COMPOUND-SPECIFIC STABLE CARBON ISOTOPE RATIOS OF THE HALOGENATED NATURAL PRODUCT Q1

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Introduction

In the past years, the halogenated natural product 2,3,3´,4,4´,5,5´-heptachloro-1´-methyl-1,2´-bipyrrole (Q1) has been detected in environmental and food samples from all six continents.[1]

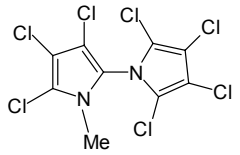

Figure 1: Structure of 2,3,3´,4,4´,5,5´-heptachloro-1´-methyl-1,2´-bipyrrole (Q1)

The natural origin of Q1 is generally accepted, however the producer has not been identified yet. Therefore, the presence and origin of Q1 still remains unclear. For instance, the high abundance of Q1 in samples from both Australia and the Antarctic, i. e. two regions with two very diverse climate indicated that more than one natural producer may exist. Stable isotope ratios of natural compounds are well established for origin assignment and may also provide insights into the origin of these polyhalogenated compounds. In this study, we used gas chromatography interfaced to a stable isotope ratio mass spectrometer (GC-IRMS) for the compound-specific determination of $\delta^{13}C$ values of lab-synthesized Q1 as well as isolates from environmental samples from Australia and the Antarctic.

Material and Methods

Standards and samples. Q1 was synthesized as previously reported.[2] In brief, 1-methylpyrrole was reacted with *N*-chlorosuccinimide and the condensed rings were perchlorinated with $PCl_5/POCl_3$.[2] The isotopic reference standards "V-PDB" and "NBS 19" were obtained from the International Atomic Energy Agency (IAEA, Vienna, Austria). The liver of Antarctic brown skua was collected in 1994 at King George Island.[3] Origin and clean-up of blubber of adult, female melon-headed whale (*Peponocephala electra*) and adult male bottlenose dolphins (*Tursiops truncatus*) were previously described in details.[4-5]

GC-IRMS. GCQ (ion trap MS) and DELTA plus XL (stable isotope ratio mass spectrometer) instruments (Thermo, Bremen, Germany) were used in combination with an HP 5890 (Agilent, Waldbronn, Germany) gas chromatograph. After the GC column (HP 5 MS, 30 m, 0.32 mm I.D., 0.25 µm film thickness), the effluent was splitt using a fixed splitter. 90% of the effluent went to the combustion furnace for isotope ratio measurement while the remaining 10 % were transferred to the ion trap MS. Details of the IRMS procedure were published elsewhere.[6-7] Isotope ratios are expressed as δ^{13} C value in permil [‰] relative to V-PDB using NBS 19 (= std).[8]
$\delta^{13}C$ value $[‰]_{V\text{-}PDB} = 1000 \times [(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{std}] / (^{13}C/^{12}C)_{std}$.

Results and Discussion

Analytical aspects of GC-IRMS. Our initial determinations of synthesized Q1 as well as abundant residues in marine biota led to some unambiguous data. The three environmental samples differed significantly from the synthetic product (more than 11 ‰ depleted in ^{12}C) and this clarified that the synthetic product cannot be the source for Q1. However, the initial $\delta^{13}C$ values of Q1 in blubber of melon-headed whale and bottlenose dolphin from the same region varied by ~6 ‰. On the other hand, the $\delta^{13}C$ value of the Australian melon-headed whale

differed only by ~1 ‰ from the $\delta^{13}C$ value in skua liver from the Antarctic. Particularly, the large difference in the two samples from Australia was conspicuous since we expected little isotopic variation for polyhalogenated organic compounds from the same source.[7]
Recently, we found that minute co-elutions (target compound interfered by <5%) can significantly falsify the $\delta^{13}C$ value of the target compound. Since the GC-IRMS system was also connected to a conventional ion trap ms (see Materials and Methods) we were able to study the respective mass spectra of the compounds (**Figure 2**). At first glance, the mass spectra were virtually identical (**Figure 2a-c**).

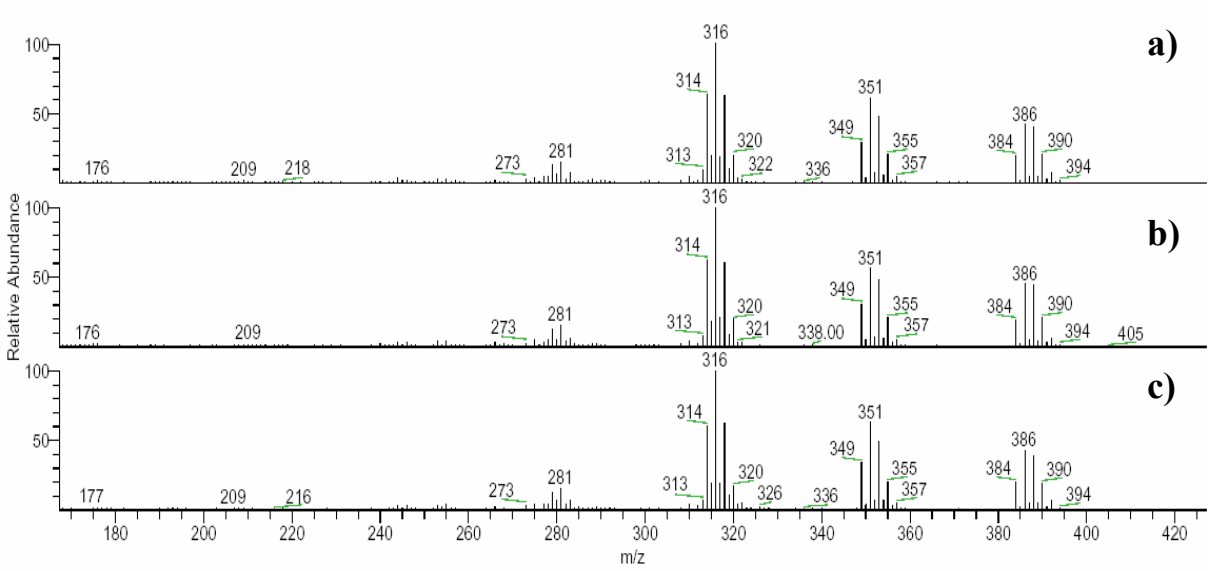

Figure 2: GC/EI-MS mass spectra of Q1 in a) Australian melon-headed whale (*P. electra*), b) Antarctic skua liver, and c) Australian bottlenose dolphin (*T. truncatus*). The mass spectra are exact matches of the sample combusted to CO_2 for the stable isotope ratio determination.

No additional fragment ion that amounted for >2% of the base peak was detected in the three mass spectra. Based on a rational additive effect we made the following calculation. The lowest individual $\delta^{13}C$ value was -21‰. According to the peak purity test, at least 95% arose from Q1, which would be -19.95 ‰. A given impurity would have to falsify the value to -16 ‰ would have to account for +60 ‰. Such a $\delta^{13}C$ value does generally not exist. Therefore, a simple additive effect can be excluded. However, a closer inspection of the mass chromatogram of the conspicuous bottlenose dolphin sample indicated a minor peak eluting slightly prior to Q1 (**Figure 3a**).

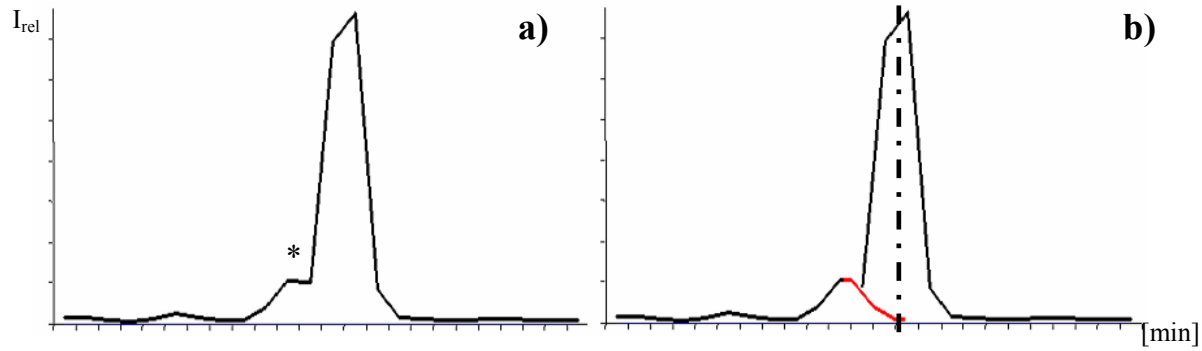

Figure 3: Enlargement of Q1 illustrating (a) the interference with PCB 101 and (b) illustrating the calculated peak shape of PCB 101 whereas the dotted line represents Q1 at the peak maximum where the mass spectrum in Figure 2c was taken

Mass spectrometric studies confirmed that the interfering peak arose from 2,2´,4,5,5´-pentachlorobiphenyl (PCB 101). On analytical DB-5 columns (0.25 mm i.d. and 30-50 m length), PCB 101 is well-spearated from Q1. However, relatively short GC oven program along with the 0.32 mm i.d. column used in the GC-IRMS system are likely responsible for the partial co-elution. PCBs do not play a role in the Antarctic skua sample and obviously were partly separated during the enrichment of Q1 in the sample cleanup (enrichment on silica). Therefore, PCB 101 was only found in the conspicuous bottlenose dolphin sample. We then zoomed into the peak and artificially mirrored the left half of the PCB peak and constructed the peak shape as expected from a non-interfered peak (**Figure 3b)**.
The illustration in **Figure 3b** clarified that >95% of PCB 101 was already eluted at the peak maximum of Q1. Therefore, it is no surprise that PCB 101 was not identified as an interference in the mass spectrum of Q1 (Figure 2c). Only a minute trace of the base ion of PCB 101 at *m/z* 326 was detected at the peak maximum of Q1 (**Figure 2c**). However, the mass spectrum at the peak maximum of PCB 101 (see asterisk in **Figure 3a**) unequivocally proved its present in the sample (**Figure 4**).

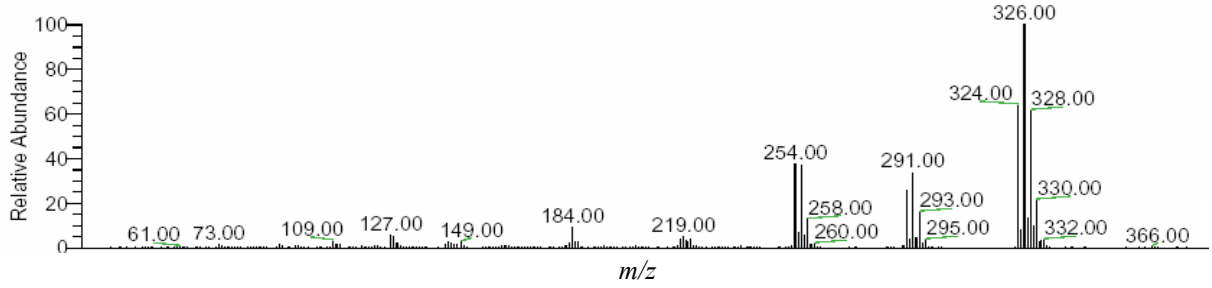

Figure 4: Mass spectrum of PCB 101 in Australian bottlenose dolphin (*Tursiops truncatus*).

Literature data on PCBs brought $\delta^{13}C$ values of about -25 ‰ to our attention.[9-10] The interference of Q1 by PCB 101 was only ~5% so that the falsification cannot be linear. This is in agreement with results obtained for toxaphene compounds.[7] It thus appears that minor impurities of fully-co-eluting signals are less relevant problems than partially co-eluting interferents. This is likely due to non-symmetric isotopic peak elutions.[11]
Unfortunately, the samples were used up and no consecutive analyses could be performed to investigate this phenomenon more thorough. Several conclusions can, however, be drawn from these investigations. First, study of the peak purity of a target compound by means of the mass spectrum at the peak maximum is not a sufficient quality criterion. Rather the mass spectrum at the offset of the peak originating from the analyte should be investigated. While the mass spectra of the target compound will not be at optimum, the absence of partly co-eluting peaks can be excluded.

$\delta^{13}C$ values of bioaccumulated naturally produced Q1 and lab-synthesized Q1. Most importantly, the identification of the analytical problem just discussed solved the problem of implausible variations in the $\delta^{13}C$ values of Q1 in two cetaceans from Australia. After exclusion of the sample where Q1 was interfered, we were able to determine the $\delta^{13}C$ values in one blubber sample of melon-headed whale from Australia and the purified extract of skua liver from the Antarctic. As mentioned above, both environmental samples significantly differed from the synthesized product (**Table 1**).

Table 1: $\delta^{13}C$ value [‰] of synthesized Q1 and Q1 residues in environmental samples

Sample	$\delta^{13}C$ value [‰]	Conditions
Q1 standard	-34.20 ± 0.27	GC-IRMS,
Q1 standard	-34.78 ± 0.02	EA-IRMS
Q1 melon-headed whale, Australia	-22.80 ± 0.33	GC-IRMS
Q1 skua liver, Antarctic	-21.47 ± 1.42	GC-IRMS

It is noteworthy that Q1 was not interfered in the environmental samples. Furthermore, the standard deviation in the case of the melon-headed whale was in the range of the standard. By contrast, the reproducibility was somewhat larger for the skua liver. This is due to the lower amount available from this sample. Thus, a lower amount (~50 ng) was injected and the resulting S/N was worse and caused a higher standard deviation. However, the $\delta^{13}C$ values of the melon-headed whale from Australia and the skua sample were similar (Δ1.3‰).

Conclusions

Since the producer(s) of Q1 is (are) unknown, our measurements could not lead to the sources of this HNP this time. There is still the option that bioproduction could occur both in the Antarctic as well as Australia where the highest concentrations of Q1 have been determined. On the other hand, Q1 could have been long-range transported from Australia to the Antarctic. Recent investigations of the toxaphene compound B8-1413 illustrated that long-range transport to polar regions and food-chain bioaccumulation will not alter the $\delta^{13}C$ value of a POP.[7] Thus, long-range transport cannot be ruled out. On the other hand, assuming bioproduction occurred on both continents with extremely different climate conditions, the very similar $\delta^{13}C$ values are surprising. Despite this unclearness, it is clear that the $\delta^{13}C$ values in the sample differed extremely from the synthesized product and the relative narrow range of $\delta^{13}C$ values increase the likelihood that the GC-IRMS technique can be used as a tracer for Q1. We also demonstrated that determination of the $\delta^{13}C$ values of polyhalogenated pollutants will be an interesting tool for investigation the source, distribution, and fate of the respective pollutants.

References

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