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An investigation of the chemical stability of arsenosugars in basic environments using IC-ICP-MS and IC-ESI-MS/MS

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This paper evaluates the chemical stability of four arsenosugars using tetramethylammonium hydroxide (TMAOH) as an extraction solvent. This solvent was chosen because of the near quantitative removal of these arsenicals from difficult to extract seafood (oysters and shellfish). Four arsenosugars (3-[5′-deoxy-5′-(dimethylarsinoyl)-β-ribofuranosyloxy]-2-hydroxypropylene glycol—As(328), 3-[5′-deoxy-5′-(dimethylarsinoyl)-β-ribofuranosyloxy]-2-hydroxypropanesulfonic acid—As(392), 3-[5′-deoxy-5′-(dimethylarsinoyl)-β-ribofuranosyloxy]-2-hydroxypropyl hydrogen sulfate—As(408), and 3-[5′-deoxy-5′-(dimethylarsinoyl)-β-ribofuranosyloxy]-2-hydroxypropyl-2,3-hydroxypropyl phosphate—As(482)) were evaluated. The stability of these four arsenosugars were studied independently in a solution of 2.5% TMAOH at 60 °C over a period of up to 8 h. Two arsenosugars, As(328) and As(392), were found to be relatively stable in this solution for up to 8 h. However, As(408) and As(482) formed detectable quantities of dimethylarsinic acid (DMAA) and As(328) within 0.5 and 2 h, respectively. It was found that 97% of As(408) degrades after 8 h of treatment producing 3.4 times as much DMAA as As(328). This is contrary to As(482), which produces 13 times as much As(328) as DMAA and only 37% of the As(482) was converted by the 8 h treatment at 60 °C. These degradation products led to the investigation of weaker TMAOH extraction solvents. Three different concentrations (2.5%, 0.83% and 0.25%) were used to determine the effect of TMAOH concentration on the degradation rate of As(408). By reducing the TMAOH concentration to 0.83%, the conversion of the arsenosugar to As(328) and DMAA is nearly eliminated (less than 5% loss). Arsenosugars, As(408) and As(482), were also studied in 253 mM NaOH to verify the degradation products. The NaOH experiments were conducted to investigate a possible hydroxide based reaction mechanism. Similar degradation plots were found for each arsenosugar when compared to the 2.5% TMAOH data. A mechanism has been proposed for the formation of As(328) from As(408) and As(482) in base *via* an S_N2 reaction (hydroxide attack) at the side chain carbon adjacent to the inorganic ester. The formation of DMAA is observed in all arsenosugars after prolonged exposure. This probably occurs *via* an S_N2 attack at the arsenic atom.

Introduction

One of the shortcomings in arsenic speciation analysis in dietary samples is the lack of a quantitative extraction prior to separation and detection. The unextracted arsenic in a non-quantitative extraction leaves a fraction of the arsenic unspeciated and in turn its toxicity undetermined. This is problematic in attempting to assess the risk from dietary exposures. Extraction conditions reported in the literature include mild extractions, mixtures of methanol and water,[1–5] and strong acid extractions.[4,6] The methanol–water system has the advantage of being a mild solvent in terms of its likelihood of introducing any chemical changes in the arsenic compounds present in the sample, but it has the disadvantage of not being able to quantitatively extract arsenicals from the sample. Acid solutions, such as hydrochloric acid or orthophosphoric acid, have also been used for extraction. These provide complete dissolution but they have the disadvantage of possibly inducing chemical changes in the arsenic compounds. In general, the more quantitative extraction often requires a more chemically aggressive set of extraction conditions. These conditions can lead to the degradation of the native arsenicals; therefore, as the extraction solvent becomes more aggressive, there is an increasing need for species-specific integrity through the entire analytical procedure (especially the extraction). Species specific preservation is important because of the dramatically different toxicities associated with the arsenicals found in dietary samples, especially in seafood. The arsenosugars, see Fig. 1 for structures, are probably the most chemically labile arsenicals (excluding the As(III)/As(V) redox couple). Edmonds and Francesconi have reported the production of DMAA from As(392) and As(328) upon treatment with aqueous sodium hydroxide.[7] Le and co-workers[8,9] and Wei *et al.*[10] have shown that the ingestion of arsenosugars produces DMAA as the primary metabolite in the urine. A more recent study by Francesconi *et al.*[11] verified that DMAA is the major metabolite in urine after the ingestion of a purified arsenosugar, As(328). How these sugars are converted to DMAA is still unclear but the treatment of the individual arsenosugars with synthetic stomach juice indicated that the major degradation by-product was not DMAA but rather As(254).[12] These findings indicated that acidic assisted extraction could lead to the degradation of native arsenosugars in dietary seafood samples. Therefore, a base-assisted extraction has been carried out in our laboratory using TMAOH. This paper investigates the stability of arsenosugar

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standards in basic solutions to support the use of a base assisted extraction for seafood. Ion chromatography–inductively coupled plasma–mass spectrometry (IC-ICP-MS) is used for trace detection of arsenicals that may be produced by the basic solvents. The structural identity of any degradation products is made using electrospray ionization-mass spectrometry/mass spectrometry (ESI-MS/MS).

This research is essential to the development of arsenic speciation methodology in seafood which quantitatively extracts all arsenicals while preserving the species-specific integrity. This approach minimizes the unextracted fraction and in doing so maximizes the potential for assessing the overall toxicity. Completely characterizing arsenic exposures in seafood is essential because small errors in estimating this exposure can exceed the entire arsenic exposure from many other food groups.

Experimental

Reagents

All solutions were prepared using deionized (18 MΩ, Millipore, Bedford, MA), distilled water. Ammonium carbonate (ACS reagent) was purchased from Aldrich (Milwaukee, WI). Ammonium hydroxide (trace metal grade), for eluent pH adjustments, was purchased from Fisher Scientific (Pittsburgh, PA). Glacial acetic acid (OPTIMA) was purchased from Fisher Scientific (Pittsburgh, PA). Methanol (OPTIMA) was purchased from Fisher Scientific (Pittsburgh, PA). Tetramethylammonium hydroxide (25% w/w aqueous solution, Electronic Grade, 99.9999%) was purchased from Alfa Aesar (Ward Hill, MA).

IC-ICP-MS analysis of arsenosugar degradation

Either a Hewlett Packard 4500 ICP-MS (Palo Alto, CA) or an Agilent 7500a ICP-MS (Palo Alto, CA) was used as the detector for the ICP-MS studies. The operating parameters for the instrument were as follows: rf power = 1200 W, carrier gas flow = 1.18 L min^{-1}, plasma gas flow = 14.0 L min^{-1}, and the spray chamber temperature was 5 °C. The mobile phase, delivered by an Agilent 1100 liquid chromatograph system (Palo Alto, CA), was 20 mM $(NH_4)_2CO_3$, pH 9 at 1 mL min^{-1}. The column used for the separation was a PRP-X100 anion exchange column (4.6 mm × 250 mm, 10 μm particle size, Hamilton (Reno, NV)). Injection volumes for IC-ICP-MS studies were 100 μL.

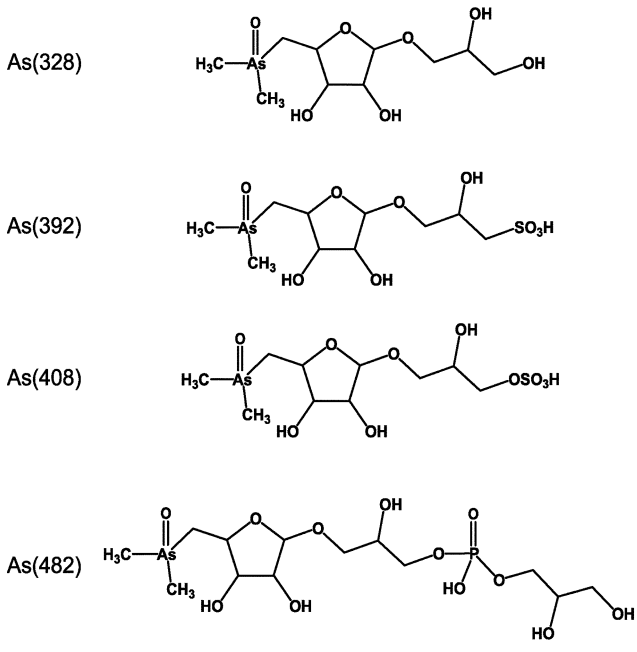

Fig. 1 Chemical structures of some arsenosugars.

IC-ESI-MS/MS analysis of arsenosugar degradation

A Finnigan LCQ Deca (ThermoQuest, San Jose, CA) ion-trap mass spectrometer was used for the electrospray studies. The parameters for the instrument were as follows: sheath gas flow at 80 units, auxiliary gas flow at 20 units, spray voltage at 4.00 kV, capillary temperature of 250 °C, capillary voltage at 3.00 V, activation amplitude at 30.0%, activation Q at 0.250, and activation time at 30 ms. The eluent, 20 mM $(NH_4)_2CO_3$, pH 9, was delivered at a flow rate of 1 mL min^{-1} using an Agilent 1100 liquid chromatograph system and split prior to the LCQ to deliver 500 μL min^{-1} to the MS detector. The splitter used was a high pressure graduated micro-splitter valve (Part no. P470, Upchurch Scientific, Oak Harbor, WA). Injection volumes for electrospray studies were 100 μL.

Purification of individual arsenosugars

Arsenosugars were purified as reported earlier.[12]

Degradation studies of arsenosugars

Solutions of arsenosugars were made in 2.5%, 0.83% or 0.25% (w/w) tetramethylammonium hydroxide (TMAOH) or 253 mM NaOH. Solutions of As(392), As(482), As(408), and As(328) were made to contain approximately 100 ppb, 100 ppb, 150 ppb, and 100 ppb, respectively. These solutions were then incubated in an oven at 60 °C for up to 24 h. Samples for analysis by ICP-MS and ESI-MS/MS were made by pH adjusting 0.5 mL of the arsenosugar solution with 8.7 M acetic acid to a pH of 4 followed by back adjusting the pH with 112 mM $(NH_4)_2CO_3$, pH 10.4, yielding a solution with an approximate pH of 9. This step was necessary to replicate the procedure used to pH adjust an actual seafood matrix sample. The concentration of the resulting sample was adjusted with 18 MΩ water.

Results and discussion

The primary focus of this investigation was to evaluate the extent of arsenosugar degradation produced by basic extraction conditions and to characterize any degradation products. This information could then be used to validate the extraction procedure in terms of species specific preservation for the arsenosugars. Fig. 2 compares the stability of four arsenosugars using single component solutions in 2.5% TMAOH at 60 °C. These conditions were chosen because they produced greater than 90% extraction efficiencies in problematic seafood.[13] In Fig. 2, As(328) and As(392) exhibit very little, if any, degradation over the 7.5 h and 8 h extraction periods at 60 °C. This is contrary to As(482) and As(408) standards which exhibit a 37% and 97% loss of the starting sugar respectively, over the 7.5 h treatment period. There are two degradation products for As(482) and As(408), with retention times of 4.7 and 7.1 min. The degradation product at 4.7 min was identified as As(328) *via* retention time using IC-ICP-MS and a spectral match *via* ESI-MS/MS. The degradation product eluting at 7.1 min was identified as DMAA by an IC-ICP-MS retention time match only. The two sugars not only have different degradation rates but they also produce a different distribution of degradation

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products. For instance, after 7.5 h, the ratio of the 4.7 min peak to the 7.1 min peak is 13.1 for As(482) and 0.3 for As(408). Although not apparent from Fig. 2, the concentration of As(328) after a 7.5 h treatment for As(408) and As(482) is 27 ng g^{-1} (22% of total arsenic) and 17 ng g^{-1} (23% of total arsenic), respectively. Therefore, the two sugars produce a similar amount of As(328), but As(408) produces a much larger percentage of DMAA. Neither the difference in degradation rates nor reaction product distribution would be predicted based on the arsenosugar structures.

Fig. 3 contains the IC-ICP-MS and ESI-MS/MS data used to characterize the starting materials and degradation products reported in Fig. 2. The data reported in Fig. 3 is for As(408) but similar characterizations were performed for all the data reported in Fig. 2. Fig. 3a contains the IC-ICP-MS chromatograms for As(408) at t = 0 and t = 7.5 h. The t = 0 chromatogram (solid line) indicates the purity of the As(408) starting material while the t = 7.5 h chromatogram (dashed line) indicates the nearly quantitative degradation of As(408) to the peaks labelled As(328) and DMAA. The ESI-MS/MS spectrum of the As(408) starting material is provided in Fig. 3b.

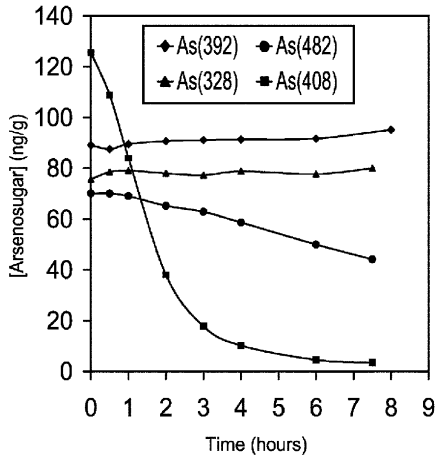

Fig. 2 Degradation of four arsenosugars in 2.5% tetramethylammonium hydroxide (TMAOH) at 60 °C.

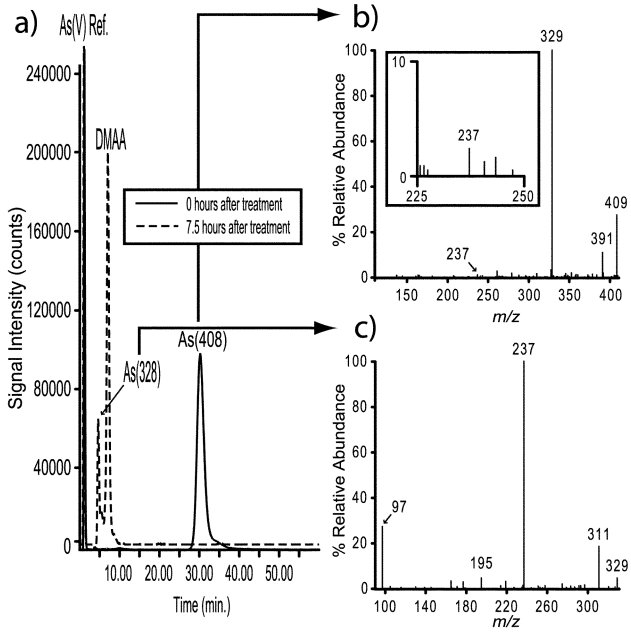

Fig. 3 Degradation of As(408) in time using IC-ICP-MS and IC-ESI-MS for structural verification. (a) IC-ICP-MS chromatogram of As(408) in 2.5% TMAOH at 0 h (solid line) and 7.5 h after treatment (dashed line) at 60 °C. (b) ESI-MS/MS spectra of starting arsenosugar, As(408). (c) ESI-MS/MS spectra of degradation product, As(328).

The mass spectrum for As(408) exhibits a $[M + H]^+$ peak at m/z 409 and product ion peaks at m/z 391 and 329. Similar spectra for As(408) have been reported by Corr and Larsen[14] and Pergantis *et al.*[15] It is important to note that the collision energy was optimised to allow for simultaneous detection of all arsenosugars at the highest sensitivity possible in MS and MS/MS mode for this study. This resulted in the incomplete fragmentation of the molecular ion for As(408). Fig. 3c contains the ESI-MS/MS spectra for the peak at 4.7 min. The product ion peaks observed at m/z 311, 237, 195, and 97 are consistent with data reported by Corr and Larsen[14] and McSheehy *et al.*[16] for As(328). In addition, the spectrum in Fig. 3c matches that of a pure As(328) standard. This spectrum was collected in order to differentiate between As(328) and As(254) which co-elute. As(254) was an acid degradation product described previously.[12] Finally, the peak at 7.1 min was identified as DMAA based on a retention time match only.

Fig. 2 indicates that extraction conditions using 2.5% TMAOH at 60 °C will produce degradation by-products from both As(408) and As(482). Lower TMAOH concentrations, with As(408) at 60 °C, were evaluated in Fig. 4 in order to minimize this effect. The TMAOH concentrations and treatment times were selected because these conditions produced near quantitative extraction on difficult to extract seafoods.[13] As(408) was chosen for this investigation because it degraded in 2.5% TMAOH faster and more quantitatively than any of the other arsenosugars. Fig. 4 indicates that the three-fold decrease in TMAOH concentration (2.5% *vs.* 0.83%) nearly eliminates the conversion of As(408) to As(328) and DMAA over the 7.5 h treatment period. This data indicates that a 0.83% TMAOH extraction solvent would produce minimal arsenosugar degradation over a 7.5 h period. The final TMAOH concentration (0.25%) was chosen because an overnight extraction using 60 °C had produced nearly quantitative extraction conditions for the difficult to extract seafoods.[13] The stability profile for 0.25% TMAOH concentration indicates minimal degradation of the As(408) over the 24 h treatment period. Therefore, these extraction conditions should also minimize unwanted extraction induced degradation products.

The data presented in Figs. 2–4 provide guidance in terms of selecting extraction conditions using TMAOH which minimize the production of extraction-induced degradation products from the arsenosugars. Fig. 5 investigates arsenosugar stability using a NaOH extraction solvent in an attempt to generalize the TMAOH degradation to "base" induced degradation. This generalization may be helpful in selecting base assisted extraction conditions for seafoods which contain arsenosugars. Fig. 5 contains the stability profile for As(408) along with the production of As(328) and DMAA. The 253 mM NaOH solution used in this figure has a pH of 11.8. Comparable results to those obtained in 2.5% TMAOH (Fig. 2) would provide some supporting data for a hydroxide driven degradation mechanism

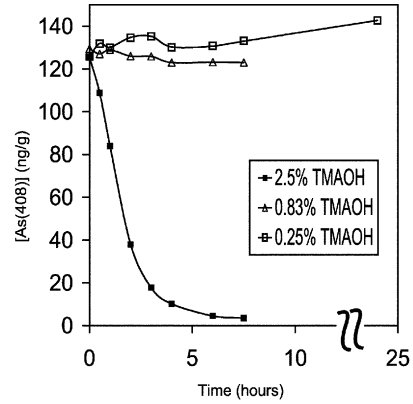

Fig. 4 Degradation of As(408) at 60 °C in time using three different concentrations of TMAOH.

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and add credence to a generalized base driven degradation of the arsenosugars. For As(408), the profiles in TMAOH and NaOH are very similar and the degradation by-products are produced at similar rates. Although not shown, a similar experiment with NaOH was carried out on As(482). In this case As(482) produced similar degradation products/rates in NaOH as in 2.5% TMAOH (see Fig. 2).

The production of As(328) in base from As(482) and As(408) suggests a hydroxide driven degradation reaction for these two sugars. The proposed mechanism for this reaction is shown in Fig. 6. The mechanism is an S_N2 reaction involving a hydroxide attack at the side-chain carbon adjacent to the inorganic ester resulting in an inorganic ester leaving group. The resulting As(328) and inorganic ester group are shown as products in the mechanism in Fig. 6. As(328) is not detected as a base generated degradation product for As(392) because it does not contain the inorganic ester linkage present in As(408) and As(482). The carbon in the sidechain of As(392) is bonded directly to the sulfur rather than an oxygen resulting in a poorer leaving group. While the S_N2 mechanism may explain how As(328) is produced by base from As(408) and As(482), it does not explain why the major degradation product from As(408) is DMAA and the major degradation product from As(482) is As(328). In all cases, DMAA is produced as a degradation product in base (S_N2 hydroxide attack at the As atom) but the accelerated rate at which As(408) degrades to DMAA would not be predicted based on the structural similarities to the other arsenosugars.

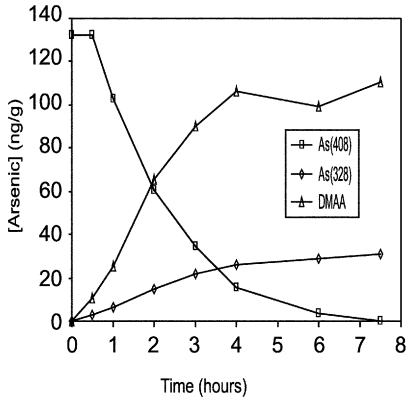

Fig. 5 Degradation of As(408) and formation of As(328) and DMAA in 253 mM NaOH at 60 °C.

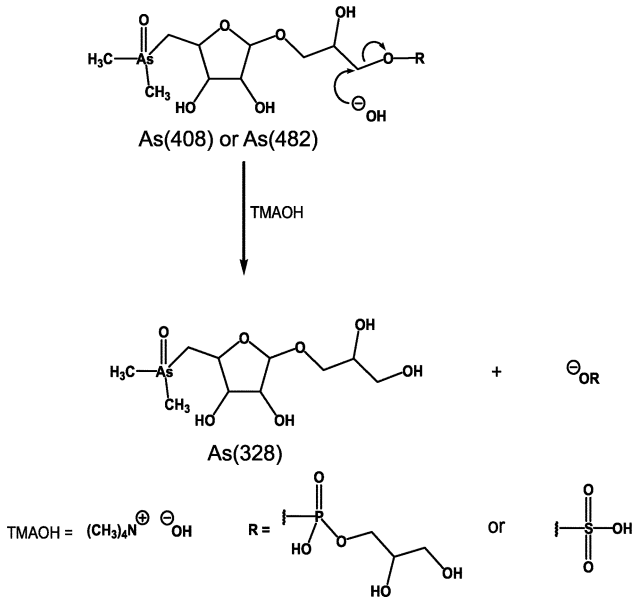

Fig. 6 Proposed mechanism for arsenosugar degradation to As(328) under basic conditions.

The purpose of this manuscript was to characterize degradation products of arsenosugars when TMAOH is used as an extraction solvent. This information can be used as a guide in selecting extraction conditions which minimize degradation and ensure species-specific integrity during the extraction process. The results of the study can be summarized as follows: (1) As(328) and As(392) are stable using 2.5% TMAOH at 60 °C for greater than 7.5 h while As(482) and As(408) both produce As(328) and DMAA. (2) The use of 0.83% TMAOH at 60 °C produces minimal degradation of As(408) (the most base labile arsenosugar) even after 7.5 h. (3) The use of 0.25% TMAOH at 60 °C does not produce a measurable amount of As(328) or DMAA in 24 h from As(408). (4) The TMAOH induced degradation is probably hydroxide driven *via* an S_N2 reaction; therefore, similar degradation would be predicted by base-assisted extraction conditions.

Disclaimer

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