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Thio arsenosugars identified as natural constituents of mussels by liquid chromatography-mass spectrometry

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Two novel thio arsenosugars have been identified by liquid chromatography-mass spectrometry as significant arsenic constituents in samples of mussels.

The first report in 1977 of the innocuous arsenobetaine [$(CH_3)_3As^+CH_2COO^-$] as the major arsenical in lobster,[1] and later in many other marine animals,[2] allayed toxicological concerns about high arsenic concentrations in seafoods. Subsequent work, however, revealed other naturally occurring organoarsenic compounds in marine samples, in particular a range of arsenic-containing carbohydrates collectively termed arsenosugars which occur in algae and organisms feeding on algae such as molluscs.[2] The toxicology of arsenosugars is yet to be fully evaluated, but it is likely to be complex because they are biotransformed in humans to many as yet unidentified arsenic metabolites.[3,4]

As part of our studies on the human health consequences of arsenic in foods, we have analysed food items with high performance liquid chromatography using inductively coupled plasma mass spectrometry as the arsenic selective detector (HPLC-ICP MS).[5] One sample, a canned mussel product,[6] was of interest, because analysis of an aqueous extract of the mussel showed the presence of an unidentified arsenic compound (U1), in addition to common arsenicals namely arsenosugars **1** and **2** (Scheme 1), arsenobetaine, trimethylarsoniopropionate, and dimethylarsinate. With time (several days at room temperature), however, peaks for **1** and **2** in the extract increased with a corresponding, although smaller, decrease in the peak for U1, and on treatment of the sample with H_2O_2 (note 7) the peak for U1 completely disappeared while peaks for both **1** and **2** increased substantially (Fig. 1). Although it first appeared as though U1 may be serving as a precursor to both arsenosugars **1** and **2**, mass balance calculations suggested that U1 was converting solely to arsenosugar **1**, and the precursor of arsenosugar **2** was a possible fourth arsenical which was being retained on the HPLC column. Indeed, when the chromatography was repeated under different conditions (pH 10.3, see note 5), an additional peak (U2) was observed, and when the extract was treated with H_2O_2 this peak disappeared with a concomitant quantitative increase in the peak for arsenosugar **2** (Fig. 1; Table 1).

The observation that the unknowns U1 and U2 were readily converted in air into arsenosugars **1** and **2** (both arsine oxides) suggested that they may be the corresponding thio arsenic species **3** and **4** (Scheme 1). Although there have been no reports of such species as natural products, a recent study[8] showed the presence of a thio arsenic species [$(Me)_2As(S)CH_2COOH$] in sheep urine. We then synthesised[9] analytical quantities of **3** and **4** to serve as standards for HPLC-ICP MS and (later) HPLC-electrospray MS. The chromatographic behaviour of the standard compounds **3** and **4** at pH 5.6 and pH 10.3 matched those for the two new arsenicals, U1 and U2 respectively, present in the mussel extract. Spiking experiments, whereby standard **3** or **4** was added to the mussel extract, produced suitably enhanced undistorted peaks for U1 (pH 5.6, t_R 16.8 min; pH 10.3, t_R 4.3 min) or U2 (pH 10.3, t_R 6.4 min), respectively.

Further evidence for the proposed new arsenicals was provided by HPLC-electrospray MS.[10] Electrospray mass spectra[11] for the two standard thio arsenicals showed clear $[M+H]^+$ protonated molecular ions (for both ^{32}S and ^{34}S) at *m*/*z* 499/501 (compound **4**) and 345/347 (compound **3**) in addition to characteristic product ions at *m*/*z* 253/255 (loss of aglycone), and signals for 107 (AsS^+), and 91 (AsO^+, formed from traces of O_2 in the N_2 drying gas[12]); these ions were selectively monitored in the HPLC-electrospray MS analyses. Analysis of the mussel extract under the optimised conditions produced the ions (*m*/*z* 501, 499, 253, 91) characteristic for compound **4** at a retention time (t_R = 13.3 min) exactly matching

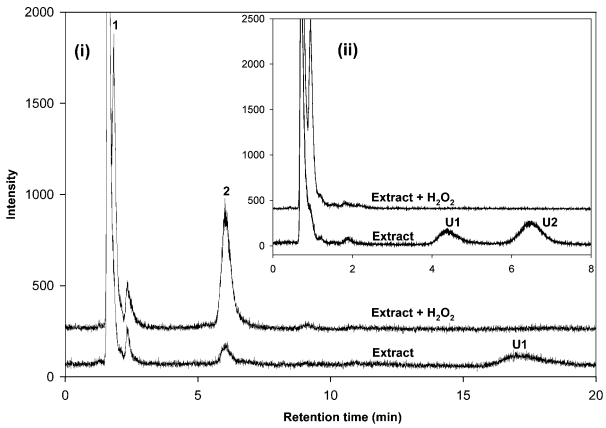
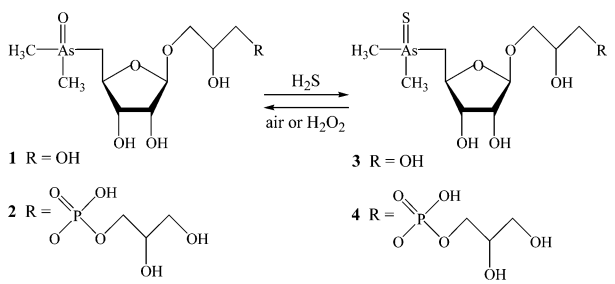


Fig. 1 HPLC-ICP MS of aqueous extract of canned mussel before and after treatment with H_2O_2 at pH 5.6 (i), and pH 10.3 (ii) (see note 5).



Scheme 1 Interconversion of novel thio arsenosugars with known arsenosugars.

Table 1 Concentrations of As species (μg As l^{-1})[a] in a fresh extract of canned mussel, and in the same extract after treatment with H_2O_2

As species	t_R[b]/min	Extract[c]	Extract + H_2O_2[c]
1	3.5 (iii)	7.9 (5.1%)	34.5 (4.3%)
U1	4.4 (ii)	27.6 (3.8%)	< 1.0
2	6.0 (i)	7.5 (6.1%)	55.2 (4.2%)
U2	6.5 (ii)	49.1 (5.0%)	< 1.0

[a] Values have been normalised to accommodate small differences in volumes due to addition of H_2O_2. [b] Number in parenthesis represents one of three HPLC conditions described in note 5. [c] Mean and relative standard deviation of three HPLC measurements.

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that of the standard (Fig. 2). Retention time (t_R = 5.1 min) matching was also achieved for compound **3** in the mussel extract, but on this occasion clear signals could be obtained only for $[M+H]^+$ at *m*/*z* 345 and for *m*/*z* 91; the product ion *m*/*z* 253 was also detected but the peak was not well resolved. Individual experiments in which the mussel extract was spiked with synthesized **3** or **4** produced single enhanced peaks for U1 and U2, respectively. On the basis of these HPLC-electrospray MS data and the HPLC-ICP MS results, the structures for the two arsenicals U1 and U2 in mussel were assigned as the novel thio arsenosugars **3** and **4** respectively.

Because our sample was a canned mussel product, it seemed possible that the new arsenic species present in the sample were artefacts from the processing procedure. HPLC-ICP MS analysis of an aqueous extract of fresh, unprocessed mussels (*Perna canaliculus*), however, similarly revealed the presence of the thio compounds **3** and **4** indicating that they are natural products (we cannot disregard the possibility of these changes being elicited between time of sample collection and analysis). We have also carried out preliminary investigations of other molluscs and a crustacean sample, and have found **3** and **4** to be present in most cases. These data suggest that thio arsenosugars are significant seafood arsenicals, in molluscs at least, which raises the question as to why they have so far eluded detection in the many previous studies investigating arsenic species in marine samples. Possibly, the unusual chromatographic behaviour of these thio arsenosugars (they are strongly retarded on the anion exchange column PRP-X100 at typically used pHs 5–6), together with their ready conversion to the corresponding oxides have contributed to their being overlooked until this study. The likelihood of other thio arsenosugars occurring as natural products, and so far escaping detection, must also be considered.

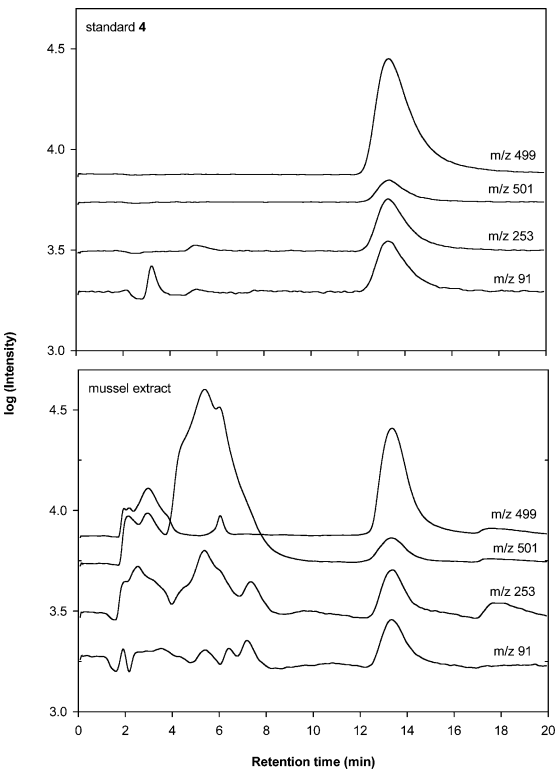

Fig. 2 HPLC-electrospray MS chromatograms for standard **4** and aqueous extract of canned mussel.

There appear to be two possible sources for the thio arsenosugars in mussels. First, these compounds may be naturally present also in algae, the major food item of the mussels examined here, and they are then accumulated unchanged by the mussels. Alternatively, the thio arsenic compounds may be formed *in vivo* by the mussels from ingested arsenosugars **1** and **2**. Although the question remains open, available data suggest that the second explanation is more likely because the thio compounds do not appear to be present in algae, and, arsenosugars, which are major arsenicals in algae, are present only as minor compounds in the mussels examined here.

The presence of thio arsenicals in marine animals including those used as human foods, raises two important issues. First, the metabolism in humans and possible toxicological properties of these new compounds needs to be assessed. Second, it would be interesting to investigate the role of these compounds in the biotransformation of arsenic, especially their possible involvement in the formation of arsenobetaine, the major form of arsenic in marine animals.

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Notes and references

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3 M. S. Ma and X. C. Le, *Clin. Chem.*, 1998, **44**, 539–550.
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5 HPLC-ICP MS was performed under three sets of chromatographic conditions: (i) Anion-exchange pH 5.6 (Hamilton PRP-X100 column, 4.1 × 250 mm; 20 mM $NH_4H_2PO_4$, pH 5.6; 40 °C); (ii) Anion-exchange pH 10.3 (PRP-X100, 4.1 × 100 mm; 20 mM NH_4HCO_3, pH 10.3, 40 °C); (iii) cation-exchange (Zorbax 300-SCX, 4.6 × 150 mm; 10 mM pyridinium formate pH 2.6, 30 °C). Flow rate of 1.5 ml min^{-1} and 10 μl or 20 μl injection volumes were used in all cases. Selective arsenic detection was performed with ICP MS (Agilent 4500 or 7500c) at *m*/*z* 75.
6 The product comprised whole mussel tissue (species unknown) bathed in an aqueous liquor. Analyses were mainly performed on an aqueous extract of the tissue, prepared with 35% extraction efficiency by shaking a portion of freeze-dried tissue (300 mg containing 11.2 μg As g^{-1} dry mass) with water (5.0 ml) overnight. The liquor (neat, untreated) was also analysed and showed the same pattern of arsenic species.
7 H_2O_2 (20 μl, 30% solution) was added to 300 μl of the extract.
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9 H_2S was bubbled for several minutes through an aqueous solution containing arsenosugar **1** (30 μg As in 10 ml) or arsenosugar **2** (1 μg As in 1 ml), which resulted in essentially quantitative conversion to the respective thio compounds (as determined by HPLC-ICP MS).
10 HPLC-electrospray MS was performed under anion-exchange conditions (PRP-X100 column, 1 × 150 mm; 4 mM NH_4HCO_3, pH 10.3 and methanol (95+5) at 40 °C). Flow rate was 0.1 ml min^{-1} and injection volume was 10 μl. Electrospray ionisation and mass detection was performed in positive ion mode with an Agilent G1946D single quadrupole MS.
11 Optimised fragmentor voltages for standard thio arsenicals **3** and **4** for protonated molecular ions and product ions were: *m*/*z* 345 & 347, 499 & 501 (100 V); 253 & 255 (150 V); 107 (250 V) 91 (400 V).
12 D. Kuehnelt, W. Goessler and K. A. Francesconi, *Rapid Commun. Mass Spectrom.*, 2002, **17**, 654–659.