Pertanika 11(3), 437-439 (1988)

Preparation of Arsenobetaine hydrobromide

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Key words: Arsenobetaine hydrobromide; arsenobetaine; marine organoarsenicals.

ABSTRAK

Habluran arsenobetaina hidrobromida telah mendap terus apabila trimetilarsina ditambahkan kepada satu larutan asid bromoasetik dalam benzena. Hasilnya adalah stabil, tak-lembab air dan boleh ditukarkan dengan mudah kepada betaina bebas dengan membenarkan ia melalui resin pertukaran ion berbes.

ABSTRACT

Crystalline arsenobetaine hydrobromide is deposited directly on addition of trimethylarsine to a benzene solution of bromoacetic acid. The product is stable, non-deliquescent and may be readily converted into the free betaine by passage through basic ion exchange resin.

INTRODUCTION

Since the first report of arsenobetaine (Edmonds *et al.* 1977) as a naturally occurring marine organoarsenical isolated initially from the flesh of the western rock lobster, *Panulirus cygnus,* numerous workers have reported this compound from a plethora of marine animals. (Ismail 1986) In addition, evidence has been obtained for the presence of arsenocholine in some marine animals (Norin & Christakopoulos, 1982) and a series of arsenosugars have been isolated from various marine algae. (Edmonds & Francesconi, 1981)

Given its occurrence in human foodstuffs, arsenobetaine has also been the subject of metabolic studies. In a preliminary report (Cannon *et al.* 1983) the compound was shown, in mice, to be non-toxic and rapidly excreted in the urine and faeces. In addition, a negative result was obtained in the Ames' test for chemical mutagens (Cannon *et al.*, 1983). Subsequent metabolic studies have utilised radiotracer techniques, in particular ^{74}As labelled arsenocompounds, but in some cases are of questionable value since the work was carried with radiochemically impure material (Goetz & Norin, 1983).

In this note, we report a modified approach to the synthesis of arsenobetaine which is suitable for adaptation to the preparation of labelled material of high chemical and radiochemical purity.

MATERIALS AND METHODS

General

Melting points were determined on a Kofler hot stage and are uncorrected. Infrared spectra were recorded on an Hitachi Model EPI-G2 instrument for KBr discs and nuclear magnetic resonance spectra were recorded on either a Bruker CXP300 (300 MHz for ^{1}H) or a JEOL FZ100 (25 MHz for ^{13}C) instrument. Chemical shifts are reported

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relative to TMS, for $CDCl_3$ solution of 3-(trimethylsilyl)propane sulphonic acid for D_2O solution. Dowex 2 x 8 (Cl^-) ion exchange resin was cycled with 5% aqueous HCl, water and 5% aqueous NaOH until the titre was constant. The resin was washed thoroughly before use.

Arsenic Analysis

Arsenic analysia of aqueous samples utilised an inductively coupled plasma-atomic emission spectrometer (Labtam Plasmalab, Melbourne, Australia) monitored at 193.7 nm. The instrument was calibrated against As standards of 0 and 100 ppm sodium arsenate.

Synthesis

Iododimethylarsine and trimethylarsine were prepared as described in the literature. (Burrows & Turner, 1920; Challenger & Ellis, 1935) Thus, sodium cacodylate (77.6 g) and Kl (160 g) were dissolved in water (300 ml). The solution was continuously saturated with SO_2 whilst HCl (10 M, 142 ml) was added dropwise. The product separated as it was formed, as a dark yellow oil. Distillation of the dried product gave iododimethylarsine as a pale yellow oil (73.7 g, 56% b.p. 80-84°C/35 mm (Burrows & Turner, 1920: 154-157°C/760 mm). Iododimethylarsine (68.4 g) in di-*n*-butyl ether (100 ml) was added dropwise to methyl magnesium iodide (prepared from methyl iodide (40.8 g) and Mg (7 g)) in di-*n*-butyl ether (100 ml) under an atmosphere of nitrogen. The mixture was stirred continuously whilst maintaining the temperature at 4°C. On completion of the addition, the solution was allowed to warm to room temperature and then heated to distil trimethylarsine (24.4 g, 69%, b.p. 54°C (Burrows & Turner, 1920: 51.9-52°C) directly. The product was stored in ampoules under nitrogen for use as required.

Arsenobetaine hydrobromide: Bromoacetic acid (0.41 g) was dissolved in benzene (2.5 ml) and trimethylarsine (0.5 ml) added dropwise under nitrogen. The solution initially turned green, then blue and finally colourless at which point a crystalline solid began to deposit. The reaction mixture was left overnight. The solvent was removed and the residue twice recrystallised from methanol/acetone to give arsenobetaine hydrobromide as prisms, 0.62 g (81%), m.p. 248°C decomp., dependent on the rate of heating). Found C, 23.16%; H, 4.60%. $C_5H_{15}AsBrO_2$ requires C, 23.10%; H, 4.90%. I.R: V_{max} 3450, 3050, 2950 2900, 2850, 1715, 1640, 1400, 1365, 1275, 1260, 1170, 940, 935, 910, 900, 850 cm^{-1}. ^1H NMR: δ 1.95 (s, 9H), 3.58 (s, 2H). ^{13}C NMR δ 8.6 (q), 31.3 (t), 170.5 (s).

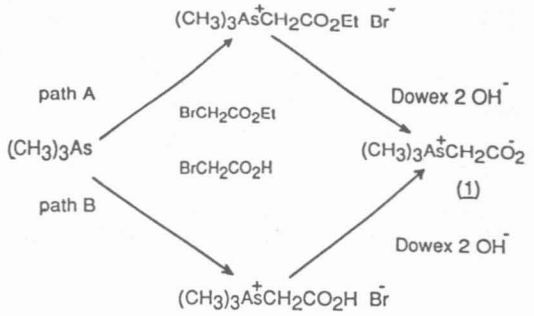
Arsenobetaine picrate: Arsenobetaine hydrobromide (0.1 g) was dissolved in hot ethanol and an ethanolic solution of picric acid (0.1 g) added. On cooling, crystals of arsenobetaine picrate were deposited and were recrystallised from hot ethanol as yellow needles, 0.15 g (94%), m.p. 177-178°C. Found C, 32.37%; H, 3.68%; N, 10.14%. $C_{11}H_{14}AsO_8N_3$ requires C, 32.43%; H, 3.44%; N. 10.14%.

Arsenobetaine reineckate: Arsenobetaine reineckate was prepared from arsenobetaine hydrobromide as described in the literature (Edmonds *et al,* 1977) for arsenobetaine. The product was found to be identical in all respects with an authentic sample.

Arsenobetaine: Arsenobetaine hydrobromide (1.0 g) was dissolved in distilled water (2 ml) and passed through a column of Dowex 2 (OH^-) (100 g). The column was eluted with distilled water and the fractions analysed for arsenic. The arsenic-rich fractions were combined and lyophillised to yield arsenobetaine as a white solid. The product was dissolved in dry methanol and crystallised, on the slow addition of acetone, as white prisms, 0.6 g (87%), identical in all respects with an authentic specimen. ^{13}C NMR: δ 8.3 (q), 34.4 (t), 172.7 (s).

RESULTS AND DISCUSSION

Arsenobetaine (1) is an extremely deliquescent, white crystalline compound which has previously been prepared (Edmond *et al,* 1977) as shown in scheme 1 path A. In the present work we have modified this preparation, scheme 1 path B, by reacting trimethylarsine with bromoacetic acid. This leads directly to arsonebetaine hydrobromide which crystallises from the reaction mixture as it forms. The hydrobromide is a non-deliquescent, white, crystalline and stable solid. As observed for arsenobetaine (Edmond *et al,* 1977) the melting point of the hydrobromide varies with the rate of heating and decomposition accompanies melting.



Scheme 1

The ^{13}C NMR spectra of arsenobetaine and the hydrobromide in D_2O solution show only minor differences in chemcial shift values as would be anticipated and these differences may be ascribed to solution pH differences. The picrate and reineckate derivatives prepared from both arsenobetaine and its hydrobromide were identical in all respects.

With respect to the preparation of radiolabelled compounds, the ease of preparation of the hyrobromide, and more importantly its stability and the ease with which it may be recrystallised, make it an ideal compound to work with. Moreover ^{14}C-labelled bromoacetic acid is commercially available although a label in the other portion of the molecule is also feasible. In particular, whilst it is possible to use the earlier strategies (Goetz & Norin, 1983) with arsenic isotopes, ^{14}C-or ^{3}H-labelled trimethylarsine is also feasible. Possible tritium labelling of trimethylarsine is particularly attractive since this may be effected, in principle, *via* direct exchange and the product may be purified by distillation.

Since this work is directed in part to a study of the metabolism of arsenobetaine in model marine ecosystems, (Ismail Hazar, 1986) the purified hydrobromide may be used directly. Howver, in studies requiring the free betaine, passage of the salt through a basic ion exchange resin readily produces arsenobetaine.

ACKNOWLEDGEMENT

One of us (HI) acknowledges the Public Service Department of Malaysia and Universiti Malaya for a scholarship.

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(Received 7 May, 1988)