MASS SPECTROMETRIC CHARACTERIZATION OF Q1, A $C_9H_3Cl_7N_2$ CONTAMINANT IN ENVIRONMENTAL SAMPLES

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Introduction

Q1, an unknown heptachloro component, was recently identified as a major contaminant in African and Antarctic seal species. The levels in these samples reached up to 350 µg Q1/kg blubber [1][2]. High levels of Q1 (up to 120 µg/kg fresh weight) were also determined in eggs of Antarctic birds [3]. Q1 was also highly abundant in human milk samples of woman from the Faeroe Islands who regularly consumed whale [4]. Furthermore, Q1 was detected in a fish sample from the Mediterranean sea, as well as harbor porpoises from Iceland [1]. Low Q1 levels were, however, determined in seal blubber from the North Sea and the Baltic, and Q1 was below the detection limit in the blubber of ringed seals from Spitsbergen (European part of the Arctic). These results suggest that Q1 is much lower abundant in the northern hemisphere than in the southern hemisphere [1][5]. Here, we discuss the low and high resolution EIMS and establish the elemental composition of Q1.

Material and methods

Gas chromatography/low resolution electron impact ionization mass spectrometry (GC/LR-EIMS). GC/LR-EIMS (mass unit resolution) studies were performed on a HP 5989B mass spectrometer, a Saturn 2000 ion trap (Varian), and a HP 5971 MSD (Hewlett-Packard). Full scan mass spectra were recorded at m/z 50-550 (HP 5989 and HP 5971) or m/z 50-650 (Saturn 2000). All measuring parameters were recently described [1][2].

Gas chromatography/ high resolution electron impact ionization mass spectrometry GC/HR-EIMS). GC/HR-EIMS were performed on a B/E-geometry double focusing MAT 95 (Finnigan MAT) mass spectrometer connected to a HP 5890 gas chromatograph (Hewlett-Packard). Splitless injections of 2µL sample (splitless time 1 min) were performed at 280°C, the MS transfer line was heated at 320°C. A DB-5ms (60m x 0.25mm x 0.25µm) GC column was installed in the GC oven. The GC oven program was the following: 95°C/min, 2 min, 20°C/min to 180°C, 2°C/min to 200°C, 6°C/min to 320°C, 5 min.

HR-EIMS (70 eV) experiments in the selected ion monitoring (SIM) mode were performed with constant B-filter. Each SIM-group (4 to 10 mass traces were monitored at resolution 16000) had adapted lock and calibration masses. The total cycle time in each SIM-group was ≤ 1s. Note that signals with a mass difference smaller than the quotient mass/resolution could not be separated totally. This explains the detectable intensities in various mass traces discussed below which were only separated by 4 mAMU (see below).

Sample and sample clean-up. GC/MS determinations of Q1 were conducted on the liver extract of a brown skua (*Catharacta antarctica lonnbergi*) sampled in 1994 at the Jubany station

(Antarctic). The sample clean-up included digestion with acids, liquid-liquid extraction with n-hexane, repeated treatment with sulfuric acid, and adsorption chromatography on silica [6].

Results

LR-EIMS of Q1. The GC/LR-EIMS of Q1 showed three major fragment ions at m/z 384 (molecular ion), m/z 349, and m/z 314. Depending on the mass spectrometer, these fragment ions were found at different ratio (see Figure 1).

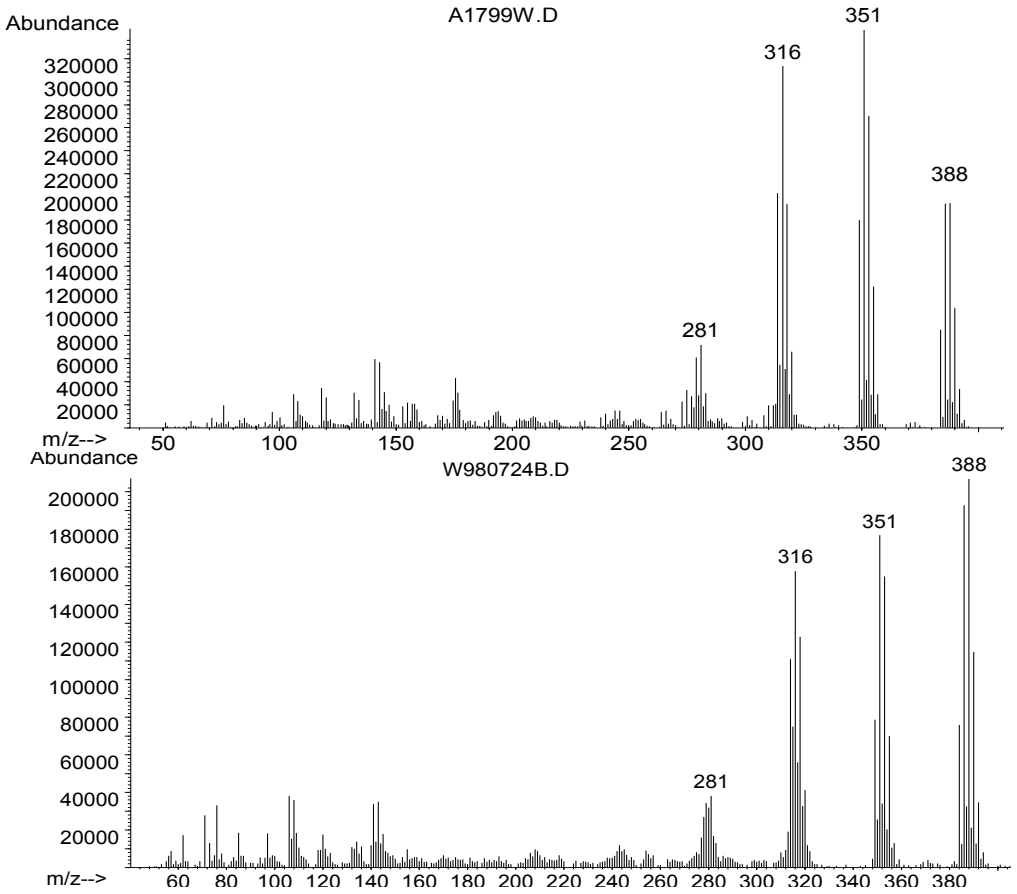

Figure 1: GC/LR-EIMS of Q1. Top: HP 5989B. Bottom: Saturn 2000 ion trap

The following discussion is based on the LR-EIMS recorded with the HP 5989 mass spectrometer (Figure 1, top). Loss of one and two Cl from the molecular ion at m/z 384 leads to m/z 349 (6 Cl) and m/z 314 (5 Cl). Furthermore, the following low abundant sequence with subsequent elimination of chlorine respectively was observed: m/z 369 (7 Cl) → m/z 334 (6 Cl) → m/z 299 (5 Cl) → m/z 264 (4 Cl). The fragment ion m/z 369 (7 Cl) is formed from m/z 384 by elimination of 15 amu (CH_3).

The fragment ion starting at m/z 174 is the doubly charged analogue of the hexachloro fragment ion at m/z 349 (the software rounds m/z values to full mass units: the correct mass of the doubly charged analogue at m/z 348.9/2 is rounded to m/z 174). Doubly charged fragment ions point toward an aromatic system.
The GC/EI full scan mass spectrum recorded on the HP 5971 and the Saturn 2000 ion trap mass spectrometer confirmed the mass fragment at m/z 369 but the doubly charged ion at m/z 174 was not observed. The number of carbons on Q1 derived from the ratio of ^{12}C (m/z 384) and ^{13}C satellites (m/z 385) was 10±1.
Subtraction of the mass of 7 Cl from m/z 384 leaves 139 amu for other elements (C, H, and hetero atoms). This leaves three possible molecular compositions for Q1, i. e. $C_{11}H_7Cl_7$, $C_{10}H_3Cl_7O$, or $C_9H_3Cl_7N_2$ [2]. Br was excluded as a further hetero atom because there was no response at m/z 79 and m/z 81 in both EI and ECNI mass spectra (see also below). One nitrogen (this would result in an odd molecular mass) and two oxygens and sulfur are theoretically not possible (139 amu - 32 amu = 107 amu which is $<C_9$). A differentiation between the three possibilities mentioned above was not possible with LR-EIMS and, therefore, HR-EIMS was applied.

HR-EIMS of Q1. The HR-EIMS in the full scan mode also showed the three abundant fragment ions at m/z 384, m/z 349, and m/z 314. Highest abundance was obtained for the ion cluster starting m/z 314 which is in agreement with the LR-EIMS recorded with HP 5971 mass spectrometer (data not shown). The HR-EIMS-SIM investigation of the molecular composition of Q1 was carried out at a resolution of 16000. The exact masses of the three structural possibilities are 383.837 ($C_{11}H_7Cl_7$), 383.812 ($C_9H_3Cl_7N_2$), and 383.800 ($C_{10}H_3Cl_7O$). The mass differences between the three possibilities are 25 mAMU and 12 mAMU, respectively. To establish the exact molecular composition of Q1, 10 m/z values with Δ = 4 mAMU were recorded (Table 1).

Table 1: High resolution mass spectrometry (SIM mode) of Q1

m/z	Abundance	Rel. Abundance	Composition
383.8000	255	7.9%	$C_{10}H_3Cl_7O$
383.8040	596	18.5%	-
383.8080	1965	61.1%	-
383.8120	3218	100%	$C_9H_3N_2Cl_7$
383.8160	1815	56.4%	$C_8H_6N_2Cl_5Br$
383.8200	362	11.2%	-
383.8240	60	1.9%	-
383.8280	<60	< 1.9%	-
383.8320	<30	< 1%	-
383.8370	<10	<< 1%	$C_{11}H_7Cl_7$

The GC/HR-EIMS experiments showed highest abundance for the variant with two nitrogens on Q1 (Table 1). The exact mass at m/z 383.812 for the $C_9H_3N_2Cl_7$ gave approx. 60% abundance at Δ4 mAMU. At a resolution of 16000, nearly baseline resolution is obtained at approx. 12 mAMU. Therefore, the response of neighbored ions in Table 1 caused by the major abundant ion could not be excluded. However, the highest abundance at m/z 383.812 clearly confirms the structure of Q1 being $C_9H_3N_2Cl_7$ and definitively excludes the two other structural variants. A further presumable structure of Q1, the combination of 2 nitrogen and Br was also lower abundant than m/z 383.812.

Exclusion of $C_8H_6N_2Cl_5Br$ was expected since carbon number (C_8) was not covered in the $^{12}C/^{13}C$ ratio determined by LR-EIMS (see above).
The exact masses of further fragment ions were investigated by HR-EIMS in the SIM mode. E. g., the fragment at m/z 369 showed highest response for m/z 368.788 which corresponds to the composition $C_8Cl_7N_2$. This fragment ion is obtained after elimination of CH_3 from the molecular ion which confirms the observation in the LR-EIMS (see above). An elemental composition of C_4Cl_2N was assigned to the fragment ion detected at m/z 132. This fragment ion was also part of the LR-EIMS of Q1 (see Figure 1). Furthermore, m/z 132 in the EIMS may be similar to the tetrachloro fragment ion at m/z 202 recorded in the ECNI-MS of Q1 which has been interpreted as a C_5Cl_4 or C_4Cl_4N fragment ion [2]. Several eliminations which correspond to the elimination CH_3CN were also discovered in the HR-EIMS of Q1.

Discussion

Our investigations confirm that the molecular composition of Q1 is no other than $C_9H_3Cl_7N_2$. Due to the presence of a CH_3 group on Q1, there is no further hydrogen on Q1, and both nitrogens on Q1 must be either tertiary amines, cyanides or cyclic tertiary nitrogens. Our CAS and Beilstein search of the literature for components composed of $C_9H_3Cl_7N_2$ revealed only one entry for 4-dichloro-N-dichloromethylene-N´-trichloromethyl-benzamidine (CAS 65866-99-1) [7]. However, this component possesses no methyl group and, therefore, Q1 and CAS 65866-99-1 cannot be identically.
To date, highly chlorinated nitrogen-containing hydrocarbons have not been reported in environmental samples. However, Tittlemier et al. recently identified a component with the molecular composition $C_{10}H_6Br_4Cl_2N_2$ which may be a marine natural product [8]. In addition to the lack of bromine, Q1 has one carbon less than $C_{10}H_6Br_4Cl_2N_2$, and these differences suggest that both compounds are not related compounds. However, further research is necessary to unequivocally clarify this.
The lack of any plausible and CAS-known structure for Q1 is curious since Q1 has been identified as an abundant contaminant in many environmental samples [1-5]. Studies of the exact structure and origin of Q1 are ongoing.

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