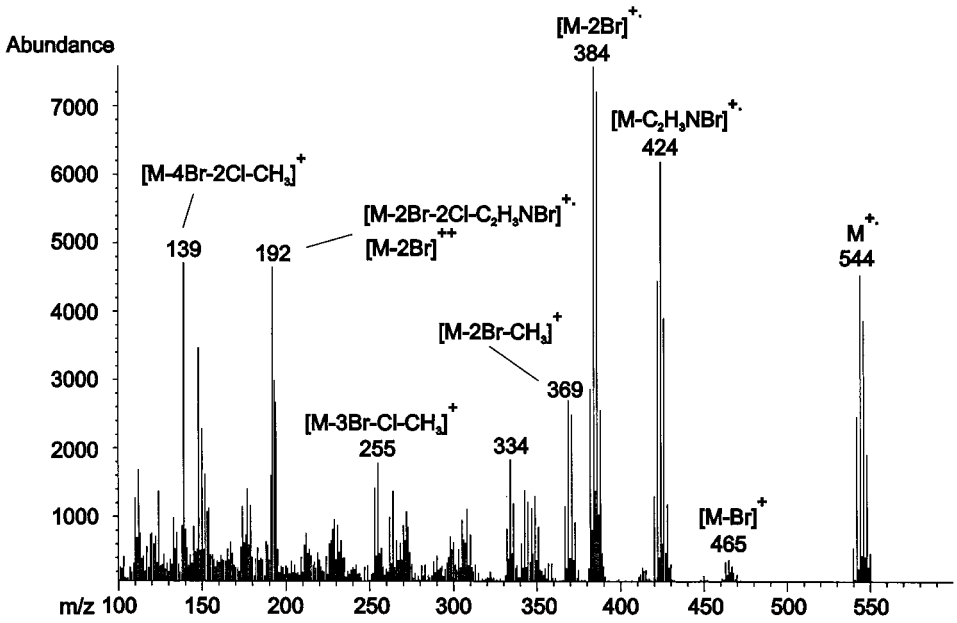


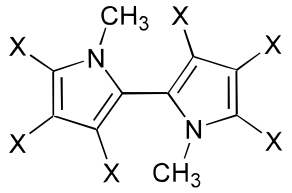
FIGURE 3. EI mass spectrum of $C_{10}H_6N_2Br_4Cl_2$ from bald eagle liver extract.

consistent with a compound containing two nitrogen atoms according to the nitrogen rule (*21*). Subsequent examination of the bald eagle liver sample revealed that a series of four mixed hexahalogenated congeners existed (Figure 2), with the halogen content ranging from Br_6 to Br_3Cl_3. The major congener, $C_{10}H_6N_2Br_4Cl_2$, was present at an amount roughly 10 times greater than the other three congeners. Therefore, the series of unknown compounds in the eagle liver have the general formula $C_{10}H_6N_2Br_mCl_n$, where $m = 3-6$, $n = 0-3$, and $m + n = 6$.

EI Mass Spectrum of $C_{10}H_6N_2Br_4Cl_2$. The low-resolution EI mass spectrum of $C_{10}H_6N_2Br_4Cl_2$ is shown in Figure 3. The molecular ion was assigned to the ion cluster starting at 540 Da. The identities of some of the fragment ions are indicated on the mass spectrum. The only fragmentations observed are successive losses of $Br^\bullet$ and $Cl^\bullet$, loss of $CH_3^\bullet$, and loss of C_2H_3NBr. The fragmentation pathways are similar for all of the congeners in the series.

One of the most likely structures for $C_{10}H_6N_2Br_4Cl_2$ is 1,1′-dimethyl-tetrabromodichloro-2,2′-bipyrrole (Figure 4). A related compound, hexabromo-2,2′-bipyrrole, is a known marine bacterial product (*22*). Loss of CHNBr from $M^{\bullet+}$ is important in the EI mass spectrum of hexabromo-2,2′-bipyrrole. This fragmentation pathway is analogous to loss of C_2H_3NBr from 1,1′-dimethyl-2,2′-bipyrrole with 5,5′-dibromo substitution, that is, loss of N and the adjacent 5-carbon with their substituents.

Also present in the EI mass spectrum is what appears to be a doubly charged ion at 191 Da, corresponding to $[M - 2Br]^{2+}$. The isotopic pattern of this cluster should be identical to that of the cluster for $[M - 2Br]^{\bullet+}$ at 382 Da; however, it is not. The presence of the doubly charged ion was confirmed



X = Br/Cl,
such that total Br = 4
and total Cl = 2

FIGURE 4. 1,1′-Dimethyltetrabromodichloro-2,2′-bipyrrole.

by lowering the ionizing electron energy to 30 eV. Doubly charged ions have high appearance potentials, thus electrons of low kinetic energy (30 eV) will not transfer an adequate amount of energy to the substrate to form the ions (*23*). When the electron energy was lowered, the isotopic cluster for $[M - 2Br]^{2+}$ disappeared, leaving only the cluster for $[M - 3Br - 2Cl - C_2H_3N]^{\bullet+}$ at 190 Da present. The overlapping isotopic pattern from $[M - 3Br - 2Cl - C_2H_3N]^{\bullet+}$ caused the isotopic patterns at 191 and 382 Da to differ.

Multiply charged ions can supply structural information on the analyte molecule, because they are formed only under certain circumstances. They are only formed from highly stabilized molecules with high π-electron density such as fused-ring aromatics (*21*) or from compounds that contain a nitrogen atom (*24*). In this case, high-resolution mass spectrometry confirmed that the doubly charged ion $[C_{10}H_6N_2Br_2Cl_2]^{2+}$ contained two nitrogen atoms. It is also probable that the doubly charged ion has an aromatic structure; the unsaturation number (*25*) of the parent compound is 6, indicating that an aromatic moiety is most likely present.

The abundance of the multiply charged ion can also provide some structural information. The doubly charged ion in the mass spectrum is about 45% of the base peak. Usually doubly charged ions are less than 10% of the base peak (*26*). An exception to this general rule occurs in the case of alkyl indoles. This suggests that an alkyl indole-type structure is another possibility.

ECNI Mass Spectrum of $C_{10}H_6N_2Br_4Cl_2$. As expected, less fragment ions are observed in the ECNI mass spectrum (Figure 5) than the EI spectrum. In ECNI, a moderating gas acts as a source of secondary electrons and creates an environment for thermalization of primary electrons and stabilization of negative molecular ions (*27*). Less energy is transferred during the capture of a thermalized electron by an analyte molecule. Less energy transferred results in less fragmentation of the analyte.

Four main clusters can be seen in the ECNI mass spectrum. The apparent molecular ion from the EI spectrum is also the base peak at 540 Da in the ECNI spectrum. A fragment corresponding to loss of $Br^{\bullet}$ is seen at 461 Da. Another fragment ion cluster occurs around 372 Da and is likely a combination of two or more overlapping patterns.

There is also evidence of bromide ion attachment in this mass spectrum. The fourth cluster at 619 Da corresponds to $[M + Br]^-$, and the isotopic pattern is indicative of an ion containing five bromine and two chlorine atoms. This seems to be the first noted instance of bromide ion attachment. The formation of the bromide ion/molecule adduct is analogous to the chloride ion attachment that has been observed for polychlorinated aromatic compounds (*28*) and hexachlorocyclopentadiene derivatives (*29*).

NH_3 PCI Mass Spectrum of $C_{10}H_6N_2Br_4Cl_2$. PCI, like ECNI, is a softer ionization technique than EI; therefore, the PCI spectrum shows less fragment ions than the EI spectrum. However, PCI is unlike ECNI in the mechanism of ionization. In ECNI, thermalized electrons ionize the analyte molecule. In PCI, the analyte molecule is ionized via addition of H^+ or $[\text{reagent gas molecule}]^+$. The difference in proton affinities of the analyte and reagent gas determine which ionized products are formed and the extent to which they will fragment.

The degree of fragmentation can be controlled by using various reagent gases. A reagent gas with a proton affinity very close to, but less than, the analyte will transfer H^+ to the analyte and keep the internal energy of MH^+ lower than the analogous parent ion in EI or ECNI. A low amount of internal energy of MH^+ will result in less fragmentation. Therefore, using a reagent gas with a relatively high proton affinity, such as NH_3, will reduce fragmentation and provide supporting evidence for the identity of the molecular ion.

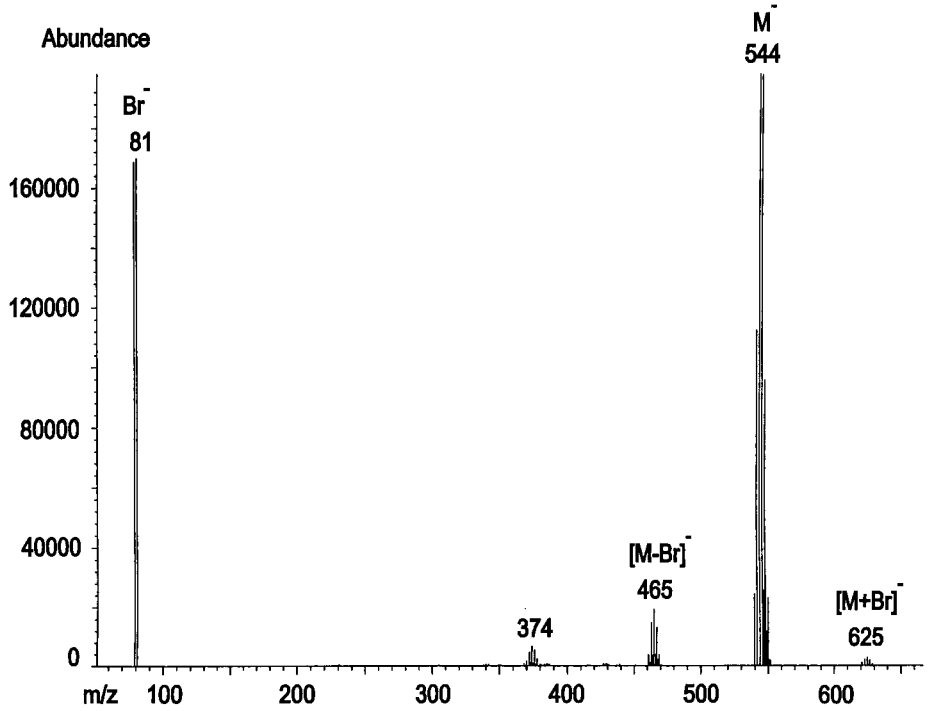


FIGURE 5. ECNI mass spectrum of $C_{10}H_6N_2Br_4Cl_2$ from bald eagle liver extract.

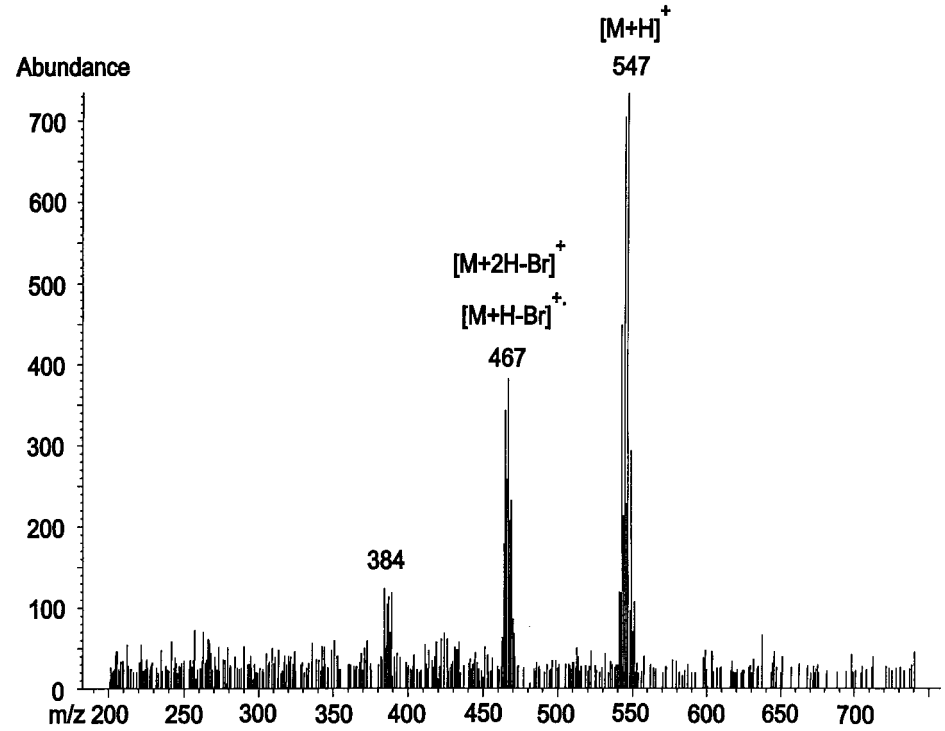


FIGURE 6. NH_3 PCI mass spectrum of $C_{10}H_6N_2Br_4Cl_2$ from bald eagle liver extract

The NH_3 PCI mass spectrum is shown in Figure 6. There are no ions of mass greater than 541 Da observed. This is strong evidence that the cluster at 541 Da is the molecular ion cluster, $[M + H]^+$. Only two other ion clusters are observed: one at 384 Da and the other at 463 Da. The identity of the first cluster is not known, while the second cluster is a composite of two overlapping clusters—$[M + H - Br]^{\bullet +}$ and $[M + 2H - Br]^+$. The pathways that form these ions must be of very low energy if they are accessible by a molecular ion produced by NH_3 PCI-MS.

Quantification of $C_{10}H_6N_2Br_4Cl_2$ in Eagle Liver Extract. The 12 standards used in the FID quantification of $C_{10}H_6N_2Br_4Cl_2$ had similar carbon weight response factors (mean: 1888 ± 165 area/g of carbon). Comparable results were found using a wider variety of compounds by Yieru et al. (*19*). Since the carbon weight response factors are similar for a variety of organic compounds regardless of their type or structure, the $C_{10}H_6N_2Br_4Cl_2$ could be quantitated using standards that are structurally dissimilar. Thus, the structure of $C_{10}H_6N_2Br_4Cl_2$ does not have to be known for adequate standards to be found. In the eagle liver extract the concentration of $C_{10}H_6N_2Br_4Cl_2$ was calculated to be 160 ± 20 ng/μL.

The ECNI total ion chromatogram of the eagle liver extract is shown in Figure 2. The four congeners of the $C_{10}H_6N_2Br_mCl_n$ series are present. However, only the major congener, $C_{10}H_6N_2Br_4Cl_2$, was present at a large enough amount to be quantitated using FID.

Distribution of $C_{10}H_6N_2Br_4Cl_2$ in Egg Samples. Absolute concentrations of $C_{10}H_6N_2Br_4Cl_2$ and concentrations relative to CB-153 are shown in Table 2. No corrections were made to the $C_{10}H_6N_2Br_4Cl_2$ concentrations because recoveries of the internal standard were all above 70%. The concentration of the mixed halogenated compound is given as a ratio to that of CB-153 to normalize its concentration to the levels of other halogenated compounds in the eggs. This ratio allows the bioaccumulation of $C_{10}H_6N_2Br_4Cl_2$ to be compared among species relative to a ubiquitous environmental contaminant.

From the data in Table 2, it is apparent that $C_{10}H_6N_2Br_4Cl_2$ is widespread in marine seabirds. By far the highest concentrations were found in Pacific Ocean surface feeders, the mid-ocean feeding albatross and Leach's storm-petrels, which feed approximately 100−200 km from the coast (*20*). Concentrations in Pacific Ocean storm-petrels were 28 times higher than in the same species in the Atlantic Ocean. Concentrations in the offshore, subsurface piscivores, rhinoceros auklet, and Atlantic puffin were similar in the Atlantic and Pacific Oceans. The bald eagle liver sample contained a higher level of $C_{10}H_6N_2Br_4Cl_2$ than each of the seabird samples because it feeds at a higher trophic level than the seabirds.

The compound was not detected in any of the herring gull samples from the Great Lakes. This absence from Great Lakes samples suggests that long-range atmospheric transport does not play a role in the distribution of these compounds. It also tends to rule out combustion sources, such as municipal incinerators. The herring gull samples are geographically representative of the Great Lakes since the year-round movements of adult herring gulls are within the Great Lakes drainage basin (*30*).

Ratios of $C_{10}H_6N_2Br_4Cl_2$ to CB-153 and absolute concentrations of $C_{10}H_6N_2Br_4Cl_2$ from the Pacific and Atlantic samples were compared using the Mann−Whitney U test. This test was used because the unequal variances of the Pacific and Atlantic data sets affected the t-test. The concentrations of $C_{10}H_6N_2Br_4Cl_2$ across the different species were much more uniform in the Atlantic samples than in the Pacific samples. The absolute concentrations and ratios were found to be significantly ($p = 0.037$) higher in the Pacific samples. The ratio of $C_{10}H_6N_2Br_4Cl_2$ to CB-153 was 24, 11, and 1.4 times greater in Pacific than Atlantic petrels, gulls, and alcids, respectively.

The ratio of $C_{10}H_6N_2Br_4Cl_2$ to CB-153 in Pacific Ocean surface feeders was 0.34−2.1, indicating that $C_{10}H_6N_2Br_4Cl_2$ ranks as a major contaminant in these species along with well-known globally distributed organohalogen pollutants. In the Pacific Ocean, Leach's storm-petrels had ratios 15 and 33 times greater than the glaucous-winged gull and rhinoceros auklets. For the Atlantic samples, the ratios were 6.8

TABLE 2. Concentrations of $C_{10}H_6N_2Br_4Cl_2$ in Various Seabird Egg Samples from Canada

sample collection location[a]	Fig. 1 map ref	ocean/ Great Lakes	no. of eggs in pool	collection date	$C_{10}H_6N_2Br_4Cl_2$ (ng/g sample, wet weight)	ratio of $C_{10}H_6N_2Br_4Cl_2$ to CB-153
Offshore Surface Feeders						
LSP, Storm Islands, BC	A	Pacific	6	02/07/94	140	2.1
LSP, Cleland Island, BC	B	Pacific	3	30/06/94	120	0.96
ALB, Sand Island, Pacific Ocean		Pacific	10	03/12/93	32	0.34
LSP, Gull Island, NF	L	Atlantic	10	22/06/96	4.6	0.097
LSP, Kent Island, NB	J	Atlantic	10	16/06/96	4.8	0.029
Offshore Subsurface Feeders						
RA, Cleland Island, BC	B	Pacific	5	29/04/90	3.1	0.050
RA, Storm Islands, BC	A	Pacific	5	04/05/90	1.8	0.043
AP, Gull Island, NF	L	Atlantic	10	15/06/96	1.7	0.048
AP, Machias Seal Island, NB	I	Atlantic	10	19/05/96	2.5	0.017
Inshore Omnivores						
GWG, Five Finger Rocks, BC	C	Pacific	10	28/06/90	5.6	0.10
HG, Silver Islet, Lake Superior	D	Great Lakes	7	18/05/96	nd	–
HG, Middle Island, Lake Erie	G	Great Lakes	13	28/04/96	nd	–
HG, Snake Island, Lake Ontario	H	Great Lakes	13	26/04/96	nd	–
HG, Chantry Island, Lake Huron	F	Great Lakes	13	23/04/96	nd	–
HG, Big Sister Island, Lake Michigan	E	Great Lakes	13	03/05/96	nd	–
HG, Sable Island, NS	K	Atlantic	10	13/05/96	1.6	0.0096
HG, Gull Island, NF	L	Atlantic	4	20/05/96	0.61	0.0088

[a] LSP = Leach's storm-petrel, RA = rhinoceros auklet, GWG = glaucous-winged gull, ALB = black-footed albatross, HG = herring gull, AP = Atlantic puffin, nd = not detected.

and 1.9 times greater for Leach's storm-petrels than the herring gull and Atlantic puffins.

Summary

The widespread occurrence of $C_{10}H_6N_2Br_4Cl_2$, in Pacific Ocean and Atlantic Ocean seabirds, coupled with its absence in the Great Lakes indicates that atmospheric transport is not involved in its distribution, nor is combustion likely to be a source. The high nitrogen and mixed chlorine/bromine content suggests a marine natural product, since synthetic organohalogens which are produced in large volume (e.g., flame retardants) are either chlorinated or brominated but not both. Sources of naturally produced organohalogens include many types of marine bacteria and algae (*6*). An extensive literature search revealed no previous mention of a compound with this molecular formula. The concentration of $C_{10}H_6N_2Br_4Cl_2$ was much higher in Pacific Ocean surface-feeding birds than in subsurface-feeding piscivorous birds from the same area and in the same species in the Atlantic Ocean. This finding suggests that the compound is produced in highest quantities in the Pacific Ocean surface layer. Another possibility is that the compound is produced and accumulated at lower depths in the Pacific Ocean and transported to the surface by organisms such as jellyfish and crustaceans. The structure suggested by the EI spectrum, 1,1′-dimethyl-tetrabromodichloro-2,2′-bipyrrole, is a good candidate. A closely related hexabrominated compound has been identified in Pacific Ocean marine chromobacteria (*21*). Furthermore, a bipyrrole with perhalogenated ring carbons and *N*,*N*′-dimethyl substitution is likely to be more resistant toward biotransformation than most possible structures and therefore has bioaccumulation potential in birds. Structures that contain primary or secondary amine groups can probably be ruled out as they are readily biotransformed in most species.

Further studies are planned to determine the structure, source, and toxicity of $C_{10}H_6N_2Br_4Cl_2$. If the source can be confirmed to be biogenic, this would be the first instance of a naturally produced organohalogen bioaccumulating in higher trophic level organisms.

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