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Research Article

One-pot synthesis of [^{14}C]arsenobetaine bromide

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Summary

A one-pot synthesis of [^{14}C]arsenobetaine from 2-dimethylarsinoylacetic acid is described. Cation- and anion-exchange columns are used in the purification of the compound and its conversion to the bromide salt. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: [^{14}C]arsenobetaine; 2-dimethylarsinoylacetic acid; reduction of tertiary arsine oxide

Introduction

Arsenobetaine was first reported as a natural constituent of lobster in 1977,[1] and although subsequent work has demonstrated its presence in a wide range of marine and terrestrial organisms,[2] its origin and possible biological role remain unclear. Most studies investigating the distribution and biotransformation of arsenobetaine and other arsenic compounds use analytical methods based on chromatography with an arsenic-selective detector, and only a few studies have employed radiolabelled compounds.[3] The use of a radiolabel, however, can provide valuable information on uptake and whole body distribution of the intact compound. The previously published synthesis for arsenobetaine bromide involves the quaternization of trimethylarsine with 2-bromoacetic acid (Scheme 1).[1] This procedure is not easily adapted to the synthesis of [^{14}C]arsenobetaine bromide, as the precursor [^{14}C]trimethylarsine is an air-sensitive, low-boiling liquid. Small quantities are difficult to handle, and loss of precious radiolabelled material would be difficult to avoid. Thus, we report the synthesis of arsenobetaine bromide by an alternative route, wherein the ^{14}C-radiolabel was introduced in the final reaction step.

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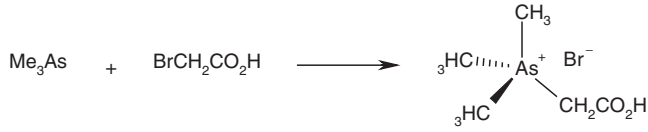
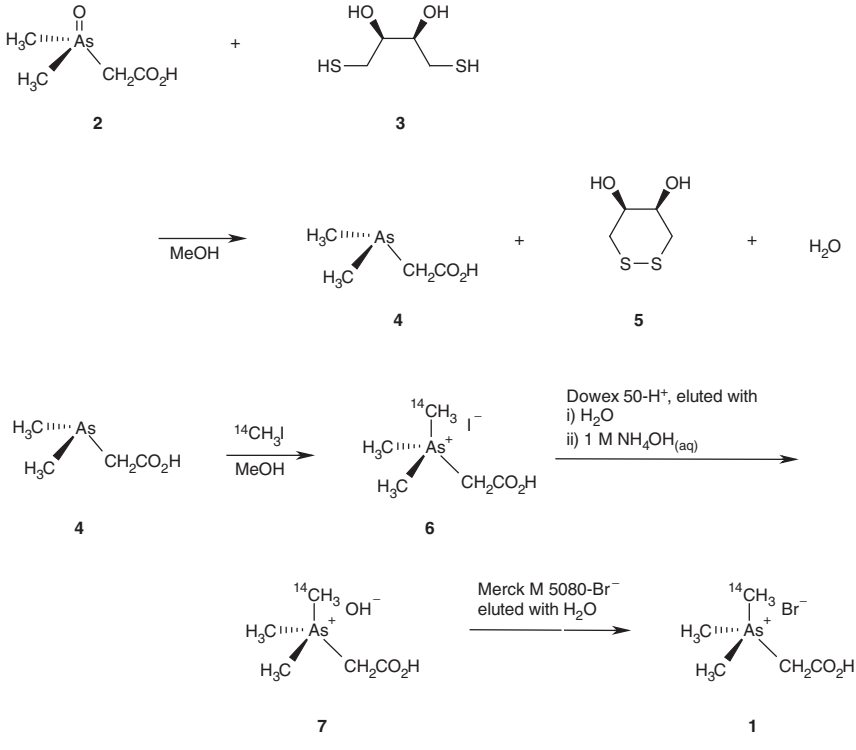


Scheme 1.



Scheme 2.

Results and discussion

[^{14}C]Arsenobetaine iodide (**6**) was synthesized in one-pot, as shown in Scheme 2. 2-Dimethlyarsinylacetic acid (**4**) was generated *in situ* by reducing 2-dimethylarsinoylacetic acid (**2**) with dithioerythritol (DTE), and then quaternized with [^{14}C]methyl iodide to give **6**.

^{1}H NMR studies in d_4-MeOH were undertaken in order to determine suitable reaction times for both the reduction and quaternization reactions. The reduction of **2** in the presence of 1.5 equiv. of DTE was found to be essentially complete within 10 min. By contrast, the quaternization of the arsine **4** occurred to the extent of 23% in the presence of 1.1 equiv. of MeI, and 58% with 10 equiv. of MeI, after 24 h. Beyond this period of time, the formation of side-products increases without increasing the yield of **6**. Thus,

although the overall yield of the reaction could be increased by adding more inactive MeI carrier, the specific activity of the product would be lower. In our final protocol, 1 mmol of **2** was reacted with 1.5 mmol DTE for 1 h, then with 1.1 mmol MeI for 24 h, followed by work-up using cation- and anion-exchange columns.

In aqueous solution, arsenobetaine exists as the hydrated cationic form, which is retained on Dowex 50 cation exchange resin. Thus, the arsenobetaine is easily separated from the by-product *cis*-4,5-dihydroxy-1,2-dithiane (**5**) and unreacted DTE by applying the reaction mixture to the cation exchange column and eluting the uncharged chemical species with water. The arsenobetaine was then displaced from the column in the hydroxide form (**7**) by eluting with aqueous ammonia. Arsenobetaine hydroxide is hygroscopic and difficult to handle; it was readily converted to the non-hygroscopic bromide **1** by passing it through an anion exchange resin in the Br^- form. This step had the added advantage of separating small quantities of **2**, which probably resulted from re-oxidation of **4**, from the final product.

In three 'cold' runs, an inactive product **1** was obtained in yields ranging from 20 to 22%. ^{1}H NMR analysis and elemental microanalysis was done on the 'cold' products, to ensure its purity and consistency, before the same protocol was applied to the actual radiolabelled compound. The ^{14}C-labelled **1** was finally obtained in 19% chemical yield.

Experimental

[^{14}C]methyl iodide was purchased from Amersham Biosciences, while other chemicals were obtained from Aldrich and used without further purification. Dowex 50W8-100 in the H^+ form (4.0 g, column 0.8 cm i.d.) and anion exchange resin M 5080-Cl^- (Merck, 3.5 g, column 1.3 cm i.d.), rendered into the Br^- form with 1 M HBr(aq), were used for cation- and anion-exchange chromatography, respectively. ^{1}H NMR spectra were measured at 25°C using a Varian Unity Inova 400; resonances were referenced to that of the solvent. Specific activity was measured by Prof. W. Sattler at the Institut für Medizinische Biochemie und Medizinische Molekularbiologie, Karl-Franzens Universität.

Dimethyliodoarsine was prepared according to the method of Burrows and Turner.[4]

2-*Dimethylarsinoyl acetic acid* (*2*)

This compound was prepared in a greater yield (previously obtained in 21% yield[5]) by a modified literature procedure. Although the synthesis was first published by Kaise *et al*. in 1988, no yield was specified.[6] Methyliodoarsine (15.0 g, 0.063 mol) was added dropwise to 10 M NaOH(aq) (6.3 ml, 0.063 mol) whilst stirring at 0°C. After 15 min, the lower aqueous layer was removed and

more 10 M NaOH(aq) (6.3 ml, 0.063 mol) was added. To this rapidly stirred suspension, a solution of sodium 2-bromoacetate (freshly prepared from 2-bromoacetic acid (9.4 g, 0.068 mol) and 1 equiv. of 1 M NaOH(aq)) in a minimum amount of water, was added at 0°C. The mixture was stirred overnight at room temperature, after which it became a homogenous solution. The pH of the solution was adjusted to 3.5 using conc. HCl, then shaken with 90% phenol–water (15 ml). The layers were separated and the aqueous layer was extracted with more phenol–water (2×10 ml) before being discarded. The phenolic layers were combined, then diluted to 250 ml with diethyl ether. The product was extracted into water (15 ml) and the layers were separated. The organic layer was extracted with more water (3×10 ml) and the aqueous layers combined. Phenol remaining in the aqueous layer was removed by washing with fresh diethyl ether (3×15 ml). The water was removed on a rotary evaporator, leaving crude **2** as a colourless syrup. Recrystallization from hot methanol–acetone gave rosettes of fine, colourless crystals; m.p. 114°C (uncorrected). Yield: 4.74 g (41.5%). ^{1}H NMR (D_2O): δ 2.31 (s, 6 H, $As(CH_3)_2$), 3.80 (s, 2 H, CH_2).

*[^{14}C]Arsenobetaine bromide (**1**)*

A solution of dithioerythritol (222 mg, 1.44 mmol) in methanol (5 ml) was added to a solution of 2-dimethylarsinoylacetic acid (176 mg, 0.98 mmol) in methanol (12 ml) at room temperature and the mixture stirred for 1 h under an argon atmosphere. The reaction flask was then attached to a vacuum manifold and frozen with liquid nitrogen prior to evacuation. [^{14}C]methyl iodide (1 mCi (37 MBq), 54.0 mCi/mmol, in 70 µl unlabelled methyl iodide, 1.08 mmol) was condensed under vacuum (2.0×10^{-1} mbar) into the flask containing the arsine, over a period of about 30 min. An atmosphere of nitrogen was restored over the mixture, which was then allowed to thaw, and left to stir at room temperature for 24 h. The solvent was then removed by freeze-drying. The residue was dissolved in water (5 ml) and applied with water washings (2×5 ml) to the cation exchange column, and the column was eluted with a further 15 ml of water. Arsenobetaine hydroxide was displaced from the column by eluting with 1 M NH_4OH(aq), discarding the first 5 ml of eluent and collecting the next 20 ml. The water and ammonia were removed by freeze-drying, the residue redissolved in water (2 ml) and applied with water washings (2×2 ml) to the anion exchange column. The column was eluted with a total of 30 ml water, all of which was collected and freeze-dried to obtain **1** as a white, amorphous solid. Yield: 48.0 mg, 19%. The product had a total activity of 0.188 mCi (6.97 MBq), specific activity: 1.02 mCi/mmol (37.7 MBq/mmol).

Unlabelled **1** prepared in an identical manner had ^{1}H NMR (D_2O): δ 1.88 (s, 9 H, As(CH_3)$_3$), 3.36 (s, 2 H, CH_2). Anal. calc. for $C_5H_{12}AsBrO_2$: H, 4.67; C, 23.19. Found: H, 4.62; C, 23.58.

References

1. Edmonds JS, Francesconi KA, Cannon JR, Raston CL, Skelton BW, White AH. *Tetrahedron Lett* 1977; **18**: 1543–1546.
2. Francesconi KA, Kuehnelt D. Arsenic compounds in the environment. In *Environmental Chemistry of Arsenic*, Frankenberger, William Jr T (ed.). Marcel Dekker, Inc.: New York, 2002; 51–94.
3. Challenger F, Lisle DB, Dransfield PB. *J Chem Soc* 1954; 1760–1761; Cullen WR, Froese CL, Lui A, McBride BC, Patmore DJ, Reimer MJ. *Organomet Chem* 1977; **139**: 61–69; Marafante E, Vahter M. *Chem-Biol Interactions* 1984; **50**: 49–57; Vahter M, Norin H. *Environ Res* 1980; **21**: 446–457; Cullen WR, Li H, Pergantis SA, Eigendorf GK, Mosi AA. *Appl Organomet Chem* 1995; **9**: 507–515.
4. Burrows GJ, Turner EE. *J Chem Soc Abs* 1920; **117**: 1373–1380.
5. Francesconi KA. Studies on the biotransformation of arsenic in the marine environment. *Ph.D Thesis*, University of Western Australia, 1991.
6. Kaise T, Yamauchi H, Hirayama T, Fukui S. *Appl Organomet Chem* 1988; 2: 339–347.

