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# ISOLATION, CRYSTAL STRUCTURE AND SYNTHESIS OF ARSENOBETAINE, THE ARSENICAL CONSTITUENT OF THE WESTERN ROCK LOBSTER *PANULIRUS LONGIPES CYGNUS* GEORGE

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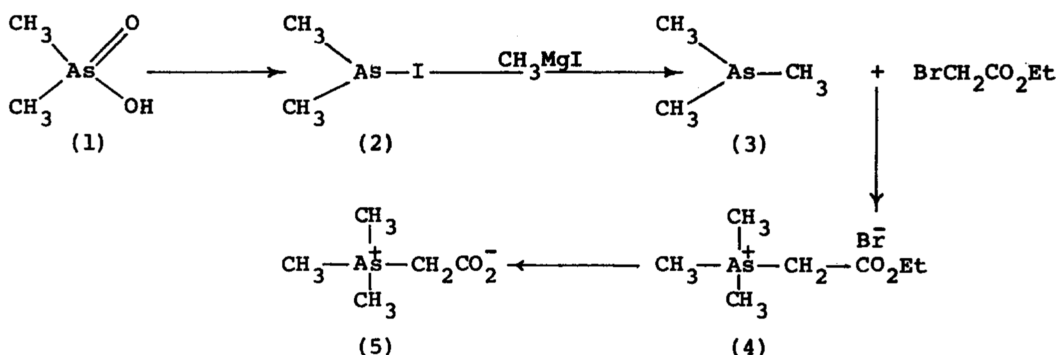
It has been recognized for many years that appreciable quantities of arsenic (up to ~100 p.p.m.) occur in some edible marine animals[1]. At present it seems that the arsenic compound(s) is acquired naturally and its presence does not necessarily reflect environmental pollution. Furthermore, the arsenic compound in sea food does not appear to be a toxic hazard for it is excreted rapidly by the human kidney[2]. Although some progress has been made[3,4] the precise structure of any organic arsenic compound present in a marine organism had not been elucidated when we began work. We now wish to report the isolation of arsenobetaine (5) from the tail muscle of the commercially-important western rock lobster (*Panulirus longipes cygnus* George). The nature of this substance has been elucidated by single crystal X-ray structure determination and its identity has been confirmed by comparison with a synthetic specimen obtained as follows.

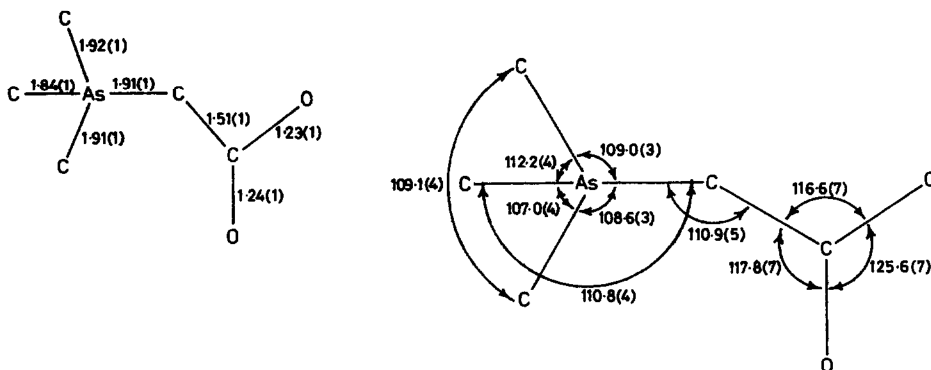



At all stages in the isolation procedure arsenic was located by vapour generation atomic absorption spectrometry[5] following digestion of the sample with a mixture of perchloric and nitric acids. Homogenized tail muscle of the rock lobster (containing 26 p.p.m. of arsenic) was extracted with methanol, the methanol evaporated and the residue dissolved in water. Some

impurities were removed by extraction with ether. The remaining aqueous solution was acidified, shaken with phenol and the arsenic compound recovered from the phenol by dilution with ether followed by extraction with water. Further fractionation was achieved by passage of the aqueous solution through columns of Dowex 2X8($OH^-$) and Amberlite IRC50($H^+$). The eluate, which contained the arsenic compound, was then applied to a column of Zeokarb 225($H^+$)(SRC8, 2% DVB, 3.5 WR, <200 mesh); repeated chromatography involving careful elution of the column with dilute ammonia eventually gave a fraction containing 12.1% arsenic. This product gave two spots $R_f$ 0.58 and 0.45 (revealed by exposure to iodine vapour) on thin layers of cellulose developed with n-butanol:acetic acid:water (60:15:25). Only the spot with $R_f$ 0.58 contained arsenic and this product was isolated by preparative layer chromatography. After further chromatography on Zeokarb 225($H^+$) this fraction crystallized from acetone containing a trace of methanol.

Despite the fact that the solid product travelled as a single spot on a thin layer chromatogram, close inspection revealed that two crystal phases were present. Each of these was deliquescent, and it was found necessary to encapsulate the crystals in Canada balsam before mounting them on a diffractometer.

The first crystals were triclinic, $\underline{P}\bar{1}$, $\underline{a}$ = 8.780(4), $\underline{b}$ = 7.088(3), $\underline{c}$ = 6.914(3)Å; $\alpha$ = 80.70(3), $\beta$ = 77.54(3), $\gamma$ = 78.96(3)°; $D_m$ = 1.59(1), $D_c$ ($\underline{Z}$ = 2) = 1.59g $cm^{-3}$. The structure was solved by the heavy atom method using 799 diffractometer reflections with $\underline{I} > 3\sigma(\underline{I})$ and refined by full matrix least squares to a residual of 0.043. The thermal motions of all non-hydrogen atoms were refined anisotropically; all hydrogen atoms were located in a difference map and their positional parameters were successfully refined. The structure determination establishes the identity of the substance as $(CH_3)_3\overset{+}{As}CH_2CO_2^-.H_2O$, the non-hydrogen molecular geometry being as follows (Å, deg):



The hydrogen atoms of the water molecule bridge the oxygen atoms of successive carboxyl groups in an infinite one-dimensional array $O(H_2O)...O$ 2.75(1), 2.77(1)Å).

The second crystalline substance isolated from this mixture was also triclinic, $\underline{P}1$ or $\underline{P}\bar{1}$, $\underline{a}$ = 10.831(4), $\underline{b}$ = 7.775(2), $\underline{c}$ = 4.884(3)Å; $\alpha$ = 104.28(4), $\beta$ = 89.66(4), $\gamma$ = 110.37(3)$^{o}$ but the diffractometer data reveal that it does not contain an arsenic atom or any other heavy atom; structural studies on this substance are proceeding.

Although arsenobetaine (5) has been the subject of a few biological studies[6] we have been unable to find details of its synthesis. In the present work cacodylic acid (1) was converted into dimethyliodoarsine (2) after the method of Burrows and Turner[7]. Treatment of (2) with methylmagnesium iodide, as recommended by Challenger and Ellis[8], gave trimethylarsine (3) which condensed smoothly with ethyl bromoacetate in benzene to afford the quaternary ester bromide (4). This substance crystallized from a large volume of acetone as prisms m.p. 143-145$^{o}$. Passage of an aqueous solution of (4) through a column of Dowex 2X8($OH^-$) followed by evaporation of the eluate gave arsenobetaine (5) which crystallized from acetone containing a little methanol as prisms of the monohydrate m.p. 204-210$^{o}$(dec.)(Found: C, 30.71; H, 6.64. $C_5H_{11}AsO_2.H_2O$ requires C, 30.63; H, 6.68%). The n.m.r. spectrum ($D_2O$) showed three singlets with $\delta$1.87 (9H,$(CH_3)_3As$), $\delta$3.28 (2H, $CH_2$) and $\delta$4.60 (HOD).

The natural and synthetic samples had the same $R_f$ value and the crystallographic cell dimensions of the synthetic product agreed with those of the natural product within experimental error. The m.p. (203-210$^{o}$ dec.) of selected crystals of the natural product was not depressed on admixture with synthetic arsenobetaine (5). The mass spectra (200$^{o}$/70eV) of the two samples were identical. No peaks with m/e >134 appeared in the spectrum and the base peak was at m/e 103. In addition, both samples yielded a mixture of trimethylarsine and dimethylarsine in the ratio of *ca.* 30:70 after treatment first with strong aqueous sodium hydroxide and then with sodium borohydride[3]. The mechanism of this degradation has not yet been elucidated but it is possible that trimethylarsine oxide and cacodylic acid (1) are intermediates in this process.

Betaine esters are conveniently hydrolysed[5] by treatment with a strongly basic anion exchange resin and as Dowex 2($OH^-$) was used during the isolation of arsenobetaine (5) from the extract of the rock lobster muscle the possibility that arsenobetaine (5) may be an artefact, derived by hydrolysis of a quaternary betaine ester or amide, was considered. However, we have

been unable to find any evidence to support this conjecture. Thin layer chromatography of a freshly-prepared methanol extract of the muscle, which had not been subjected to treatment with base, revealed that the arsenic-containing compound had the same $R_f$ value (0.58) as synthetic arsenobetaine (5). Furthermore, chromatography of the quaternary betaine ester (4) under the same conditions showed it to be significantly more mobile ($R_f$ 0.75) in this system.

Much work remains to be done on the biosynthesis and metabolism of arsenobetaine (5) in *Panulirus longipes cygnus* George, and possibly other marine organisms. However, the way is now clear for these studies to be undertaken and for a detailed toxicological assessment of this naturally occurring organic arsenic compound to be made.

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