A new arsenobetaine from marine organisms identified by liquid chromatography–mass spectrometry

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A new arsenic-containing betaine, trimethyl(2-carboxyethyl)arsonium inner salt 2, has been identified in fish muscle tissue by liquid chromatography–mass spectrometry.

Arsenic is a common constituent of marine organisms where it occurs in a wide range of chemical forms.[1] The major form in marine animals is arsenobetaine **1**, while arsenic containing carbohydrates (dimethylarsinoylribosides) are the predominant forms in marine algae. The biogenesis of the various arsenic compounds is still not known, although plausible pathways have been proposed.[2,3] Unknown arsenic compounds have also been reported in marine samples, the most widespread of these being a cationic arsenical present in fish, molluscs and crustaceans.[4–9] This unknown compound was detected in aqueous (or aqueous methanol) extracts of tissues by HPLC with arsenic-specific detectors, such as an inductively coupled plasma mass spectrometer (ICPMS),[4–8] or an atomic fluorescence spectrometer.[9] Determination of the precise chemical structure of this compound may help elucidate biogenetic

$Me_3As^+CH_2COO^-$ **1** $Me_3As^+CH_2CH_2COO^-$ **2**

pathways for arsenic.

Recently, we analysed (HPLC–ICPMS) aqueous extracts of a range of tropical marine organisms, and found that a coral reef fish, *Abudefduf vaigiensis* (Pomacentridae), contained significant amounts of the unknown compound (8% of the total arsenic), in addition to arsenobetaine, which constituted *ca*. 90% of the arsenic. The extract from *A. vaigiensis* was subjected to TLC (cellulose on a glass plate; butan-1-ol–acetic acid–water 60 : 15 : 25); the cellulose support was then cut into narrow bands (5 mm), each of which was extracted with water (2.0 mL) and a portion (20 μL) of the extract analysed for arsenic by graphite furnace atomic absorption spectrometry. Arsenic was detected in three adjacent fractions in an apparently homogeneous band centred at R_f 0.54, matching the R_f for arsenobetaine. Subsequent HPLC–ICPMS analyses, however, showed that the two slower running TLC fractions contained all their arsenic as arsenobetaine, whereas the faster running fraction contained both arsenobetaine and the unknown arsenical (2 : 1 in terms of arsenic). The concentration of the unknown in this solution (175 ng As cm^{-3}) was now sufficient to enable detection of arsenic (*m/z* 75, As^+) by positive ion LC electrospray MS.[10]

Cation-exchange LC electrospray MS of the solution at 200 V fragmentor voltage and measuring *m/z* 75 yielded only two peaks with retention time 1.83 (arsenobetaine) and 4.31 min (unknown). The analysis was then repeated at 70 V fragmentor voltage and the eluent was analysed in scan mode measuring *m/z* 141–160 (we made the initial assumption that, under these conditions, we were seeing predominantly the $[M + H]^+$ molecular species). This process was repeated scanning from *m/z* 161–180, and so forth, up to *m/z* 400. Only one ion (*m/z* 193), eluting at 4.33 min matched the retention time for the unknown arsenic peak; the closest other ions had retention times of 4.02 (*m/z* 162) and 4.74 min (*m/z* 151). The procedure was then carried out at variable fragmentor voltages,[10] which enabled simultaneous detection of *m/z* 75 (arsenic) and 193 (presumed $[M + H]^+$ for the unknown); the retention times for the two peaks agreed exactly (Fig. 1).

The chromatographic properties and mass spectral data for the unknown were consistent with the structure of the arsenic betaine **2**. An authentic specimen of **2**, prepared[11] by reacting trimethylarsine with 1-bromopropionic acid, was then analysed by LC electrospray MS. It produced retention time and mass spectral data (Fig. 1) matching those obtained for the unknown. Our previous experience with the unknown had revealed a

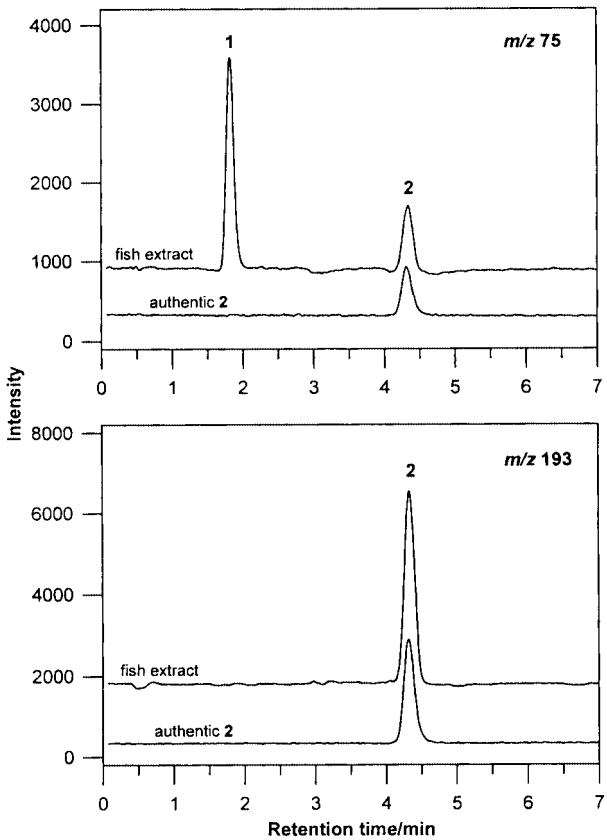

Fig. 1 LC electrospray mass chromatograms of partially purified extract from coral reef fish and authentic compound **2** at *m/z* 75 and 193. Intensity baselines for the fish extract have been offset (600 units for *m/z* 75 and 1500 units for *m/z* 193) to facilitate comparison with chromatograms for authentic compound **2**. LC conditions were: Ionospher C cation-exchange column (3 × 100 mm, 5 μm) from Chrompack; mobile phase was 20 mM aqueous pyridine pH 2.6 (adjusted with HCOOH) mixed with methanol (9 : 1) at 30 °C and flow rate 1.0 mL min^{-1}; 5 μL injection; ions were detected with a G1946A MSD single quadrupole mass spectrometer (Hewlett Packard, Waldbronn, Germany) equipped with an atmospheric pressure ionization (API) LC-MS interface operating at variable fragmentor voltages 70 (*m/z* 193) and 200 V (*m/z* 75).

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marked decrease in retention time (cation-exchange) as the pH of the mobile phase was increased, behaviour consistent with a bipolar compound. In the present study, when the chromatography was repeated at pH 4.0, the peaks (*m/z* 75 and 193) for the unknown and for authentic compound **2** both moved to retention time 1.65 min. Characteristic *m/z* fragments for compound **2** were then determined, and the optimal fragmentor voltage was found for the major fragments at *m/z* 120 (Me_3As^+) and 105 (Me_2As^+). LC electrospray MS with variable fragmentor voltages enabling simultaneous detection of the four ions characteristic of compound **2** (*m/z* 75, 105, 120 and 193), was performed on the fish extract, authentic compound **2**, and a co-injected mixture of the two; the mass chromatograms showed coincidence of peaks in all cases (Fig. 2). On the basis of these data, we assigned the new arsenic species from *A. vaigiensis* as trimethyl(2-carboxyethyl)arsonium inner salt **2**. Subsequent HPLC–ICPMS analyses of the sample and authentic material produced data entirely consistent with this assignment.

An unknown cationic arsenic compound (considered to be the same compound) has been reported as a constituent of several certified reference materials[4,5,9] including DORM-2[5] (dogfish muscle tissue from the National Research Council of Canada, Ottawa, Ontario, Canada). LC electrospray MS of an aqueous extract of DORM-2 indicated the presence of compound **2**. However, although the *m/z* 193 molecular species gave a clear signal, the low concentrations of **2** resulted in only a small *m/z* 75 peak. Verification of the new arsenic betaine **2** in DORM-2 was provided by HPLC–ICPMS (a more sensitive technique for determining arsenic ions) by co-chromatography with the authentic standard. The concentration found in this work (0.17 ± 0.05 mg As kg^{-1} dry mass, $n = 3$) is in good agreement with previously published data.[5] Possibly, in future studies on the new arsenic betaine, DORM-2 can serve as a reference sample for those laboratories that do not have access to synthetic material.

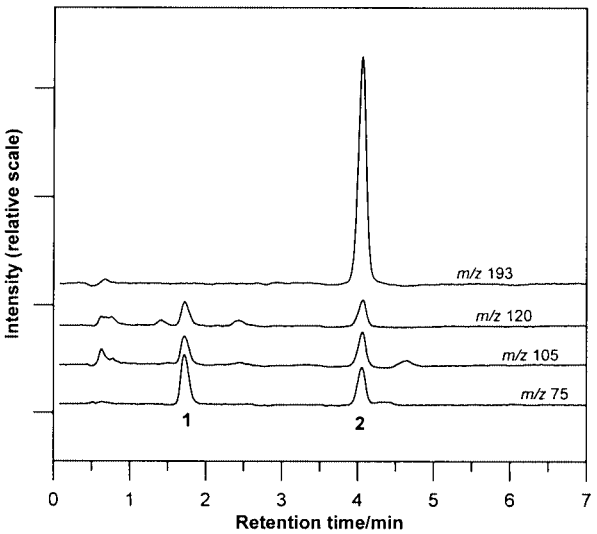

Fig. 2 LC electrospray mass chromatograms of partially purified extract from coral reef fish (5 μL) co-injected with a solution (10 μL) of authentic compound **2** (mixture was 1:1 in terms of concentration of **2**). LC-MS conditions were as described in Fig. 1, except that variable fragmentor voltages were 70 (*m/z* 193), 150 (*m/z* 120, 105), and 200 V (*m/z* 75).

The chromatographic properties of the arsenic betaine **2** match those for the unknown arsenic cation reported in certified reference materials and in a range of marine organisms.[4–9] Consequently, we consider it likely that **2** will prove to be a common constituent of marine animals, in many cases occurring together with arsenobetaine **1**, albeit at much lower concentrations. The presence of the two arsenic betaines **1** and **2** in marine animals offers some insight into the biogenesis of arsenic-containing natural products. A previously proposed pathway[2] suggested that arsenobetaine **1** might be derived from dimethylarsinoylribosides (common metabolites of algae), with the carboxymethyl group of **1** being formed from C4 and C5 of the ribose. Dimethylarsinoylribosides, however, seem less likely to be implicated in the biogenesis of **2** (*i.e.* as a source of the carboxyethyl group) and hence their involvement in the biogenesis of arsenobetaine **1** might also be questioned. A scheme recently proposed by Edmonds[3] may more readily account for the presence of both **1** and **2** in marine organisms. In that scheme, the possible 'arsenylation' of 2-oxo acids by a process analogous to their amination to amino acids was used to explain the origin of several naturally occurring arsenic compounds, including arsenobetaine. The proposed agent for 'arsenylation' was dimethylarsinous acid (Me_2AsOH), the reduced form of the common arsenic metabolite dimethylarsinic acid. Reaction of dimethylarsinous acid with the common 2-oxo compound oxaloacetic acid in the general scheme outlined by Edmonds[3] could give rise to compound **2**.

Identification of other unknown arsenic compounds detected in marine samples will improve our understanding of arsenic-containing natural products. Possibly, these compounds are also arsenic betaines, and structural information might quickly be obtained by mass spectrometric studies (LC–MS and MS/MS) focussing on such compounds.

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