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Synthesis and Characterization of Arsenobetaine and Arsenocholine Derivatives

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Multigram quantities of arsenobetaine bromide and arsenocholine iodide were synthesized from trimethylarsine using uncomplicated techniques. Arsenobetaine bromide and arsenocholine iodide are both non-hygroscopic. Arsenocholine iodide is, however, light-sensitive and should be used with actinic glassware. Both compounds were characterized by elemental and spectroscopic techniques and found to be suitable for use as primary analytical standards. © 1998 John Wiley & Sons, Ltd.

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INTRODUCTION

Arsenic occurs in the environment in a variety of forms, including arsenite(III), arsenate(V), monomethylarsonic acid, dimethylarsinic acid, arsenobetaine and arsenocholine. Both organic and inorganic trivalent arsenic compounds are far more toxic than pentavalent arsenic compounds[1–3] due to the high affinity of As(III) for thiol groups commonly present in enzyme systems. Compounds containing pentavalent arsenic probably interfere with oxidative phosphorylation.[3]

While marine species have been known for many years[4] to contain high levels of arsenic (up to 100 ppm), the lack of observable toxic effects among seafood consumers indicated that the arsenic was present in a non-toxic form. However, it was not until 1977 that the arsenic compound was identified as arsenobetaine[5] and later determined to be the major organic arsenic compound present in marine species such as fish,[1,2,6–8] crustaceans[1,2,9] and molluscs.[1,2,10] Arsenobetaine is essentially non-toxic[6,11] and is rapidly excreted in the urine of mice, rats and rabbits.[12] Organic arsenic present in flounder (probably arsenobetaine) is also rapidly excreted in the urine of human volunteers.[13,14] Arsenocholine is found only occasionally in marine species[9,15] but is of interest because of its probable biogenic relationship to arsenobetaine.[2,3,16]

Only a few reports[5,17,18] and two theses[19,20] provide experimental details for synthesizing arsenobetaine and arsenocholine compounds.

The purpose of this study was to synthesize commercially unavailable, non-hygroscopic derivatives of arsenobetaine and arsenocholine on a preparative scale using uncomplicated synthetic procedures. This paper details the synthesis of these compounds along with their complete analytical characterization.

MATERIALS AND METHODS

Reagents

Distilled-in-glass grade solvents (BDH Inc., Toronto, Canada) and ACS reagent chemicals were used. Trimethylarsine (Me_3As) was purchased from Strem Chemicals Inc. (Newburyport, MA, USA) and was stored in a Teflon joint ampoule under N_2 at low temperature (approx 4 °C). Bromoacetic acid, bromoethanol and sodium iodide were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA) and used as received.

Instrumentation

All NMR spectra were recorded on a Bruker

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Table 1 ICP MS operating conditions

Forward power	1350W
Reflected power	0–2W
Nebulizer flow rate	0.95 l min^{-1}
Cooling flow rate	13.0 l min^{-1}
Auxiliary flow rate	0.90 l min^{-1}
Spray-chamber temperature	5 °C
Mass monitored	75 amu
Acquisition mode	Dual scanning
Dwell times	320 μs/640 μs

AM400 spectrometer equipped with an Aspect 3000 computer and a 16-bit Analog to Digital Converter (ADC). The ^{13}C and ^{1}H spectra were acquired at 400.13 and 100.61 MHz respectively. The spectra were run in D_2O at 24 °C, using sodium 2,2-dimethyl-2-silapentane sulfonate (DSS) as the external chemical shift reference standard (0.00 ppm). Elemental analyses were carried out using a Perkin-Elmer Series (II) CHNO 2400 analyzer. Total arsenic was determined with a VG Plasma Quad II ICP mass spectrometer. System operating conditions are listed in Table 1. Quantification was by comparison with external arsenic (As) standards. Both standards and samples were prepared in 2% HNO_3 with 10 ng ml^{-1} indium added as an internal standard. FAB mass spectra were recorded on a Kratos Concept IIH instrument. The cesium ion gun was operated with an 8 kV accelerating voltage and glycerol (3-nitrobenzyl alcohol) was the calibration standard.

Synthesis procedures

Most of the reactions were carried out either under an N_2 flow or in an Atmosbag^{TM} (Aldrich Chemical Co., Milwaukee, WI, USA) connected to an N_2/vacuum system in a well-ventilated fumehood.

Arsenobetaine bromide, $[Me_3As^+CH_2COOH]Br^-$

Me_3As (2.1 ml, 19.7 mmol) was placed in a 50 ml flask containing 20 ml of toluene and cooled to 5 °C. Bromoacetic acid (2.8 g, 20.1 mmol) was added to this solution; precipitation started immediately after mixing the two reagents. The reaction mixture was stirred for 6 h and then cooled at (− 20 °C for half an hour. The solid was collected by filtration, dissolved in hot ethanol and filtered again. The filtrate was cooled to 5 °C, upon which white shiny crystals started to separate. The product (4.9 g, 18. 9 mmol, 96% yield) was collected and dried.

Arsenocholine bromide, $[Me_3As^+CH_2CH_2OH]Br^-$

Me_3As (2.0 ml, 18.7 mmol) was transferred to a reacti-vial (5.0 ml) inside an Atmosbag^{TM} and $BrCH_2CH_2OH$ (1.3 ml, 18.3 mmol) was added to it. The reacti-vial was closed, taken out of the Atmosbag^{TM} and sealed with Teflon tape. The mixture was heated at 75 °C (using a block heater) for 72 h until the two separate layers of reagents homogenized. Everything solidified upon cooling. Inside the Atmosbag^{TM}, the solid was dissolved in warm acetonitrile and filtered. The product (arsenocholine bromide) separated at room temperature. The product (4.0 g, 16.3 mmol, 89% yield) was collected and dried under vacuum in a desiccator. Arsenocholine bromide is white, crystalline and very hygroscopic.

Arsenocholine iodide, $[Me_3As^+CH_2CH_2OH]I^-$

Arsenocholine bromide (4.0 g, 16.3 mmol) was placed in a flask (wrapped in aluminum foil) containing acetonitrile (50.0 ml). Sodium iodide (2.5 g, 16.7 mmol) was added to it. The flask was closed and the solution was stirred for 2 hours. The synthesis was performed in the absence of light. Sodium bromide, a by-product of the synthesis, was filtered off at the end of the reaction. White, non-hygroscopic crystals of arsenocholine iodide (3.8 g, 13.0 mmol, 80% yield) formed upon cooling the filtrate to 5 °C. The crystals were collected and dried under vacuum in a desiccator.

RESULTS AND DISCUSSION

Reaction yields were significantly improved if trimethylarsine was handled under an inert atmosphere rather than in an N_2 flow, thus minimizing evaporative losses of the compound during reactions. Previously reported procedures for arsenocholine iodide[17,19,20] resulted in a low yield of a yellow semi-solid product instead of the expected white crystalline material. However, when the reaction conditions were modified to exclude light, high yields of arsenocholine iodide were obtained. Initial attempts at drying the product at 100 °C resulted in decomposition. All further drying was performed under vacuum at room temperature. Arsenocholine iodide must, therefore, be protected from light and excessive heat during synthesis and storage, to achieve and maintain high purity of the complex. Both reported compounds can be readily synthesized on a multigram scale.

Table 2 ^{13}C NMR parameters

	δ (ppm)			
Compound	Me_3As	$AsCH_2$	COOH	CH_2OH
$[Me_3As^+CH_2COOH]Br^-$	10.50	33.28	172.46	—
$Me_3As^+CH_2CH_2OH]I^-$	10.56	31.19	—	58.51

NMR spectra

The ^{1}H and ^{13}C spectra for the compounds are consistent with the structures. The proton spectra are shown in Figs 1 and 2 and the carbon-13 chemical shifts are given in Table 2. As far as we are aware, no NMR spectral data have been reported in any previous journal publication. However, data for these compounds were reported in two theses.[19,20] The general appearance of the spectra in these reports and in the present work is similar. The chemical shift values reported previously for arsenocholine iodide differ significantly from each other and from the present work. However, the relative proton chemical shift values are very similar, with one report[19] listing proton

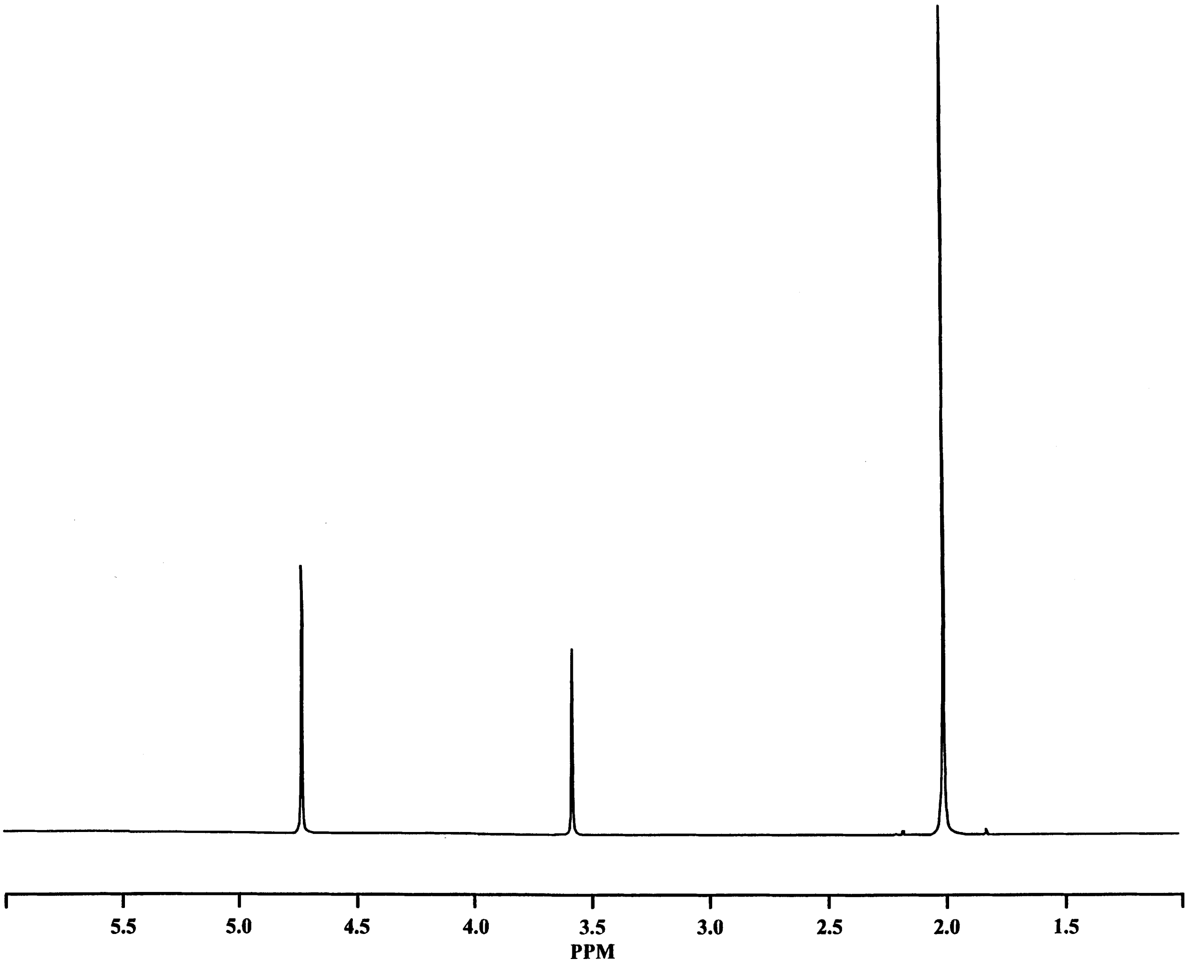

Figure 1 ^{1}H NMR spectrum of arsenobetaine bromide.

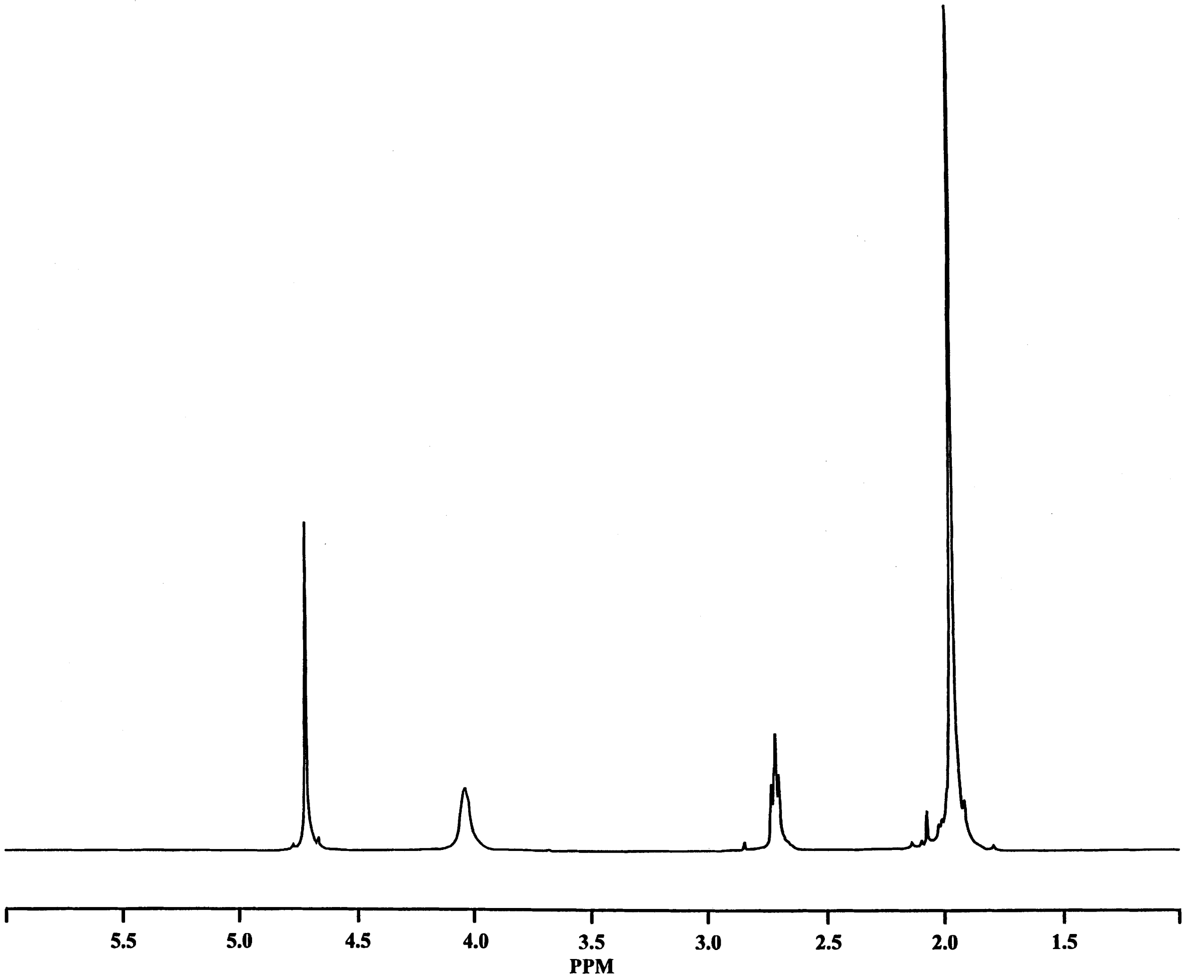

Figure 2 ^{1}H NMR spectrum of arsenocholine iodide.

chemical shifts that were 0.4 ppm larger than ours and the other[20] 0.8 ppm smaller. It would appear that these other workers had problems in setting the chemical shift reference value. For the arsenobetaine bromide, the two reported sets of ^{1}H chemical shifts differed greatly from each other,[19,20] even though they were run in the same solvent (DMSO-d_6). The ^{1}H chemical shifts we found (using D_2O as solvent) agree fairly well with one report,[20] indicating that there was probably a referencing problem in the other study.[19] The chemical shift assignments (see below) of the resonances to the protons and carbons for both compounds are straightforward and agree with the previous reports.

Arsenobetaine bromide

In the ^{1}H NMR spectra (Fig. 1), the singlet at 1.997 ppm is attributed to nine equivalent protons of the Me_3As group and the second singlet at 3.577 ppm is assigned to the two protons of the $AsCH_2$ group. The proton ratio as calculated from the integration of peaks is 8.98:2 (9:2). The signal at 4.726 ppm is caused by an HOD impurity in the D_2O solvent.

The signals for Me_3As group C-atoms in the ^{13}C NMR spectra resonate at 10.504 ppm. The singlets at 33.28 and 172.46 ppm correspond to $AsCH_2$ and COOH groups respectively (Table 2).

Arsenocholine iodide

The ^{1}H NMR spectrum of arsenocholine iodide (Fig. 2) is characterized by a methyl singlet at 1.967 ppm, a sharp triplet at 2.718 ppm from the methylene group attached to the arsenic atom and a

Table 3 Elemental composition of arsenobetaine bromide and arsenocholine Iodide (calculated/found)

	Analysis (%)			
Compound	C	H	As	As (ppb)[a]
$[Me_3As^+CH_2COOH]Br^-$	23.2/23.2	4.67/4.63	28.9/29.0	50.0/49.2
$Me_3As^+CH_2CH_2OH]I^-$	20.6/20.6	4.83/4.79	25.7/25.7	51.3/49.5

[a] Determined by ICP MS, compounds prepared as 50 ppb (as As) solutions in 2% HNO_3.

broad signal at 4.039 ppm due to the CH_2 group attached to OH.

The two high-field signals at 10.56 and 31.19 ppm in the ^{13}C NMR spectrum are assigned to the Me_3As methyl group and the $AsCH_2$ group respectively. The resonance at 58.51 ppm is from the CH_2OH group (Table 2).

Mass spectra

Arsenobetaine bromide

The base peak at *m/z* 179 corresponds to $[Me_3As^+CH_2COOH]$, which is formed by cleavage of Br (Fig. 3). Fragmentation proceeds by the loss of methyl groups and cleavage of acetate groups to give rise to the fragment compounds with masses 535, 357, 135 and 120 corresponding to $2[Me_3As^+CH_2COO]Br^- + H_2O$, $2[Me_3AsCH_2COO] + H^+$, Me_4As^+ and Me_3As^+, respectively.

Arsenocholine iodide

The base peak at *m/z* 165 corresponds to $[Me_3As^+CH_2CH_2OH]$ (Fig. 4). Aside from this

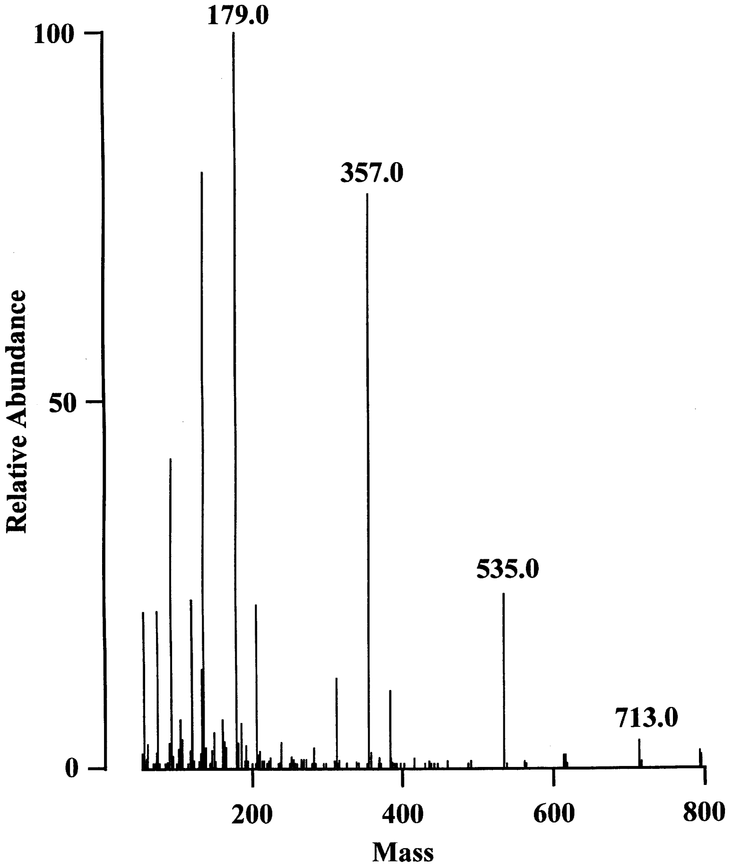

Figure 3 FAB mass spectrum of arsenobetaine bromide.

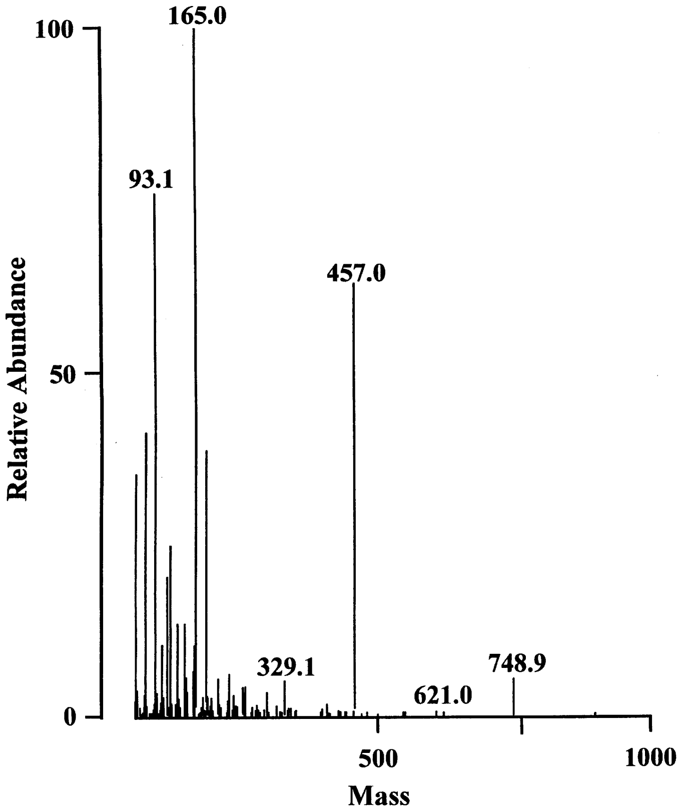

Figure 4 FAB mass spectrum of arsenocholine iodide.

peak, a few other fragments are observed at *m/z* values of 457, 120, 105 and 57. These are assignable to $2[Me_3As^+CH_2CH_2O] + I^-$, Me_3As^+, Me_2As^+ and As^+, respectively.

Elemental analysis

The expected percentages of C, H and As were very similar to those found (Table 3) by elemental analysis. Further, the actual levels of As found in 50 ppb (as As) solutions of each compound examined by ICP MS (Table 3) were 96.5 – 98.4% of the expected values.

CONCLUSIONS

The arsenobetaine and arsenocholine derivatives described in this study can be readily synthesized in multigram quantities using the reported procedures. Both compounds are non-hygroscopic, stable (if the precautions outlined for arsenocholine iodide are noted) and suitable for use as primary analytical standards.

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