**SHORT PAPER**

# Arsenocholine from anaerobic decomposition of a trimethylarsonioriboside

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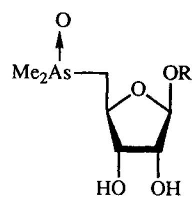
**When subjected to conditions supporting anaerobic microbial activity, the naturally occurring trimethylarsonioriboside, (2′*S*)-2′-hydroxy-3′-(sulpho-oxy)propyl 5-deoxy-5-trimethylarsonio-β-D-riboside 4 was converted to arsenocholine 5 in virtually quantitative yield.**

**Keywords: Trimethylarsonioriboside, anaerobic decomposition, algae, arsenocholine**

$Me_3\overset{+}{As}CH_2CO_2^-$

**1**



**2**

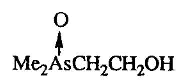
**a** R = $CH_2CHOHCH_2OH$

**b** R = $CH_2CHOHCH_2SO_3H$

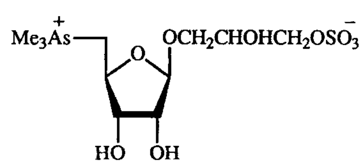
**c** R = $CH_2CHOHCH_2OPO(OH)OCH_2CHOHCH_2OH$

**d** R = $CH_2CHOHCH_2OSO_3H$

**e** R = $CH_2CHNH_2CH_2SO_3H$



**3**



**4**

## INTRODUCTION

Arsenic occurs in seawater mainly as inorganic arsenate, at levels of 2–3 $\mu g\ dm^{-3}$ and in marine biota at levels of up to 100 $mg\ kg^{-1}$ (wet weight). The majority of arsenic in marine animals is present as arsenobetaine **1**,[1] whereas in marine algae the major forms of arsenic are dimethylarsinylribosides **2a–2e**, and arsenobetaine **1** is absent.[2] It has been proposed[2] that dimethylarsinylribosides are transformed into arsenobetaine, at least partly, by microbial activity in sediments, and support[3] for this view has come from the facile transformation of algal dimethylarsinylribosides into 2-dimethylarsinylethanol, **3**. However, attempts in our laboratory to convert **3** into arsenobetaine **1** by anaerobic or aerobic microbial activity have proved unsuccessful.

The trimethylarsonioriboside **4** is also present in marine algae,[4] and although it has been reported as only a minor constituent it may serve as a precursor to arsenobetaine, **1**. This paper reports on the anaerobic decomposition of compound **4**.

## EXPERIMENTAL

Duplicate anaerobic environments were prepared as follows: beach sand (220 $cm^3$, <1 mm particle size) from the surf zone near the Western Australian Marine Research Laboratories was mixed with fresh, chopped brown alga (*Ecklonia radiata*, 2 g, 10 $\mu g\ g^{-1}$ As) and transferred to a

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250 cm$^3$ separatory funnel with deoxygenated seawater (10 cm$^3$). The *Ecklonia* served as a natural source of nutrients; the quantity of arsenic it contributed was small (5%) in comparison with that of the introduced arsenic compounds. Compound **2d** (400 µg As, natural product previously isolated from an algal source[5]) in deoxygenated seawater was added to the first funnel; compound **4** (400 µg As, prepared[6] by reducing **2d** with 2,3-dimercaptopropanol and treatment of the resultant arsine with methyliodide) was similarly added to the second funnel.

The funnels were allowed to become anaerobic over 20 days. By this time the beach sand had turned black and the contents of the funnels smelt strongly of hydrogen sulphide. Each of the funnels was then treated as follows: it was drained and the contents washed with methanol (4 × 50 cm$^3$, the last methanol wash contained negligible arsenic); the effluent and washings were combined, evaporated and the resultant residue extracted with methanol (50 cm$^3$). Half of the extract (1 g total solids) was then subjected to buffered cation-exchange chromatography on CM Sephadex C-25 [26 × 300 mm, 0.1 mol dm$^{-3}$ ammonium formate (pH 6.5) buffer, void volume 100 cm$^3$].

## RESULTS

For the first funnel (compound **2d**) about 5% of the arsenic (as determined by graphite furnace atomic absorption spectroscopy) eluted at the void volume, the position expected for unchanged starting material. The rest of the arsenic eluted in the region (140 cm$^3$) expected for the weakly basic 2-dimethylarsinylethanol, **3**, and was not further examined. Previous work[3] on the anaerobic degradation of dimethylarsinylribosides present in the brown alga *Ecklonia radiata* (compounds **2a**, **2b** and **2c**) showed virtually quantitative conversion to 2-dimethylarsinylethanol, **3**. The observation that the dimethylarsinylriboside **2d** behaved similarly served as a check that the anaerobic conditions achieved in the current experiment were similar to those in the previous experiment.

When the extract from the second funnel (initially containing compound **4**) was subjected to chromatography on CM Sephadex (conditions as above), most of the arsenic (>90%) was greatly retarded, suggesting the presence of a strongly basic arsenic compound. The buffer was removed from the arsenic-containing fraction by gel permeation chromatography on Sephadex LH-20/methanol and the arsenical residue was rechromatographed on CM Sephadex. More than 90% of the arsenic eluted as a single band peaking at 540 cm$^3$ which, on removal of buffer (Sephadex LH-20/methanol) yielded a solid (0.5 mg, 150 µg As) shown to be arsenocholine, **5** (present as the formate), by $^1$H NMR spectroscopy at 300 MHz.

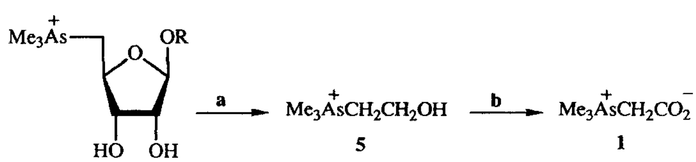
$$Me_3\overset{+}{As}CH_2CH_2OH$$

**5**

## DISCUSSION

The trimethylarsonioriboside **4** degrades, under conditions of anaerobic microbial activity, in a manner analogous to that observed for dimethylarsinylribosides, undergoing cleavage at C3–C4 of the ribose ring. However, the decomposition product, arsenocholine **5**, is an immediate precursor to arsenobetaine **1**, requiring only oxidation of the primary alcohol group.

A simple pathway for the biosynthesis of arsenobetaine from trimethylarsonioribosides may be proposed (Scheme 1). This pathway, unlike that proposed earlier based on dimethylarsinylribosides,[2] does not require that further methylation of arsenic occur outside the alga. Both steps proposed in Scheme 1 have been shown to occur




**Scheme 1** Proposed biosynthetic pathway for arsenobetaine from trimethylarsonioribosides: **a**, anaerboic decomposition; **b**, oxidation.

under conditions likely to be found in the natural marine environment—the first step, described in this paper, could occur in anaerobic sediments, and the second step has been shown[7] to occur readily within the fish *Aldrichetta forsteri* (yellow-eye mullet) when arsenocholine **5** was included in their diet.

Compound **4** is so far the only trimethylarsonioriboside reported[4] in algae, where it occurs at levels of a little less than 1% of that of its dimethylarsinyl analogue **2d**. Other trimethylarsonioribosides may possibly also occur in algae but, if they do, they have so far escaped detection and are not likely to be present at levels much greater than about 1% of the level of the dimethylarsinyl compounds. Although trimethylarsonioribosides may well form arsenocholine **5** in natural systems, it remains to be determined if they occur in algae in sufficient quantities to account for the high levels of arsenobetaine **1** found in marine animals. Nevertheless, the ease with which the steps outlined in Scheme 1 have been shown to occur suggests that trimethylarsonioribosides in algae may at least contribute to the arsenobetaine content of marine animals.

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