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Refractory Methylated Arsenic Compounds in Estuarine Waters: Tracing Back Elusive Species

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Water from the Tagus estuary, Portugal, was concentrated and purified through evaporation, solvent extraction, ion exchange and HPLC, and peaks of refractory arsenicals were detected by difference between total arsenic (GF AA) and hydride-forming arsenic species (HG QF AA). DCI mass spectra of these fractions presented peaks at *m/z* 139, 157 and 159; the proportion of *m/z* 157 and 159 peaks, approx. 3:1, suggested a chlorinated moiety. DCI MS/MS daughter-ion fragmentation of these peaks seems compatible with dimethylarsenic (cacodylic) acid and structures of the type $Me_2As(O)Cl$ or $Me_3As(OH)F$. The refractory character of these fractions, however, cannot be explained by these structures. Further work with mixtures of halogen and arsenic species injected in the HPLC system showed that fluoride and iodide can shift DMA (dimethylarsenic) and TMAO (trimethylarsine oxide) to shorter retention times but not to R_f values similar to refractory arsenicals. These latter are attained by mixtures of sodium chloride + arsenobetaine, and sodium fluoride and chloride + arsenocholine. We suggest that peaks at *m/z* 139 and 157 correspond to fragments of a heavier refractory molecule mainly formed by halogenated betaines including chloro-arsenobetaine and chloro- and fluoro-arsenocholine.

Keywords: Arsenic, identification, DCI MS/MS, hydride generation, arsenobetaine, arsenocholine, methylarsenic, chlorine, fluorine.

INTRODUCTION

Hidden fractions of organoarsenical compounds refractory to hydride generation (HG QF AA), and eventually also to sodium hydroxide digestion, have recently become evident in estuarine waters.[1–3]

The development of suitable analytical methods for the fractions isolated in the Tagus proved, however, to be unexpectedly difficult and cumbersome; it was hampered by problems arising from the complexity of the matrices in which they appear, including salts and other organic compounds in interfering concentrations.[4]

The complete identification of these fractions seems to be nevertheless of the utmost importance, given the toxic potential and the relative weight of the reservoir of environmental arsenic that they represent within the estuarine water column compartment (between 19 and 25% of the total arsenic stock). An important link for the future understanding of the marine biogeochemical cycling of arsenic may also have emerged from these preliminary results.[3]

Although a complete identification of these compounds has not been possible so far, it is our view that these preliminary results should be presented and discussed.

EXPERIMENTAL

A variety of different analytical techniques is now available for the identification of organometallic compounds. These include different versions of gas and liquid chromatography, coupled or not with mass spectrometry (MS) and also nuclear magnetic resonance (NMR) techniques. In an attempt to obtain more structural information, strong emphasis has been put, in this work, on mass spectrometry techniques. Preliminary purification steps use different versions of liquid chromatography (LC) and a need remains for the

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quantification of the different arsenic fractions throughout the extraction/purification procedures; this has been assured by atomic absorption spectrophotometry (AA).

Collection and preservation of samples

Surface water samples were collected, for identification purposes, at Praia do Alfeite (sample I_{00}, 40 dm^3, 5 August 1990) as in previous work.[5] The samples were collected by hand, using 'Brand' polyethylene gloves, in 1 dm^3 Kartell bottles which had been presoaked in acid and rinsed. The samples were kept chilled in cold boxes. Immediately upon arrival in the laboratory, the samples were filtered through pretreated 0.45 μm AH Millipore filters. The filtrate was acidified with Suprapur hydrochloric acid (HCl) (up to 0.05 M) and stored at 4 °C until analysis.[6]

All glass and plastic ware was kept clean as indicated elsewhere.[5]

Sample processing—extraction and purification methods

Filtrates were evaporated to dryness at low temperature (below 40 °C) and the dry residue (about 2000 g) was extracted with 4300 cm^3 of methanol. These extracts were subsequently evaporated to dryness and the residue was recovered with approximately 900 cm^3 of de-ionized, double-distilled Milli-Q water, acidified up to 0.05 M with Suprapur HCl and kept cold like the original sample (I_{01}). Fraction I_{01} was passed through a weak cationic ion-exchange resin (Lewatit-CNP, Na^+) and the leakage (I_{04}) through a strong cationic ion-exchange resin (Amberlite IR-120, H^+). The pH was adjusted to approximately 8.5 and 2.9 before the weak and strong cationic columns, respectively. Bed volumes and the conditioning of the resins were as indicated previously.[5] The Lewatit column was then eluted with 1200 cm^3 of Milli-Q water and the extract (I_{02}) freeze-dried, recovered with methanol and concentrated to a volume of about 1500 μl ($I_{02/M}$); an aliquot of that fraction (<150 μL) was injected into a high-performance liquid chromatrograph (HPLC) and the arsenic peak detected was submitted to analysis by fast atom bombardment (FAB)–tandem mass spectrometry.

The strong cationic resin, on the other hand, was extracted with 20 × 250 cm^3 of 0.5 M Suprapur HCl. The first five fractions of this extract (I_{05}^{1-5}) were discarded, given the high concentration of organics; the last fraction of the 0.5 M HCl eluate (I_{05}^{20}) was also discarded due to the low arsenic content detected (GF AA); the remainder (I_{05}^{6-19}) was taken to dryness in a Heidolph VV 2001 rotary evaporator; part of the solid residue (about 400 mg), was recovered with 1 cm^3 of Milli-Q water and a fraction of 150 μl was injected into the HPLC system (30 January 1991). One of the peaks detected when total arsenic was quantified with GF AA was kept at ambient temperature and re-analysed for hydride-forming species (by HG QF AA) three months later (12 April 1991). Another fraction of that residue, about 1400 mg, was recovered with water, and 10 fractions of 150 μl of the solution thus obtained were injected into the HPLC system. The peaks detected by GF AA were assembled according to retention times and evaporated under vacuum to give volumes of 1–2 cm^3 prior to desorption chemical ionization mass spectrometry (DCI MS) and desorption chemical ionization tandem mass spectrometry (DCI MS/MS) (June 1991). Subsamples of these arsenic peaks (100 μl) were diluted in 25 cm^3 of Milli-Q water and digested with sodium hydroxide according to the usual procedure.

The remainder of the solid residue of I_{05}^{6-19} was dissolved in Milli-Q water and injected into the HPLC system. Aliquots (100 μl) of the fractions in which arsenic peaks were detected were totally digested and total arsenic quantified by the hydride method (HG QF AA). Arsenic speciation was also determined by the same analytical method in undigested aliquots (50 μl) of the same fractions. The remainder of the fractions corresponding to those peaks were further concentrated with the help of a nitrogen jet passing through a Pasteur pipette, at 40 °C, and the concentrate (50 μl) was analysed once more by DCI MS (9–11 July 1992). These same samples were later submitted to thermospray mass spectrometry (TMS).

The general scheme is depicted in Fig. 1.

Digestion procedures

Alkaline digestions were performed making the solutions 0.5 M in sodium hydroxide and by heating at 80 °C for 24 h. Total digestions were obtained by adapting the dry-ashing classical procedure of Uthe[7] to water samples. In short, an adequate volume (2 cm^3 per 50 cm^3 of sample) of ashing-aid (a slurry of 7 g of MgO + 10.5 g $Mg(NO_3)_2.H_2O$, in 100 cm^3 of Milli-Q water) was added to the sample, then the mixture was dried at 80 °C overnight and heated (1 h/200 °C +

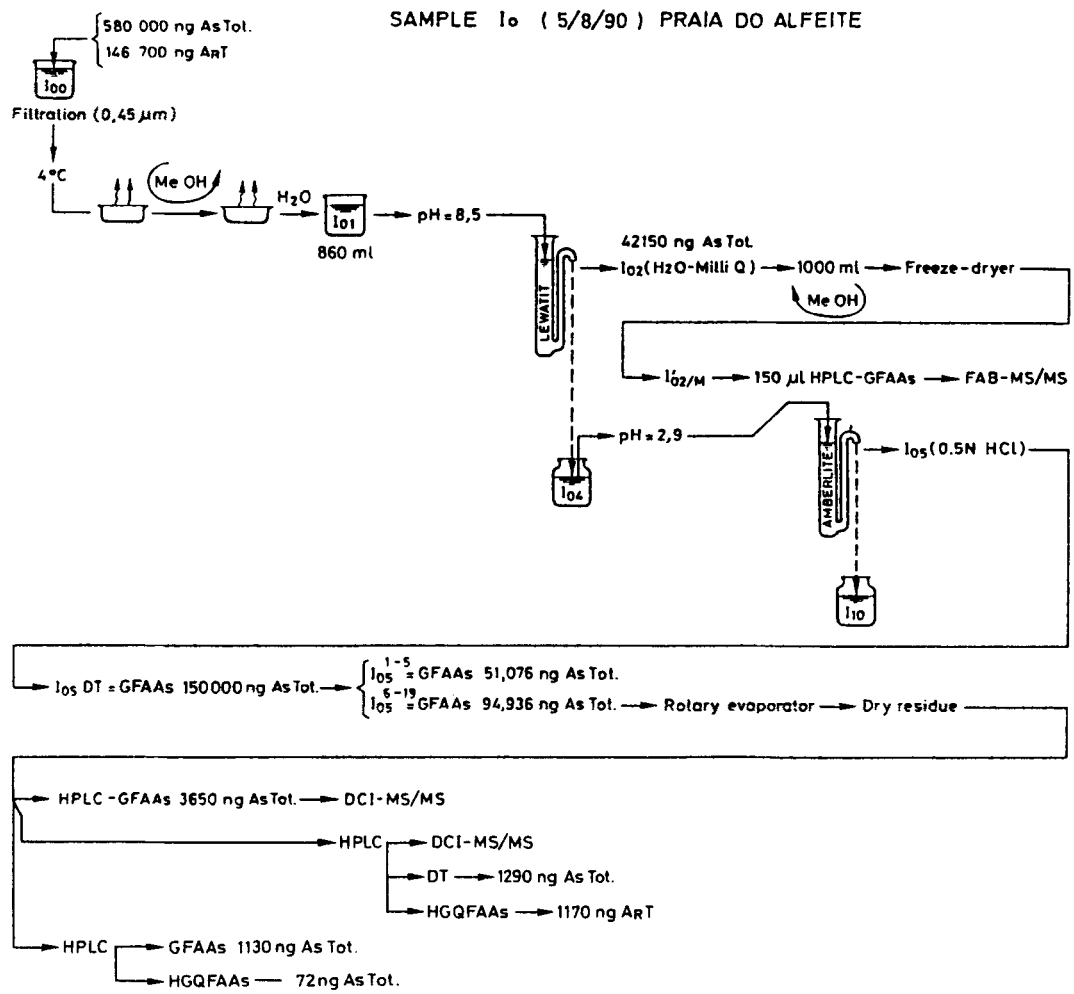


Figure 1 Flowsheet of concentration/purification process prior to mass spectrometry; As_{Tot}, total arsenic content measured by GF AA or HG QF AA after total digestion; A_{RT}, total refractory arsenic.

1 h/300 °C + 12 h/460 °C) in a muffle furnace. The dry residue was recovered with 2 M Suprapur HCl and made up to 50 cm^3 with Milli-Q water, prior to analysis. Blanks of Milli-Q water were run in the same way and arsenocholine $[(CH_3)_3AsCH_2CH_2OH^-]$ or tetramethylarsonium iodide $[(CH_3)_4As^+I^-]$ was used as internal standard to determine the yield of the digestions.

Atomic absorption spectrophotometry

Arsenic hydride-forming species were detected and quantified by hydride generation quartz-furnace atomic absorption spectrophotometry (HG QF AA) in a Perkin–Elmer 5000 AA spectrophotometer equipped with deuterium background correction, EDL source and lamp; the wavelength used was 193.7 nm; the reactor vessel was 25 cm^3 in volume and glass surfaces were passivated with silanization agents. Details are given elsewhere.[5,6,8]

Total arsenic contents were obtained through the application of the same analytical method (HG QF AA), after total digestion of water fractions, or by use of graphite-furnace atomic absorption spectrophotometry (GF AA). These latter determinations were performed in a Perkin–Elmer 2380 AA spectrophotometer equipped with an HG-500 graphite furnace; L'Vov platforms and pyrolytic tubes were used; strong matrix effects led, in some concentrated extracts, to the use of $Ni(NO_3)_2$ matrix correction. The heating programme was the following: drying at 120 °C, 50 s (inc. 15 s ramp); charring at

500 °C, 10 s (inc. 5 s ramp); gas interruption 500 °C, 5 s; atomizing at 2300 °C, 11 s (inc. 3 s ramp); ashing at 2650 °C, 6 s (inc. 2 s ramp). Details are given elsewhere.[9]

High-performance liquid chromatography (HPLC)

HPLC was used as a final polishing and detection step before mass spectrometry determinations. An LKB instrument was used throughout; the loop was 150 µl; to avoid losses of sample, aliquots of less than 150 µl were injected directly into the loop with a gas-tight syringe; the HPLC semipreparative column was a Merck Lichosorb RP-18, 250 mm long, 10 mm i.d. (7 µm, grain size of packing material); the flow of eluent was 2.0 cm^3 min^{-1}, fractions of 1 cm^3 being collected each 30 s. The eluent was a mixture of methanol and water buffered at pH 3.5 with glacial acetic acid; the elution programme was the following: 3% methanol for 10 min; linear increase up to 10% methanol over 2 min; 12 min at 10% methanol; increase to 30% methanol over 6 min; elute from 30 min to the end at 30% methanol. The collected fractions were analysed for arsenic by GF AA, the HPLC instrument not being, however, interfaced directly with the atomic absorption instrument.

Mass spectrometry

Mass spectra were recorded by use of fast atom bombardment (FAB) including fast atom bombardment tandem mass spectrometry (FAB MS/MS). FAB mass spectra, obtained with glycerol as matrix, were performed on a Kratos Concept II HH four-sector mass spectrometer. Mass spectra observations obtained by FAB mass spectrometry with 2-hydroxyethyl disulphide (HEDS) as matrix as well as desorption chemical ionization (DCI) probe measurements and B/E linked scan MS/MS results were performed on a JEOL HX110/HX119 tandem mass spectrometer equipped with Datasystem DA5000. Operational conditions were as follows: DCI filament heated 1/8 A min^{-1}, from 0 to 0.8 A; ionizing current, 100 µA; source temperature, 220 °C; CI gas, methane. Mass spectra were also obtained using a MAT 95 mass spectrometer equipped with thermospray and a Finigan–MAT interface, without an LC column. The samples were diluted in a 0.05 M ammonium acetate solution in HPLC-grade water. Operational conditions were as follows: mass range *m/z* 120–500; scan rate, 2 s $decade^{-1}$; ion source temperature 250 °C; resolution 600; thermospray vaporizer temperature, 120 °C; operation mode, positive ion.

RESULTS

In Fig. 2 and Table 1 we present inorganic arsenic ($As^{3+} + As^{5+}$) and total arsenic content of a typical HPLC peak, obtained from an aliquot of fraction I_{05}^{6-19}, prior to DCI MS analysis. The concentration of common methylated species ($MMA^- + DMA^- + TMAO$) was below detection level (in samples diluted 1 : 10).

In Fig. 3 we present, on the other hand, arsenic speciation of a 'degraded' HPLC peak obtained, as mentioned, from a different aliquot of the same fraction I_{05}^{6-19}. In this peak total inorganic arsenic ($As^{3+} + As^{5+}$) amounts to 17 ng, MMA^- arsenic to 3.5 ng, DMA^- arsenic to 1.5 ng and TMA^- arsenic to 50 ng. Total arsenic determined by GF AA, however, is 1.130 ng.

Full identification of the refractory organoarsenicals present in Tagus water was first attempted, in this study, by means of FAB mass spectrometry and FAB MS/MS both in the positive and negative modes with glyceerol as matrix and in the positive mode with a 2-hydroxyethyl disulphide (HEDS) matrix as well. The results obtained have shown that FAB mass spectrometry was not the most appropriate technique for the analysis of arsenic anions [arsenate, arsenite, monomethylarsonate, dimethylarsenate (cacodylate)], in contrast to successful analysis of quaternary arsenic compounds. From the MS/MS daughter-ion spectra of these peaks, the presence of an organoarsenic compound at *m/z* 133 was suspected, but unequivocal evidence was not obtained. This inconclusive result may be due to reduction processes occurring in the radiation-damaged matrix: these are often considered as one of the major difficulties when dealing with FAB mass spectra of metal-containing species.

On the other hand, DCI MS has been used successfully in the analysis of thermally labile compounds.[10] Moreover, DCI seems to be one of the MS techniques less affected by the presence of inorganic impurities in the sample[13] (including sodium) that may mask sample peaks when using FAB. DCI MS was therefore used as an alternative method and successfully produced a number of spectra, one of which is reproduced in Fig. 4.

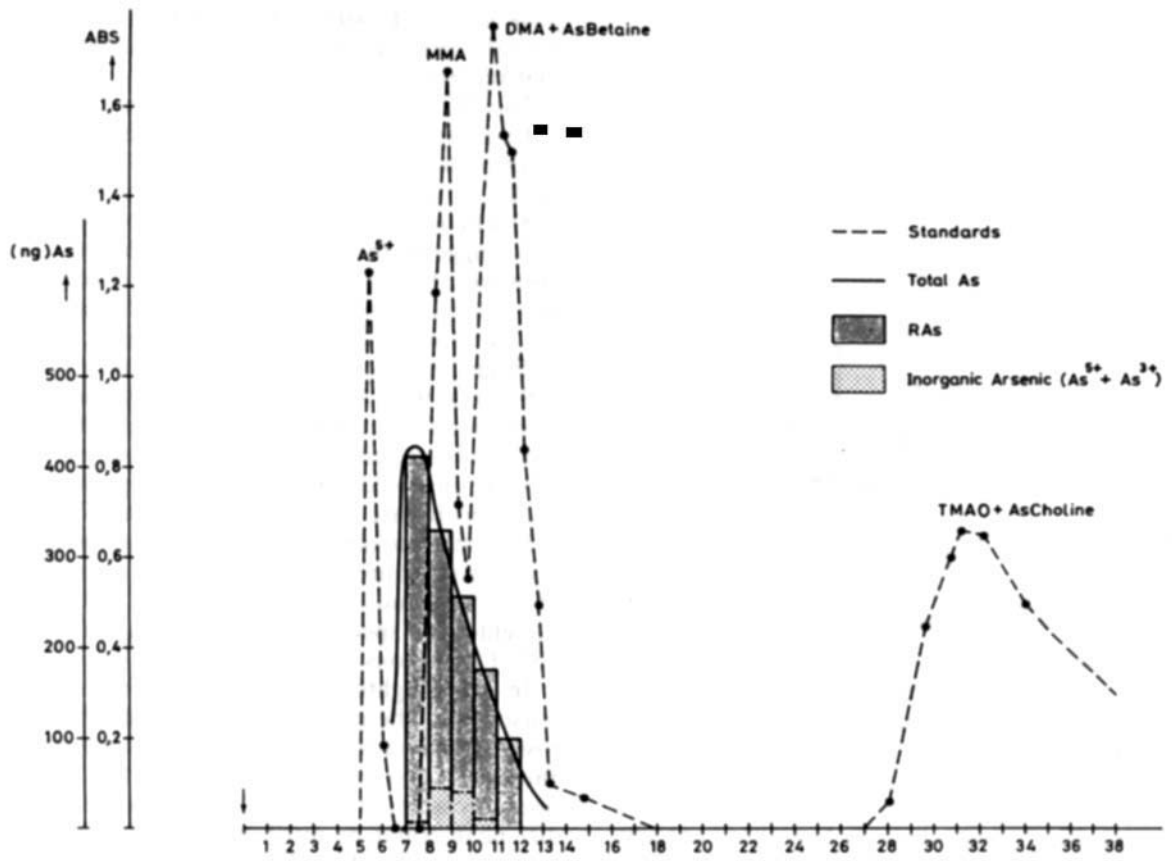


Figure 2 HPLC peak of refractory arsenic corresponding to Table 1. ABS = absorbance.

Table 1 Total arsenic, inorganic arsenic and refractory arsenic (RAs) contents of an HPLC peak (ng As)

	Total As[a] (±S.D.)	Corrected Total As[b] (±S.D.)	$As^{5+} + As^{3+}$ [c] (±S.D.)	RAs[d] (±S.D.)
Blank	45 ± 30			
Yield of standard digestion = 69% (arsenocholine)				
Tube 15	329 ± 106	413 ± 160	14 ± 8	399 ± 160
Tube 16	273 ± 91	331 ± 138	48 ± 17	283 ± 139
Tube 17	221 ± 77	256 ± 119	44 ± 19	213 ± 119
Tube 18	174 ± 64	180 ± 102	13 ± 11	175 ± 102
Tube 19	114 ± 48	100 ± 81	N.D.	100 ± 81
Total peak		1.289 ± 275	118 ± 28	1.171 ± 271

[a] Total As, total arsenic determined after total digestion. [b] Corrected total As, total arsenic content corrected for blank and yield of the digestion. [c] $As^{5+} + As^{3+}$, As-arsenate + As-arsenite. RAs, refractory arsenic.

DISCUSSION

It is known from previous works[5,9] that the chromatographic properties [thin layer chromatography (TLC), HPLC] of refractory arsenicals, as they result from our extraction/purification procedures, do not agree with the properties of available standards [arsenobetaine, arsenocholine, acetylarsenocholine, dimethyloxarsylethanol, trimethylarsine oxide, monomethylarsonate, dimethylarsinate, arsenite and arsenate]. Moreover, the TLC properties of Me_4As^+ do not seem compatible with what is known from refractory arsenic fractions.[11]

Figure 2 confirms these distinctive properties of refractory arsenic in RP-18 HPLC columns, as the refractory peak does not co-chromatograph with any of the standards of arsenic compounds assayed.

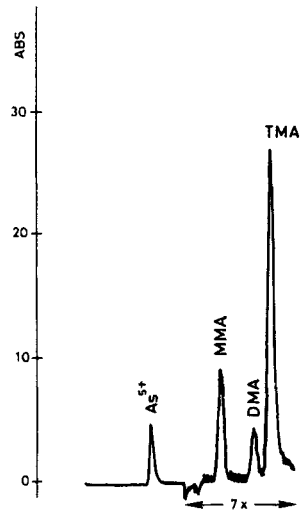


Figure 3 HG QF AA record of a 'degrading' HPLC fraction of refractory arsenic; As^{5+}, inorganic arsenic (arsenate + arsenite); MMA, monomethylarsenic; DMA, dimethylarsenic; TMA, trimethylarsenic.

The pattern of Fig. 3 suggests that, although these arsenic species are distinct from all the standard compounds mentioned, they are actually at least trimethylated. The retention time of 7.5 min is quite distinct from the retention time of TMAO in the same HPLC system (usually about 30 min), which contradicts the hypothesis of background contamination. In addition, the TMA peak obtained, with 50 ng in a total of 72 ng of hydride-forming arsenic, is clearly dominant in that record.

The DCI mass spectra first obtained for three different fractions of an HPLC peak from I_{05} (Fig. 4) presented reduced fragmentation but intense peaks, of which the relative intensity of *m/z* 157 (100%) and *m/z* 159 (35%) peaks, approximately 3:1, suggests the presence of a chlorine atom. However, this proportion could not be confirmed in a later determination of a different fraction of the same water sample (Fig. 8, below). Instead different relative abundances of the *m/z* 157 and 159 peaks, 90% and 40% respectively, along with abundant fragmentation of low intensity, were observed, stressing the complex character of the mixture involved. Other significant peaks in the mass spectra are *m/z* 139, 121, 105 and 75.

In order to obtain more information on the possible structure of the presumable *m/z* 138 and 156 compounds, we investigated further the daughter-ion spectra of *m/z* 139 (Fig. 5) and *m/z* 157 (Fig. 6) ions.

In the daughter-ion spectrum of *m/z* 139 precursor ions, the peak at *m/z* 121 argues for water loss, while *m/z* 109 could very well correspond to the loss of two methyl radicals. The loss of methane may account for *m/z* 123 ions while the *m/z* 107 peak could be attributed to the loss of two methane molecules. The presence of the rela-

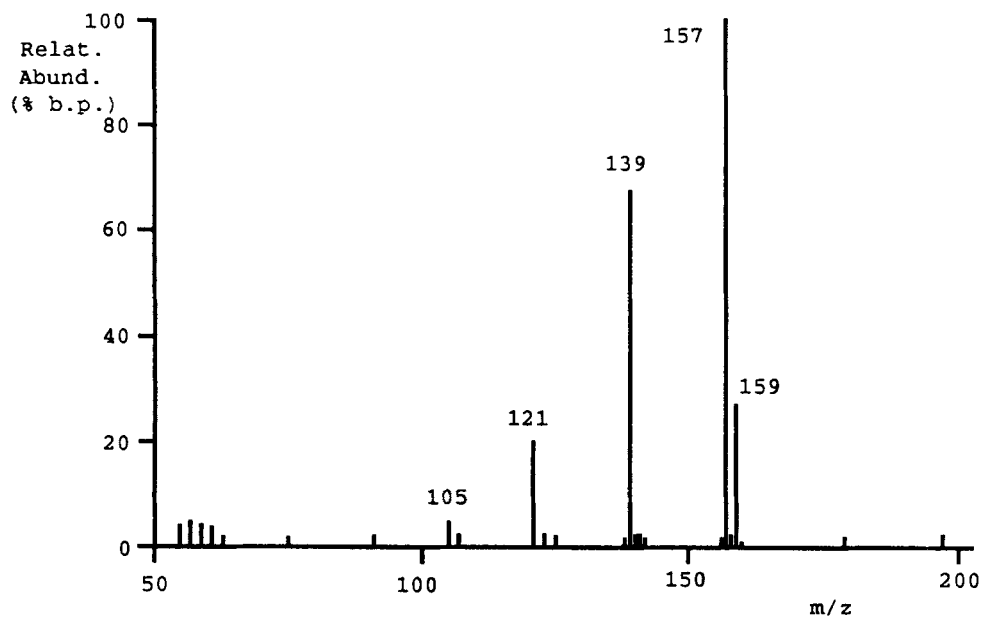


Figure 4 DCI mass spectrum in the positive mode of an extract of estuarine water using methane as reagent gas (4 June 1991).

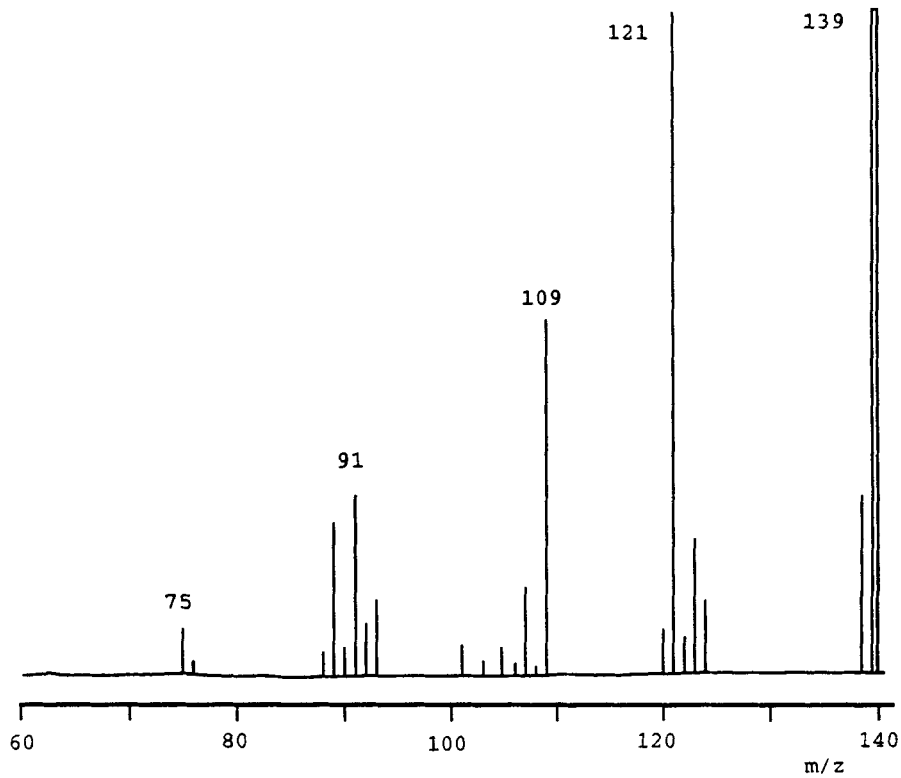


Figure 5 MS/MS spectrum (B/E linked scan) of *m/z* 139 precursor ions from DCI spectrum in the positive mode of an extract of estuarine water using methane as reagent gas.

tively abundance *m/z* 75 ions with respect to the very low abundance of *m/z* 76 and almost non-existence of *m/z* 77 ions seems to exclude the presence of an aromatic compound and to confirm the presence of arsenic in the precursor ion moiety, a conclusion which confirms what was already known from elemental analysis (AA). The *m/z* 91 ions could be assigned to $MeAsH^+/AsO^{+\bullet}$, and the other peaks in the spectrum could easily be explained in terms of arsenic-containing fragment ions. These observations seem to suggest that a protonated form of dimethylarsenic acid may be responsible for the appearance of *m/z* 139 ions. Moreover, our findings seem to be in reasonable agreement with the results reported by Siu *et al.*,[12] taking into account the difference of techniques used.

The interpretation of the daughter-ion spectrum of *m/z* 157 precursor ions (the presence of a chlorine atom having been indicated but not confirmed) seems to suggest that a protonated form of a $Me_2(O)AsCl$ species is conceivable and could be responsible for the *m/z* 157 peak observed. On this assumption the presence of *m/z* 142 and 127 peaks could be attributed to the loss of one methyl radical and two methyl radicals, respectively, while *m/z* 121 and 107 peaks could be due to the elimination of hydrogen chloride (HCl) and methyl chloride (CH_3Cl) respectively. Moreover, if *m/z* 157 ions did correspond to a protonated form of a $Me_2(O)AsCl$ species, *m/z* 76 and 77 peaks could be attributed to AsH^+ and AsH_2^+ structures respectively, while the arsenic ion would be responsible for the appearance of the *m/z* 75 peak. In addition, *m/z* 91 and 92 peaks could very well be attributed to $AsO^{+\bullet}$ and $AsOH^+$ respectively, while $AsCH_2^+$ could be assigned to *m/z* 89 ions.

We should emphasize, however, that in addition to chlorinated species the presence of a fluorinated species, such as $Me_3(OH)AsF$, seems possible and entirely compatible with the fragmentation pattern observed. In this case the presence of *m/z* 142, 127, 121 and 107 peaks could be attributed to the loss of one methyl radical, two methyl radicals, fluorine hydroxide (FOH) and $(2CH_3^{\bullet} + FH)$, respectively. Moreover, if *m/z* 157 ions did correspond to a protonated form of a

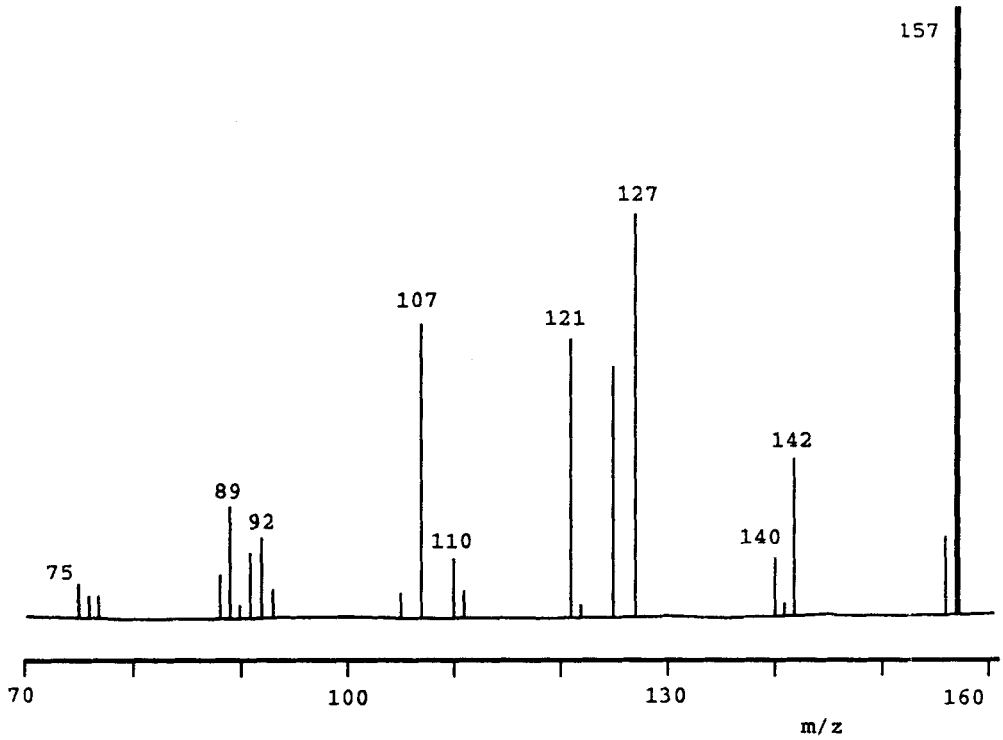


Figure 6 MS/MS spectrum (B/E linked scan) of *m/z* 157 precursor ions from the DCI spectrum in the positive mode of an extract of estuarine water using methane as reagent gas.

$Me_3(OH)AsF$ species, the presence of the two hydrogen atoms could be responsible for the appearance of *m/z* 76 and 77 peaks, for which AsH^+ and AsH_2^+ structures, respectively, would be conceivable, while the *m/z* 75 peak is likely to be the arsenic ion. In addition, *m/z* 91 and 92 peaks could very well be assigned to $MeAsH^+/AsO^+$ and $AsOH^+$, respectively, and $AsCH_2^+$ be assigned to *m/z* 89 ions; *m/z* 159 ions would therefore correspond to Me_3AsF_2. It has not therefore been found possible to put forward a satisfactory explanation for the MS/MS fragmentation pattern observed for the peak at *m/z* 157 without the presence of a bonded halogen.

It is noteworthy that in measurements performed later on the same fractions we have not been able to reproduce these spectra. Nevertheless, they were again observed in DCI MS determinations on another fraction of the same extracts (Fig. 7).

The considerable dependence of DCI spectra on the surface properties of the wire as well as on the thermal processes occurring there is well known.[13] Therefore the rapid decay of total ion current together with the ion currents associated with ions *m/z* 157, 139 and 75 (Fig. 8) suggest a rapid poisoning of the DCI probe's filament, and may be responsible for the non-reproducible appearance of the spectra. It might also be that, by using 1 μl subsamples in the DCI probe, we are operating near the absolute detection limit of these species for this technique.

Attempts to confirm, by high-resolution mass spectrometry (HR MS), the presence of such structures in the water fractions were not successful, apparently because of the minute quantities involved. Thermospray mass spectrometry (TMS)—which has proved successul in the detection of molecular ions of organoarsenic compounds[14]—was not encouraging either, apparently because of similar difficulties, although good-quality spectra were obtained for a number of synthetic standards (e.g. dimethylarsinate and monomethylarsonate).

Refractory (or resistant) nature of the compounds

We are conscious that these findings raise a number of questions that are difficult to answer at present.

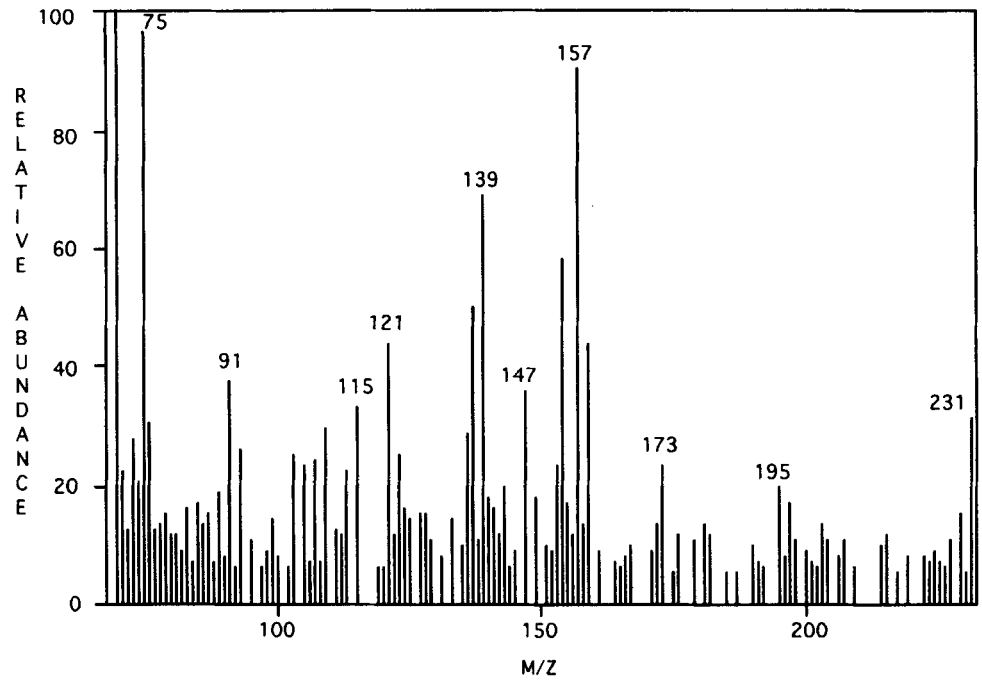


Figure 7 DCI mass spectrum in the positive ion mode of another aliquot of the same extract as Fig. 4, using methane as reagent gas (9 July 1992).

An inescapable question is why are these compounds refractory (both to $NaBH_4$ hydride production and NaOH digestion, as is the case with the I_{05}^{6-19} fraction used to produce DCI MS spectra) if the suggested structures are the true structures of the original (refractory) arsenic fraction

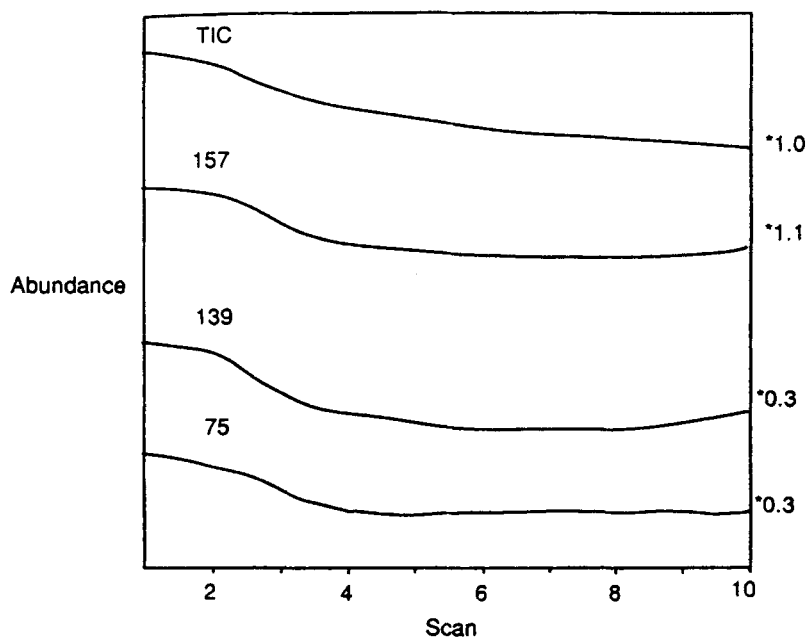


Figure 8 Mass chromatogram showing the decay of the total ion current (TIC), *m/z* 157, 139 and 75 ion abundances, with scan number.

in estuarine waters?

Indeed, nothing in the proposed structures seems to predispose them to such a refractory character. If some doubts persist for the halogenated species proposed for *m/z* 157, it is more than clear that cacodylic acid cannot be present (in significant quantities) in the samples analysed by DCI MS; otherwise they would have produced dimethylarsine on $NaBH_4$ attack. That is, however, not the case (Table 1). We observed also that an NaOH digestion has not been able to change this behaviour immediately prior to MS/MS determinations.

It is also not yet entirely clear why the halogenated structure proposed for *m/z* 157 is not hydrolysed to cacodylic acid in marine water.

HPLC experiments

The spectra obtained may not be understood without some form of strong association between the halogen and the arsenical moieties.

Following this idea, halogen hydrides were added to different arsenic species and the resulting solutions were passed through the HPLC system.[18] These experiments proved that the addition of F^-, Br^- and I^-, but not Cl^-, shifts the peaks of DMAA and TMAO to shorter retention times. This shift is particularly significant in the case of HF + TMAO and suggests some form of association between both types of compounds that could eventually result in the formation of haloarsenic compounds.

In fact there is experimental evidence[16,17] showing that HF added to $(CH_3)_3AsO$ forms complexes of the type $(CH_3)_3AsO \cdot HF$. The retention times of these experimental HPLC peaks (TMAO + F^-) were not coincident, however, with our refractory peaks (15–20 min instead of 6–10 min). Moreover, they were shown to produce hydrides with $NaBH_4$. One would therefore expect the HPLC refractory arsenic peaks isolated immediately before MS determinations, if they correspond to the chemical structure of these hydrates, to behave in a similar way producing arsines with $NaBH_4$ and eventually also trimethylarsine oxide with NaOH. Once more, this has not been the case—which suggests that in our refractory moieties the halogen is attached to a distinct point of the molecule, possibly directly to the arsenic atom.

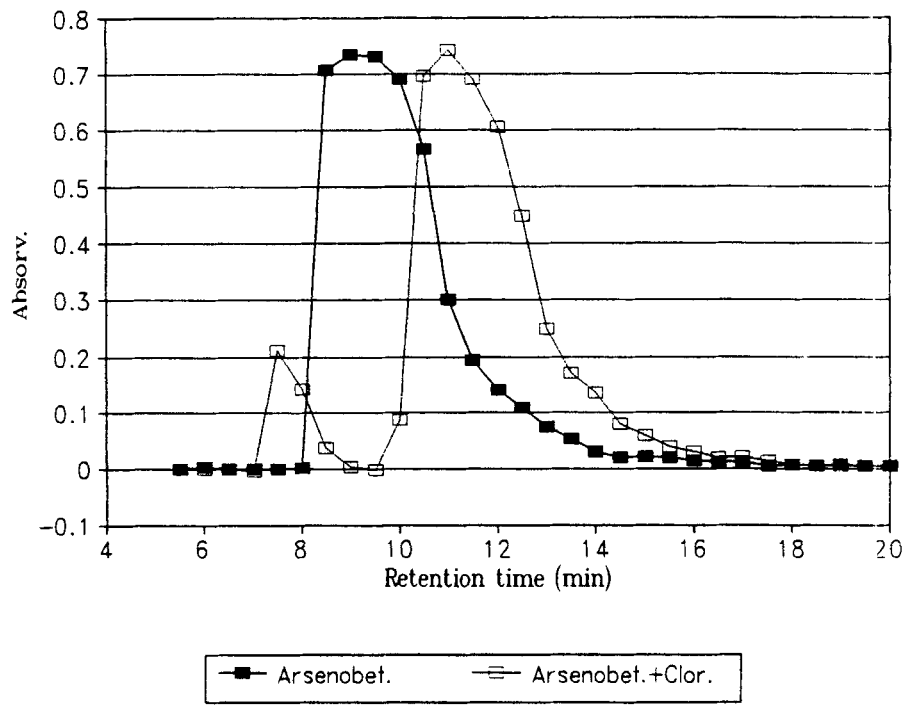


Figure 9 Shift of arsenobetaine HPLC R_f with added sodium chloride: ■, arsenobetaine; □, arsenobetaine plus NaCl.

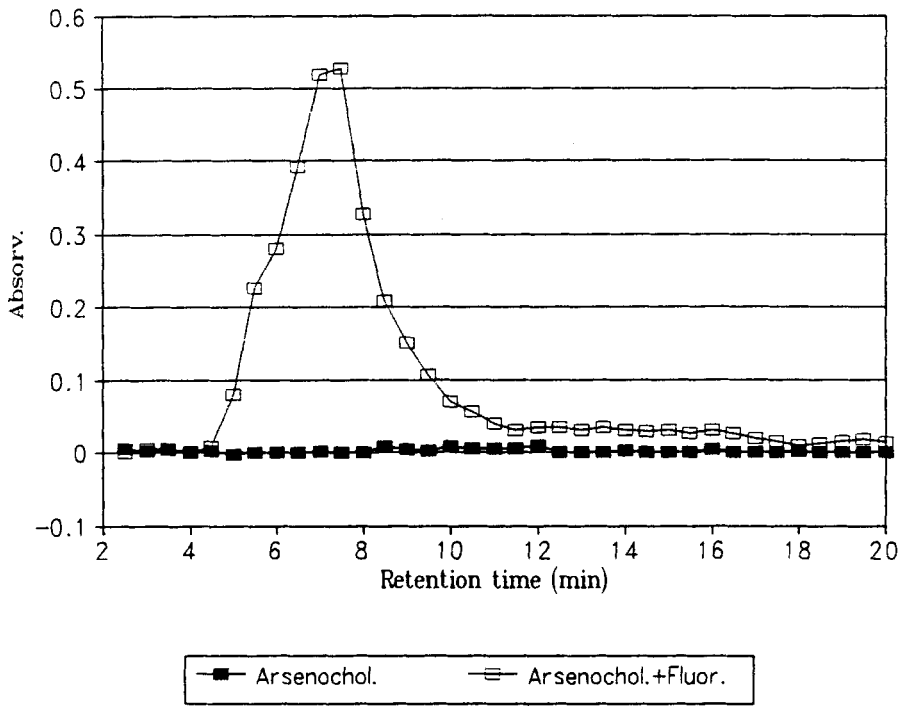
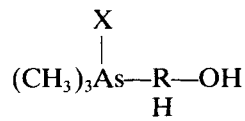


Figure 10 Shift of arsenocholine HPLC R_f with sodium fluoride: ■, arsenocholine; □, arsenocholine plus NaF.

The hypothesis of a refractory precursor

A possible explanation for a great deal of the collected evidence is the hypothesis that peaks at *m/z* 139 and 157 account, not for molecular ions, but for ion fragments of the molecule of a precursor ion—as well as some of the other peaks present. This precursor would be refractory to borohydride reduction, and eventually also to sodium hydroxide digestion, while that would not be the case for the moieties produced by DCI MS fragmentation. In fact, a structure such as, for instance:

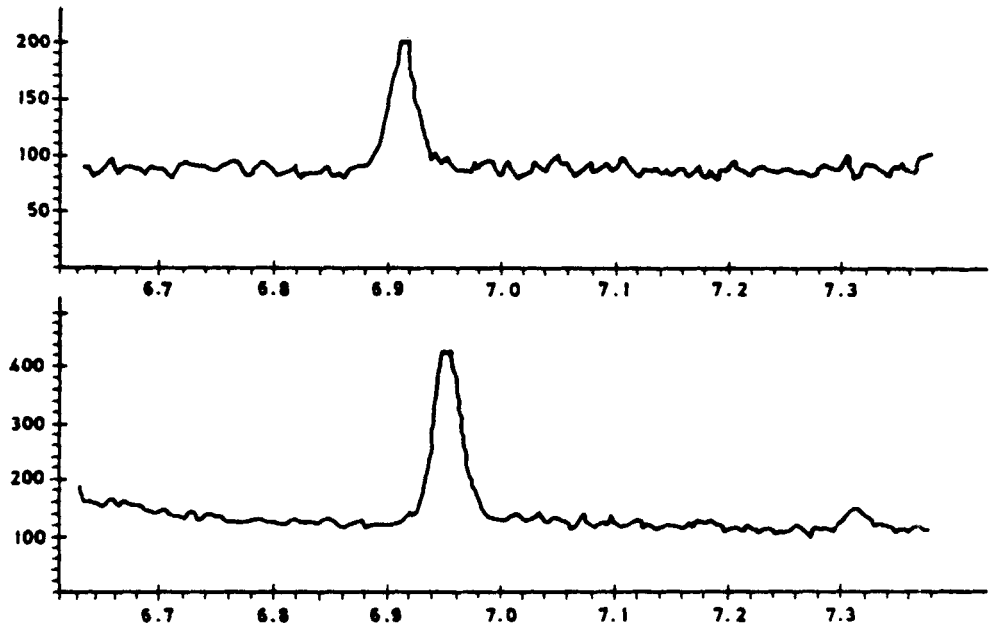


(X = Cl, F; R = carbon chain with or without =O)

might easily explain the observed DCI MS fragmentation. The peak at *m/z* 139 would be, in this case, preferentially assigned to $Me_3As(OH)H^{+\cdot}$ and not to cacodylic acid as the DCI MS/MS mass spectra observed seemed to suggest.

There is apparently no theoretical objection to the existence of such a compound and some mild empirical evidence is even reported by Goetz and Norin[18] in support of the occurrence of a chlorine-bonded arsenobetaine. Furthermore, fluorine–arsenic and chlorine–arsenic bonds are believed to be of an intermediate nature, between ionic and covalent, in the related trimerthylarsonium hydroxyhalides,[19] fluorine–arsenic bonds being the most stable, thermally, in the dihalides.[17] Moreover it is known, from preparative chemistry, that covalent fluorine–arsenic bonds can be obtained by oxidative direct fluorination of organoarsanes.[20,21]

Such a moiety, protected by the halogen against the attack of heterotrophs, would tend to be relatively stable in the environment.[22] Moreover, the mechanism for alkali attack on this molecule seems likely to give low yields, as the halogen-arsenic bond will reduce the positive charge of the arsenic atom. Also, such a compound could quite probably be refractory to $NaBH_4$ reduction, in our operating conditions, because the arsines produced would probably be too heavy for volatility. According to previous ultrafiltration findings,[1,3] it is unlikely that the molecular weight of the entire molecule will exceed 210 Da (RMM).



ION 163.00 amu from TMA.M
TMA.M.

peak #	ret time	area	start time	end time
1	6.952	6.216	6.900	7.061

ION 166.00 amu from TMA.M.

peak #	ret time	area	start time	end time
1	6.913	2.820	6.820	7.017

Figure 11 Pyrolysis mass fragmentogram of an estuarine water extract (22 June 1987) showing typical fragments of derivatized arsenocholine (dimethylarsinoethyl propionate), *m/z* 163, and its deuterated variant, *m/z* 166.

In an attempt to test this hypopthesis, we have prepared mixed solutions of synthetic arsenobetaine [carboxymethyl (trimethyl) arsonium bromide] and arsenocholine [2-hydroxyethyl (trimethyl) arsonium bromide] with excess sodium chloride and sodium fluoride, in pure double-distilled Milli-Q water. After a period of storage, intended to favour the completion of any eventual reaction between the compounds present, these solutions were injected into the HPLC system and the arsenic peaks were detected by GF AA as usual.

The most significant findings of these experiments are depicted in Figs 9 and 10. Here one can observe that the arsenobetaine standard is split into two peaks on reaction with sodium chloride, the lower peak being coincident with the refractory arsenic peaks (tube 15; 7.5 min). One may observe also that the association of fluorine with arsenocholine produces a single peak and significantly shortens the retention times. This compound also occurs with R_f values identical to those of refractory arsenicals. Moreover, chloride + arsenocholine shifts the peak of this compound to R_f values close to that refractory arsenic (tube 14; 7 min). Fluoride added to arsenobetaine does not change the retention time of this compound in our HPLC system. Some HPLC refractory arsenic peaks also suggest the presence of two compounds instead of one.

Therefore, refractory arsenicals could be the result of the halogenation of rather common

arsenical moieties leading to halo-arsenobetaines or halo-arsenocholines, or eventually both. The stability required for a strong halogen–arsenic bond will favour fluorine within the halogens, and strong cations, such as arsenocholine, within the betaines. The often observed indigestible behaviour with alkalis will also favour arsenocholine. It is also known that fluorine displaces chlorine in the related trimethylarsonium dichlorides.[19] The DCI MS spectra observed are in favour of a chlorinated species, however. The possibility that fluorinated and chlorinated arsenic species are present in the same samples should not, therefore be ruled out.

In this connection, one should remember that, in a previous occasion, typical arsenocholine fragments (Fig. 11), equivalent to 211 ng of As-arsenocholine, have been observed in a Tagus water extract submitted to pyrolysis–mass fragmentometry.[23] This earlier observation seems coherent with the evidence reported here as the halogen–arsenic bond will probably be broken down by pyrolysis at 600 °C.

The possibility of an artifact?

A fact that may hinder these conclusions from a study in which estuarine water samples—a rather complex mixture—are concentrated by a factor of at least 40 000:1, is the possibility of an artifact that would change the nature of the componds in question. This might occur during the long stepwise processing of the samples, or even in the mass spectrometer chamber. One cannot exclude the possibility, therefore, that an artifact might be at the origin of the halogenated species detected.

CONCLUSIONS

Refractory organoarsenic species present in the complex estuarine waters of the Tagus appear to be, at least in part, trimethylated.

On the grounds of DCI MS spectrometry results, DCI MS/MS fragmentation patterns and the RP-18 HPLC retention times observed, we suggest that the refractory arsenic fraction is mainly formed by halogenated arsenobetaines. Those may include chloroarsenoglycine-betaine [$(CH_3)_3As(Cl)CH_2COO^-$], chloroarsenocholine [$(CH_3)_3As(Cl)CH_2CH_2OH$] and fluoroarsenocholine [$(CH_3)_3As(F)CH_2CH_2OH$] or other haloarsenobetaines.

Several questions still remain unanswered, especially concerning the non-reproducible character of the DCI MS spectra, the possibility of an artifact during the concentration/purification process and the geochemical mechanisms that might be responsible for the formation of such compounds in the marine environment.

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