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Identification of the new arsenic-containing betaine, trimethylarsoniopropionate, in tissues of a stranded sperm whale *Physeter catodon*

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Arsenic compounds in aqueous extracts of muscle, liver, kidney, and lung tissue taken from a sperm whale *Physeter catodon* (Mammalia: Cetacea) beached on a small island near Phuket in the Andaman Sea were determined by high performance liquid chromatography using an inductively coupled plasma mass spectrometer as the arsenic specific detector. The total arsenic concentrations in the tissues (dry mass) were low, ranging from 0.3 μg g^{-1} for liver and lung, to 1.0 μg g^{-1} for muscle and 3.0 μg g^{-1} for kidney. Most of the arsenic (>60%) was extracted from the tissue into water, and the bulk (>80%) of this arsenic was present as arsenobetaine. Dimethylarsinate was present at low levels in all four tissues whereas arsenocholine was detected in kidney, liver and lung, but not in the muscle tissue. Another arsenic containing betaine, trimethylarsoniopropionate, was also present in all four tissues. This is only the second report of trimethylarsoniopropionate as a naturally-occurring arsenic compound.

INTRODUCTION

Interest in the chemical form of organoarsenic compounds present in marine animals increased after the identification (Edmonds et al., 1977) of arsenobetaine [trimethylarsonioacetate, $(CH_3)_3As^+CH_2COO^-$] in the western rock lobster, and there is now a considerable amount of data on the distribution of arsenic compounds in marine animals (Francesconi & Edmonds, 1997). The data collected so far have come largely from the application of modern analytical techniques capable of identifying the different organoarsenic compounds at environmental concentrations. Foremost among these techniques has been the combination of high performance liquid chromatography (HPLC) and inductively coupled plasma mass spectrometry (ICPMS). This technique provides good separation of the arsenic compounds, and, because the ICPMS can serve as an element specific detector, the separated compounds can be detected and quantified at low concentrations and in complex matrices (Larsen, 1998).

An overview of the data collected so far on arsenic in marine animals shows that arsenobetaine is by far the dominant arsenic compound, and other arsenicals such as the tetramethylarsonium ion [$(CH_3)_4As^+$], arsenocholine [$(CH_3)_3As^+CH_2CH_2OH$], and arsenosugars are also common albeit less abundant arsenic forms (Francesconi & Edmonds, 1997). Perusal of the data, however, reveals deficiencies in some areas, namely in the range of species and the type of tissues examined. The majority of studies so far have dealt with fish, crustaceans and molluscs, and have usually examined only the muscle tissue. The situation regarding other marine animals and other tissues is less well covered, although the few studies in this area have produced interesting data. For example, the single report (Edmonds et al., 1994) of arsenic compounds in a marine reptile has shown that the turtle, *Dermochelys coriacea*, is unique among marine animals because it contains arsenocholine at significant concentrations in some tissues. Similarly, there are large differences in the distribution of arsenic compounds in the various tissues of the fish *Mugil cephalus* (Maher et al., 1999). Information on the arsenic compounds in a broad range of species and tissues may help elucidate the origin of organoarsenic compounds in marine organisms. Accordingly, we report here an investigation, with HPLC/ICPMS, of the arsenic compounds in tissues from a stranded sperm whale, *Physeter catodon* (L.).

MATERIALS AND METHODS

Tissues (muscle, liver, kidney, and lung) were collected from a single adult female sperm whale (8.0 m total length and 5400 kg wet mass), which beached itself near Phuket in the Andaman Sea in October 1996. The tissues were taken within hours of the whale's death and immediately frozen and freeze-dried. The dried tissues were then ground to a powder and their arsenic content determined by hydride generation atomic absorption spectrophotometry (HGAAS) (Bachmann et al., 1999).

The dry tissues (100 mg) were extracted with (2×5.0 ml) water with the assistance of a sonication probe as previously described for other marine tissues (Bachmann et al., 1999); for each tissue extract two identical portions were evaporated to dryness on a centrifugal lyophilizer and individually stored at −18°C until their analysis by

HPLC/ICPMS using anion-exchange and cation-exchange chromatographic columns (Kuehnelt et al., 2000). These analyses were performed on two separate occasions, in June 1998 and in March 2001.

RESULTS AND DISCUSSION

The tissues contained only low concentrations of arsenic: 0.3 $\mu g\,g^{-1}$ (lung and liver), 1.0 $\mu g\,g^{-1}$ (muscle), and 3.0 $\mu g\,g^{-1}$ (kidney) (all values are on a dry mass basis). These values are lower than those found in other whales, e.g. 0.17–1.27 μg As g^{-1} wet mass in liver of pilot and beluga whale (Goessler et al., 1998), or 1.66 μg As g^{-1} wet mass in kidney and 4.35 μg As g^{-1} wet mass in liver of the common whale (Fernandez et al., 2000), and very much lower than those reported (Francesconi & Edmonds, 1997) for most other marine animals, some of which can contain >300 μg As g^{-1} (dry mass).

Aqueous extraction removed most of the arsenic in the tissues: ~90% for muscle and kidney, and >60% for lung and liver (low arsenic concentrations precluded more precise extraction data for lung and liver). The HPLC/ICPMS analyses carried out in June 1998 clearly showed that arsenobetaine was the major arsenic compound for all tissues, and that small quantities of dimethylarsinate $[(CH_3)_2AsOO^-]$ and arsenocholine were also present. These assignments were made by matching chromatographic retention times with those of standard arsenic compounds. A fourth arsenic compound was also present in all samples, but it could not be identified as its retention time did not match that of any of the available standards. Because the arsenic standards available to us included essentially all of the approximately 25 naturally-occurring arsenic compounds reported (Francesconi & Edmonds, 1997) at that time, it appeared likely that the unidentified arsenic compound in whale tissues was a new natural product.

Subsequent work (Francesconi et al., 2000) on the arsenic compounds in a coral reef fish, also taken from the Andaman Sea, reported the presence of a new arsenic natural product, namely trimethylarsoniopropionate $[(CH_3)_3As^+CH_2CH_2COO^-]$. The structure of the new compound was proposed following analysis with electrospray ionization mass spectrometry, and confirmed by comparison with synthetic material. Following the identification of trimethylarsoniopropionate in coral reef fish, we re-examined the whale tissue extracts. On this occasion, the analysis was performed on freeze-dried extracts that had been stored at −18°C for almost three years; it produced chromatograms identical with those from the earlier analysis.

The peak which could not be identified in the earlier analysis had a retention time (3.0 min, cation exchange pH 2.6) identical with trimethylarsoniopropionate. Spiking the tissue extracts with authentic compound produced a single homogeneous peak at the 3.0 min (see Figure 1 for a representative chromatogram). A characteristic of trimethylarsoniopropionate is that its retention time is markedly influenced by the pH of the mobile phase. At lower pH, the carboxy group is protonated and the resultant cationic compound is strongly retarded on cation-exchange columns. This interaction is weakened at higher pH because the molecule loses its acidic proton and exists as a zwitterion. When the cation-exchange chromatography was repeated at pH 4.0, the arsenic compound in the whale tissue extracts had a retention time of 2.1 min, identical with the standard trimethylarsoniopropionate. Again, spiking experiments performed at pH 4.0 produced a homogeneous peak at 2.1 min (Figure 1). On the basis of these chromatographic data, the fourth arsenic compound in the whale tissue extracts was identified as the new arsenic betaine, trimethylarsoniopropionate.

The quantities of the arsenic compounds in the aqueous extracts of the whale tissues are summarized in Table 1. The major compound in all samples was arsenobetaine (80.3–93.6% of total arsenic compounds), while trimethylarsoniopropionate was a minor constituent (2.9–4.6%) along with dimethylarsinate (0.9–10.5%) and arsenocholine (0.9–2.8%). Additionally, a trace cationic

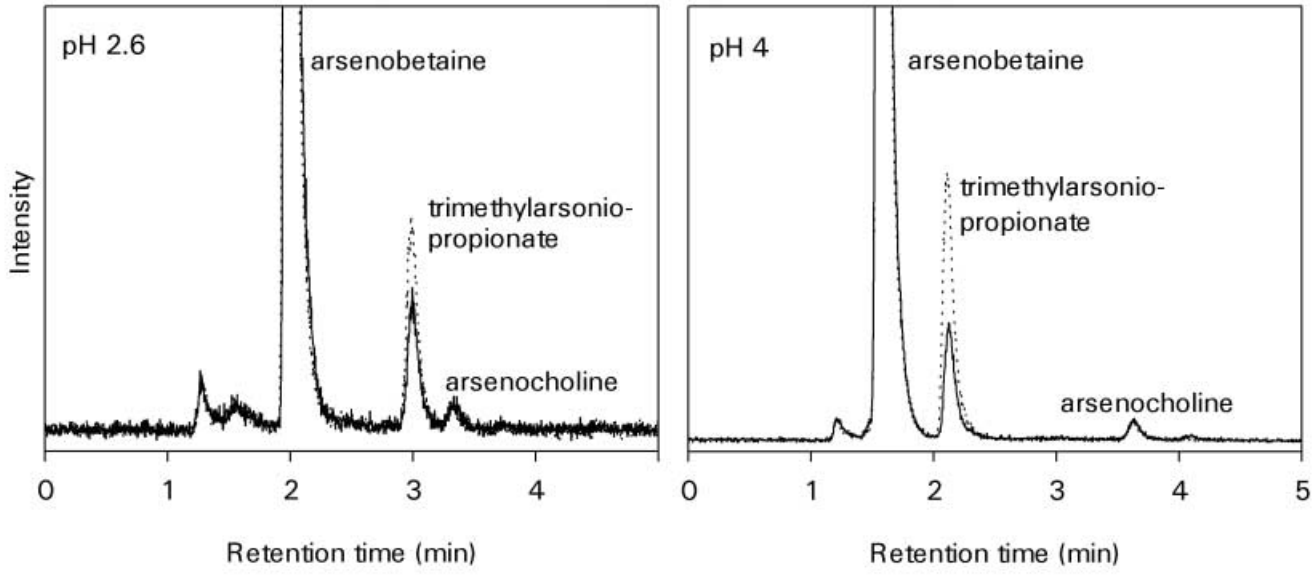

Figure 1. The HPLC/ICPMS cation-exchange chromatograms at pH 2.6 or pH 4.0 of an aqueous extract of kidney (solid line) and an aqueous extract of kidney spiked with authentic trimethylarsoniopropionate (dotted line). Conditions were: cation column Zorbax LC-SCX, 20 mM pyridine, pH 2.6 or 4.0, 1.5 ml min^{-1}, 30°C, 20 μl injected.

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Table 1. *Arsenic compounds in tissues of the sperm whale* Physeter catodon. *Quantities are expressed in g As g^{-1} dry mass. Values in parentheses represent, as a percentage, the contribution of each compound to the total extractable arsenic.*

	Arsenic compound in μg As g^{-1} dry mass and (% of total)					
Whale tissue	arsenobetaine	trimethylarsonio-propionate	dimethylarsinate	arsenocholine	unidentified cation	unidentified arsenic
kidney	2.65 (93.6%)	0.11 (3.9%)	0.03 (0.9%)	0.03 (0.9%)	0.006 (0.2%)	0.02 (0.6%)
liver	0.22 (80.3%)	0.01 (3.5%)	0.03 (10.5%)	0.01 (1.9%)	0.004 (1.4%)	0.01 (2.3%)
lung	0.28 (85.8%)	0.01 (2.9%)	0.01 (3.0%)	0.01 (2.8%)	trace	0.02 (5.5%)
muscle	0.92 (91.5%)	0.05 (4.6%)	0.01 (1.2%)	<0.005	0.005 (0.6%)	0.01 (1.0%)

arsenical (retention time 3.7 min at pH 2.6 and 4.1 min at pH 4) occurred in all tissues but it could not be identified. There was also a small quantity of arsenic which eluted at the void volume of the cation-exchange column. This arsenic was not detected when anion-exchange chromatography was performed; it may exist as a neutral species (e.g. arsenous acid) under the chromatographic conditions employed (pH 5.6) and thus came at the void volume together with the cationic arsenicals (e.g. arsenobetaine). Alternatively, this arsenic may have comprised several anionic compounds which, when separated, were at concentrations below the detection limit.

The data reported here for the tissues of the sperm whale agree closely, in terms of arsenobetaine, dimethylarsinate and arsenocholine, with the results from the study of Goessler et al., (1998) on arsenic compounds in whale livers. In that study, an unknown arsenic compound was also detected in all samples. Possibly, that unknown compound was also trimethylarsoniopropionate. The absence of tetramethylarsonium ion in the extracts of the sperm whale is of interest—this arsenical is a common constituent of marine organisms and usually occurs at appreciable concentrations (Francesconi & Edmonds, 1997). Tetramethylarsonium ion was also absent in the livers of whales (two species, three individuals) in the study of Goessler et al. (1998), but was detected in all the samples of seal livers (two species, 11 individuals) examined in that same study.

The identification of trimethylarsoniopropionate in the whale extracts illustrates two interesting analytical points. The first relates to the use of HPLC/ICPMS as a technique for arsenic speciation analyses. Because HPLC/ICPMS is unable to provide structural information, it cannot directly be used to identify novel compounds. Thus, molecular mass spectrometry with electrospray ionization was necessary for the first identification of trimethylarsoniopropionate as a natural product (Francesconi et al., 2000). However, the samples examined in the current study were not amenable to liquid chromatography (LC) electrospray mass spectrometry (MS) analysis because the arsenic concentrations were far too low. The great sensitivity of HPLC/ICPMS and its ability to analyse crude extracts enabled the determination of the new compound, trimethylarsoniopropionate, in whale tissues at concentrations of 0.01 μg As g^{-1} (dry mass). The second point deals with sample storage. In our study, aqueous extracts of each sample were freeze-dried and stored at -18°C. It is worth noting that the arsenic compounds in the samples stored in this manner were stable and remained unchanged after almost three years. We believe that the sample preparation and storage reported here is efficient and practical, and could be used to provide representative samples for archive purposes.

It is of interest that the arsenic concentrations are so low in the sperm whale tissues. Sperm whales can be found from the tropics to the pack-ice edges of both hemispheres. They are deep divers and inhabit oceanic waters, but can also come close to shore. Sperm whales feed on a variety of fish and cephalopods, the latter being considered to be the major prey items (Jefferson et al., 1993). Cephalopods contain high concentrations of arsenic, most of which is arsenobetaine (Shiomi et al., 1983; Morita & Shibata, 1987). Thus the diet of the sperm whale is high in arsenobetaine, and yet this compound is present at low (absolute) concentrations (0.9 μg As g^{-1} in muscle). This represents another example of the lack of correlation between trophic position and arsenic content in marine animals.

Trimethylarsoniopropionate was first reported (Francesconi et al., 2000) in fish muscle tissue, and it was suggested that this new arsenic betaine could be a common marine arsenic compound occurring together with the ubiquitous arsenobetaine. The current study showing the presence of trimethylarsoniopropionate in various tissues of a whale supports that view. It is also relevant that trimethylarsoniopropionate was present in the whale tissues even though the total arsenic concentrations were very low. The origin of arsenic compounds in organisms is not known although the recently proposed pathway of Edmonds (2000) seems to account for much of the data available so far. In that pathway, it is proposed that dimethylarsinous acid, the reduced form of dimethylarsinic acid, 'arsenylates' 2-oxo acids to give a range of naturally-occurring arsenic compounds. Trimethylarsoniopropionate could be formed from oxaloacetate following the general scheme of arsenylation proposed by Edmonds, and thus its presence in the whale tissue is consistent with that pathway.

Further support for arsenic biosynthetic pathways might come from the identification of other unknown arsenic compounds detected in marine environmental samples. Because these compounds are present in low concentrations, HPLC/ICPMS together with molecular mass spectrometric methods will play an important role in their structural elucidation.

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