Bio-Analytical Mass Spectrometry

2-Dimethylarsinothioyl Acetic Acid Identified in a Biological Sample: The First Occurrence of a Mammalian Arsinothioyl Metabolite**

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Arsenic is an element with a particularly strong affinity for sulfur; as biological systems are rich in sulfydryl groups it is likely that compounds with this combination of elements are created in vivo. In 1886 it was shown that trialkyl arsine oxides are converted into trialkyl arsine sulfides when treated with hydrogen sulfide in the laboratory.[1] Because of their potential therapeutic applications, many studies on thio-arsenicals were performed in the nineteen thirties.[2–5] Since then, it has been established that thiol groups play an essential role in the enzymatic pathway for biotransformation of arsenic,[6] and that arsenite binds to a variety of sulfur-rich peptides and proteins, such as glutathione (GSH),[7,8] metallothionein,[9] actin and tubulin,[10] galectin I thioredoxin peroxidase II,[11] and other macromolecular constituents of tissues. Despite the fact that arsenic binds to sulfur-rich proteins, As–S and As=S compounds have not been identified in natural samples. Consequently the main focus of arsenic speciation has been on oxo-arsenicals, as these compounds appear to be more abundant in nature. To our knowledge there has only been one report (unpublished) of a thioarsenical (2-dimethylarsinothioyl ethanol) occurring in a natural sample.[12] The response of thio-arsenicals to standard analytical procedures used in the speciation field is unknown, which may result in misleading conclusions. The presence and underestimation of thio-arsenicals in nature has previously been suggested.[13,14]

Herein we report the first characterization of a thio-organoarsenate (2-dimethylarsinothioyl acetic acid (**1**)) compound isolated from a biological source (sheep's urine). Urine samples were obtained from a primitive breed of wild sheep, which lives on the beaches of North Ronaldsay (Orkney

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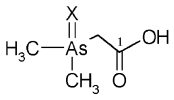

1 X = S
2 X = O

Islands, UK) and naturally consumes large amounts of arsenosugars (30 mg As daily) through their major food source, seaweed.[15] Arsenosugars have to date been classified as nontoxic.[16,17] However, as long as the metabolic intermediates are unknown, the real toxicity of a compound cannot be established. The discovery of a thio-organoarsenate compound in the urine samples shows that ingestion of arsenosugars can lead to formation of As–S and As=S compounds in vivo, which may cause interference with metabolic processes.

During the analytical work performed we found **1** very prone to degradation, especially at low pH values. Its elution from chromatographic columns was pH dependent; when using a mobile phase of pH 5.3, the compound was permanently adsorbed on the anion-exchange column. We emphasize the importance of combining a range of complementary analytical methods for the successful structural elucidation of this novel structure.

Urine from North Ronaldsay sheep was analyzed using anion-exchange-HPLC coupled simultaneously to inductively coupled plasma(ICP)-MS and ES-MS. The species giving rise to the main signal in the ES-mass spectrum (*m*/*z* 197), co-eluted with arsenic (giving a signal at *m*/*z* 75) in the ICP-mass spectrum (Figure 1 A, B). Ions giving less intense signals at

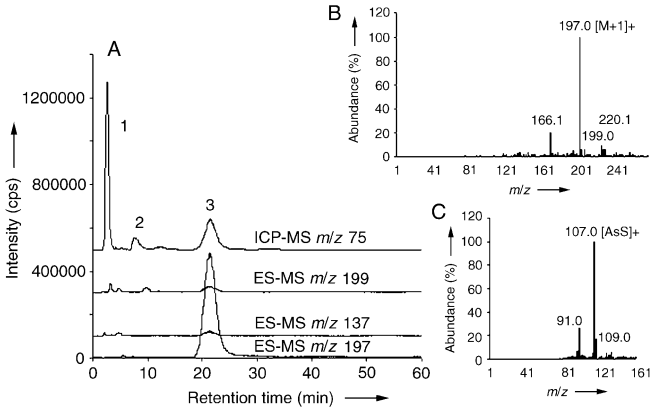

Figure 1. Anion-exchange chromatograms of a urine sample, detected by ICP-MS (*m*/*z* 75) and by ESI-MS (*m*/*z* 199, 198, 137, 197) using scan mode (A). Peak 1 consists mainly of dimethylarsinoyl ethanol, dimethylarsinic acid and dimethylarsinoyl acetic acid, peak 2 and 3 are unknown arsenicals.[20] Mass spectrum of peak 3 at fragmenter voltage B) 100 V and C) 240 V.

m/*z* 199, and 137 were also observed to co-elute (Figure 1 A). When increasing the cone voltage (to 240 V) to induce in-source fragmentation of the ES-generated ions the dominant peak was at *m*/*z* 107 (Figure 1 C). The ICP-MS and ES-MS data provide various clues to the identity of the compound; the relative intensities of the ions at *m*/*z* 197 and 199 were consistent with the isotope pattern of a compound containing one sulfur atom (relative isotopic contributions of ^{32}S and ^{34}S of 95.018 and 4.215%, respectively). Storage and sample treatment slowly resulted in a reduction in the intensity of the ion at *m*/*z* 197 and a concomitant increase in the intensity of an ion at *m*/*z* 181. Dimethylarsinoyl acetic acid (**2**) (which yields a protonated molecule at *m*/*z* 181) has previously been identified as a natural product in shellfish[18,19] and as a breakdown product of arsenosugars in urine.[20] A **2** standard was treated with hydrogen sulfide to produce **1**, which showed matching retention time to the unknown on an anion exchange column, the eluent of which was analyzed by ICP-MS (Figure 2 A), and showed a strong signal at *m*/*z* 197 (Figure 2 B) in the ES mass spectrum. Except for *m*/*z* 166, the ions generated from in-source fragmentation at *m*/*z* 91, 107, and 137 (Figure 1) are consistent with the proposed structure of **1**.

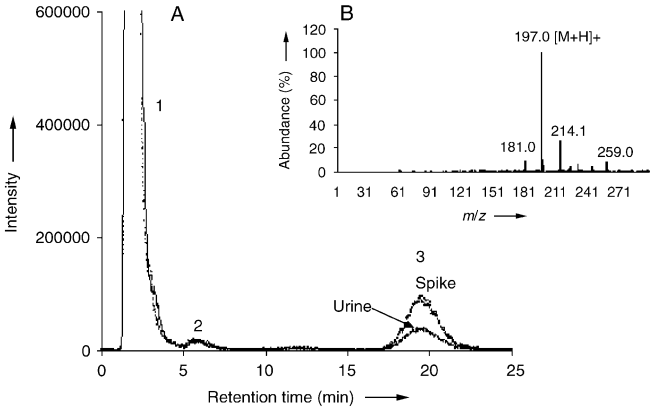

Figure 2. A) Anion-exchange chromatograms with ICP-MS (*m*/*z* 75) detection of a urine sample (solid line) and urine sample spiked with **1** standard (dotted line). Peak 1, 2, and 3 as described in Figure 1. B) Mass spectrum of standard **1** (**2** + hydrogen sulfide).

The urine was subjected to HPLC fractionation, and the fraction containing the unknown arsenical analyzed using ES-IT-MS^n. ES mass spectra obtained from the synthetically prepared **1** (**2** + H_2S) and the fractionated urine contained low-intensity signals at both *m*/*z* 181 and 197. Since the postulated structure contains sulfur, an ion at *m*/*z* 199 corresponding to the ^{34}S isotope is to be expected. However, this species was not obvious above background in the ES mass spectra owing to the low abundance of the parent compound. Collision-induced dissociation (CID) tandem mass spectra of the ions at *m*/*z* 197 in both the synthetically prepared **1** and the fractionated urine were obtained, which yield structurally diagnostic fragment ions (Figure 3). Product ions were observed at *m*/*z* 165, 151, and 137 from both samples. Additionally, the ion at *m*/*z* 197 derived from the urine sample yielded a product ion at *m*/*z* 105. These product ions are consistent with the proposed structure of **1**. Both spectra contain background ions arising from the co-selection and fragmentation of isobaric matrix ions. The structures of the product ions assigned in Figure 3 are supported by the results of further stages of tandem mass spectrometry. The MS^2 ion at *m*/*z* 151 yielded third generation ions at *m*/*z* 123 and 105. The MS^2 ion at *m*/*z* 137 yielded third generation ions at *m*/*z* 121 and 109.

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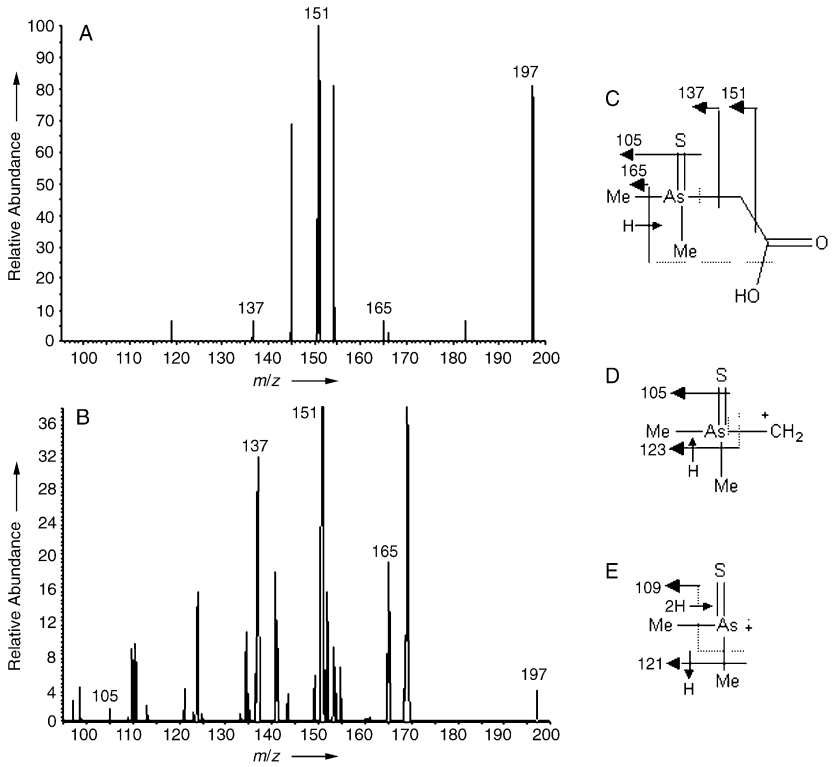

Figure 3. CID MS^2 data obtained from the ion at *m/z* 197 in A) the synthetically prepared **1** and B) the fractionated urine. C) The fragment ions produced from CID MS^2 of the $[M+H]^+$ ion at *m/z* 197, D) The fragment ions produced from CID MS^3 of the MS^2 product ion at *m/z* 151, and E) The fragment ions produced from CID MS^3 of the MS^2 product ion at *m/z* 137.

Despite the fact that the ^{34}S isotopic ion did not stand out above background in the ES mass spectra, structurally diagnostic product ions were obtained from CID tandem mass spectrometric analysis of the ions at *m/z* 199 generated from both samples. The ions are consistent with species containing the ^{34}S isotope of **1** in both samples. Comparison of data obtained from the *m/z* 197 and 199 species facilitated interpretation of the product-ion spectra, particularly localization of the sulfur to the arsenic. The product ions generated from *m/z* 197 previously observed at *m/z* 151 and *m/z* 137 were shifted to *m/z* 153 and *m/z* 139, respectively.

^1H NMR data were obtained on **2** and the **1** standard using a standard presaturation pulse sequence. Owing to the low concentration of the samples, the ^{13}C NMR spectra were obtained indirectly from the HSQC and HMBC spectra (Table 1). In both cases the ^1H methyl singlets correlated in the HSQC to highfield signals in the ^{13}C NMR spectrum. The methylene group in both cases was identified from the 2H integral in the ^1H NMR and correlated in the HSQC to a ^{13}C signal at approximately 40 ppm. The chemical shift of the carboxylate carbon atom at approximately 170 ppm was determined from its HMBC correlation to the methylene proton signal.

Table 1: Chemical shift values [ppm] from ^1H NMR and ^{13}C NMR spectroscopy for **1** and **2**

Group	**1**		**2**	
	δH	δC	δH	δC
C=O	–	170.5	–	172.8
-CH_2-	3.19	38.1	2.99	40.0
-CH_3	1.87	18.0	1.69	14.0

In conclusion, we report the first characterization of a thio-organoarsenate (2-dimethylarsinothioyl acetic acid (**1**)) compound isolated from a biological source. The finding is novel but not unexpected. We emphasize that more sulfur-containing arsenicals are likely to be found in biological samples in the future—if the right analytical techniques are applied. Since **1** is the first compound of a new class of arsenic metabolites, it indicates that mammalian arsenic metabolism is much more complex than has been claimed for a long time, which opens up new questions about the toxicity of such organoarsenothio(y)l compounds.

Experimental Section

Separation was performed on a PRP-X100 anion-exchange column (150 × 4.6 mm) with a Hamilton PEEK pre-column (11.2 mm, 12–20 μm) from Hamilton Company. The mobile phase used was 30 mM ammonium carbonate (1 mL min^{-1}). Before injection (loop volume 100 μL) onto the HPLC column all samples were filtered through a 0.45-μm nylon filter. For flow injection measurements the sample volume was 20 μL. A HP1100 HPLC system (Agilent Technologies, USA) with cooled autosampler was used throughout the experiments. The autosampler was cooled to 4 °C and the column kept at ambient temperature. Post-column the flow was split in a ratio of 1:4 (1 part into the ICP-MS, 4 parts into the ES-MS) using a microsplitter (Upchurch, UK).

An ICP-MS 7500 ((Agilent Technologies, USA)) was used for element specific detection of arsenic. The instrument was equipped with either a Babington nebulizer or a micro-concentric nebulizer (flow rate < 100 μL min^{-1}) and a peltier cooled spray chamber. The instrument was mostly used in the soft extraction mode without oxygen. The instrument settings were checked daily for arsenic sensitivity and optimized when necessary. The elements monitored were arsenic (*m/z* 75), tellurium (*m/z* 130), sulfur (*m/z* 34), and sulfur oxide (*m/z* 48).

The HP1100 series LC/MSD (quadrupole mass spectrometric detector) instrument (Agilent Technologies, USA) was used as a molecule-specific detector. The MSD was used in the positive-ionization mode from *m/z* 70 to *m/z* 1000 or in the single-ion mode with the API-electrospray head. The settings chosen were: capillary voltage of 4000 V, nebulizer pressure of 40 psi, drying gas flow of 12 L min^{-1} at 350 °C, quadrupole temperature 100 °C, and cone voltage of 100 V or 240 V. The cone voltage set at 100 V allowed the mass spectrometric analysis of intact protonated species, whereas an increased cone voltage of 240 V generated fragments of electrospray-generated ions by collision-induced dissociation with resident atmospheric gases in the source of the mass spectrometer. Mass calibration was controlled regularly and when necessary optimized using the calibration solution supplied by Agilent.

The unknown arsenical in urine samples was isolated by HPLC fractionation (sample treatment and conditions of mobile phase and column as above). The HPLC system consisted of a LKB Bromma

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model 2150 pump with a Rheodyne 6 port sample injector (loop volume of 100 μL). The standard **1** was synthesized when an aqueous solution of standard **2** was purged with gaseous H_2S. The reaction was carried out for 2 h.

Arsenic-containing HPLC fractions were analyzed using a ThermoFinnigan LCQ DECA XP Plus ion-trap mass spectrometer with an electrospray source. Fractions were infused into the source at 1 μL min^{-1} using the built-in syringe driver. The electrospray capillary was held at 5800 V. The buffer gas used was nitrogen. Data were collected over the *m*/*z* range 50–200. Collision-induced dissociation (CID) tandem mass spectrometry was performed with a normalized collision energy setting of 30%, activation *q* setting of 0.250 and an activation time of 30 ms.

Presaturated ^1H, HSQC, and HMBC NMR spectroscopy experiments were recorded on a Varian Unity Inova 400 MHz spectrometer at 299 K in D_2O solution. Chemical shifts are reported in ppm (δ) downfield relative to residual HOD at 4.60 ppm. Samples of compounds (38–48 μg) were prepared in degassed D_2O, capped immediately and used within 2 h of preparation.

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[1] A. Michaelis, U. Paetow, *Justus Liebigs Ann. Chem.* **1886**, *233*, 60.
[2] H. J. Barber, *J. Chem. Soc.* **1929**, 1020.
[3] A. Cohen, H. King, W. I. Strangeways, *J. Chem. Soc.* **1931**, 3043.
[4] A. Cohen, H. King, W. I. Strangeways, *J. Chem. Soc.* **1932**, 2505.
[5] A. Cohen, H. King, W. I. Strangeways, *J. Chem. Soc.* **1932**, 2866.
[6] M. Styblo, D. J. Thomas, *Toxicol. Appl. Pharmacol.* **1997**, *147*, 1.
[7] K. T. Suzuki, B. K. Mandal, Y. Ogra, *Talanta* **2002**, *58*, 111.
[8] G. M. Bogdan, A. Sampayo-Reyes, H. V. Aposhian, *Toxicology* **1993**, *93*, 175.
[9] M. Toyama, M. Yamashita, N. Hirayama, Y. Murooka, *J. Biochem.* **2002**, *132*, 217.
[10] D. B. Menzel, H. K. Hamadeh, E. Lee, D. M. Meacher, V. Said, R. E. Rasmussen, H. Greene, R. N. Roth, *Toxicol. Lett.* **1999**, *105*, 89.
[11] K. N. Chang, T. C. Lee, M. F. Tam, Y. C. Chen, L. W. Lee, S. Y. Lee, P. J. Lin, R. N. Huang, *Biochem. J.* **2003**, *371*, 495.
[12] K. A. Francesconi, **2003**, personal communication.
[13] W. R. Cullen, B. C. McBride, J. Reglinski, *J. Inorg. Biochem.* **1984**, *21*, 45.
[14] W. R. Cullen, B. C. McBride, J. Reglinski, *J. Inorg. Biochem.* **1984**, *21*, 179.
[15] H. R. Hansen, A. Raab, K. A. Francesconi, J. Feldmann, *Environ. Sci. Technol.* **2003**, *37*, 845.
[16] T. Sakurai, T. Kaise, T. Ochi, T. Saitoh, C. Matsubara, *Toxicology* **1997**, *122*, 205.
[17] T. Kaise, Y. Oya-Ohta, T. Ochi, T. Okubu, K. Hanaoka, K. J. Irgolic, T. Sakurai, C. Matsubara, *J. Food Hyg. Soc. Jpn.* **1996**, *37*, 135.
[18] E. H. Larsen, *Fresenius J. Anal. Chem.* **1995**, *352*, 582.
[19] S. McSheehy, J. Spunzar, R. Lobinski, V. Haldys, J. Tortajada, J. S. Edmonds, *Anal. Chem.* **2002**, *74*, 2370.
[20] H. R. Hansen, A. Raab, J. Feldmann, *J. Anal. At. Spectrom.* **2003**, *18*, 474.

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