Arsenic Compounds from the Kidney of the Giant Clam *Tridacna maxima*: Isolation and Identification of an Arsenic-containing Nucleoside†

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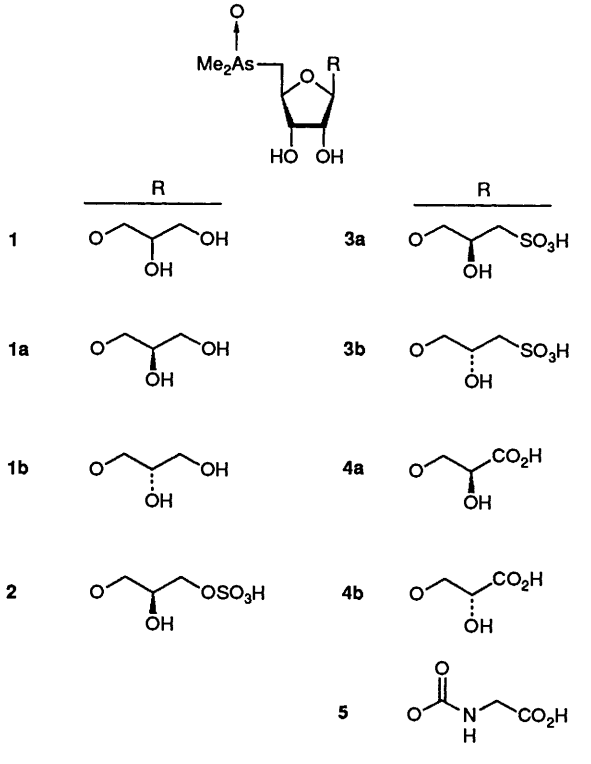
The kidney of the giant clam *Tridacna maxima* contains algal arsenic compounds, probably as a consequence of the clam's symbiotic relationship with unicellular algae (zooxanthallae). Extraction of *Tridacna* kidneys yielded three novel arsenic compounds, *N*-(5'-deoxy-5'-dimethylarsinoyl-β-D-ribosyloxycarbonyl)glycine **5**, (2*S*)-3-(5'-deoxy-5'-dimethylarsinoyl-β-D-ribosyloxy)-2-hydroxypropanoic acid **4a**, and (2*R*)-3-(5'-deoxy-5'-dimethylarsinoyl-β-D-ribosyloxy)-2-hydroxypropanoic acid **4b**, in addition to four previously reported dimethylarsinoylribosides. The structures of the three new compounds were assigned chiefly from NMR data, and those for compounds **4a** and **4b** were confirmed by synthesis. Extraction of a second batch of *Tridacna* kidneys gave, in addition to compounds identified in the first extraction, (2*S*)-3-[(5'-deoxy-5'-trimethylarsonio-β-D-ribosyloxy)-2-hydroxypropyl]sulfate **18** and two novel compounds: an arsenic-containing nucleoside, 9-(5'-deoxy-5'-dimethylarsinoyl)-9*H*-adenosine **16** and *N*-[4-(dimethylarsinoyl)butanoyl]taurine **21**. Syntheses of compounds **16** and **21** are reported. The presence of the nucleoside **16** in *Tridacna*, as a consequence of algal metabolism, supports a proposed pathway for the biogenesis of arsenic-containing ribosides by algae involving methylation and adenosylation by *S*-adenosylmethionine. A biogenetic pathway for compound **21** involving donation of the 3-amino-3-carboxypropyl moiety of *S*-adenosylmethionine is also proposed. The presence of both compounds **16** and **21** in *Tridacna* may represent the first example of donation, by *S*-adenosylmethionine, of all three of its alkyl groups to a single acceptor (arsenic) within one organism.

Arsenic-containing ribosides have been isolated from a number of macroalgae,[1b] in particular from brown algae where arsenic occurs typically at levels of 10–40 mg kg^{-1}. Arsenic-containing ribosides have also been identified in some bivalve molluscs[2] where their presence presumably results from the consumption of unicellular algae by the molluscs. In the case of the giant clam *Tridacna maxima* the presence of high concentrations of arsenic-containing ribosides in the kidney probably results from the presence of symbiotic unicellular algae (zooanthallae) in the clam tissues.[3,4] An earlier study[4] identified the major compounds in the kidney of *Tridacna* as the arsenic-containing ribosides **1** (now known to have the stereochemistry depicted in **1a**)[5] and **2**; other arsenicals were reported as being present but they remained unidentified.

The biogenesis of arsenic-containing ribosides possibly involves the novel transfer of the adenosyl group from *S*-adenosylmethionine (AdoMet) to arsenic, and identification of additional algal arsenic compounds may reveal intermediates in the proposed biogenetic pathway.[1b] The kidney of the giant clam *Tridacna maxima* is a rich source of algal arsenicals, and these compounds are further examined here.

Results and Discussion

Isolation of Arsenic Compounds from the First Extraction of Tridacna *Kidneys.*—In our previous study[4] of the arsenic constituents of *Tridacna* kidneys, in which the two major arsenic compounds present were identified, anion-exchange chromatography on DEAE-Sephadex gave a fraction containing a mixture of three minor arsenic compounds. This mixture was set aside and not examined in the earlier work. In the current study, repeated chromatography on DEAE-Sephadex separated this arsenic into three fractions of increasing acidity.



The arsenic material from the most acidic of the fractions was isolated as a syrup (3.9 mg). The ^{1}H NMR spectrum of this material indicated, by a doubling of some signals, the presence of two diastereoisomers in the ratio 4:1. The ^{1}H and ^{13}C NMR signals of the major component of this mixture were identical with those of (2*S*)-3-(5'-deoxy-5'-dimethylarsinoyl-β-D-ribosyloxy)-2-hydroxypropanesulfonic acid, compound **3a**, previously isolated from the brown macroalga *Ecklonia radiata*.[6] The ^{1}H

† A preliminary account of part of this work has been published: see ref. 1(*a*).

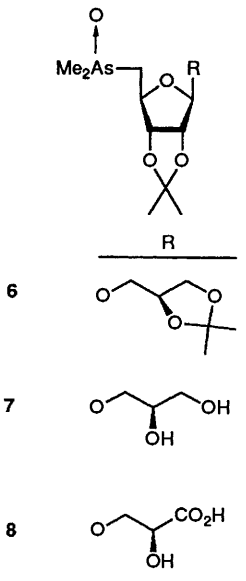
NMR signals of the minor component were consistent with its being the C-2 diastereoisomer of the major component, *viz.* the acid **3b**. Previous work has shown the presence of both these C-2 diastereoisomers (3:1) in the brown algae *Hizikia fusiforme*[7] and *Laminaria japonica*.[8] The configuration at C-2 of acids **3a** and **3b** has been recently established by comparison of their ^{1}H NMR data with those of model compounds.[9]

The arsenic constituent from the least acidic of the three fractions from DEAE-Sephadex was isolated as a syrup (5.3 mg). The ^{1}H NMR spectrum of this material again showed the presence of a dimethylarsinoylriboside. The only substantial difference from previously isolated arsinoylribosides was in the aglycone region of the spectrum, which contained only three non-exchangeable protons. Some of the signals in the spectrum were poorly resolved, and this, together with the presence of three signals (one broadened) at δ 1.9 for the two methyl groups on arsenic, suggested that the syrup was again a mixture of diastereoisomers.

The ^{13}C NMR spectrum supported this view, with many of the signals appearing as double resonances in the ratio 2:3. Signals for the 5-dimethylarsinoylribosyl moiety could be readily assigned, and the remaining signals revealed that the aglycone comprised three carbons with resonances at δ_C 70.2 (CH_2), 71.8 (CH) and 178.2 (C=O) for the major isomer, and at δ_C 71.0, 72.1 and 178.2 for the minor isomer. The presence of the carboxylate ion suggested by the signal at δ_C 178.2 was supported by the IR spectrum, which showed a strong absorbance at 1597 cm^{-1}. On the basis of these spectral data the compounds were assigned as (2*S*)-3-(5′-deoxy-5′-dimethylarsinoyl-β-D-ribosyloxy)-2-hydroxypropanoic acid **4a** and the 2*R*-diastereoisomer **4b**. The structures of these compounds have been drawn as the free acids although, as a consequence of the isolation procedure, they were isolated as salts.

Confirmation of the structures proposed for the naturally occurring diastereoisomers **4a** and **4b** was sought by synthesis. The approach taken was the oxidation of the primary alcohol group in the diol **7** and in the tetraol **1b**. The diol **7** was obtained by selective deprotection (pyridinium toluene-*p*-sulfonate (PPTS)–methanol) of the acetonide **6**, prepared by the method of McAdam *et al.*[5] Treatment of the diol **7** with oxygen and a platinum catalyst under basic conditions achieved a fairly selective oxidation of the primary alcohol group, giving the carboxylic acid **8** as the major product. One of several minor products separated from acid **8** on gel permeation chromatography (GPC) and was shown to be dimethylarsinic acid (^{1}H NMR); the formation of dimethylarsinic acid following treatment of dimethylarsinoylribosides with aqueous sodium hydroxide has been reported.[6] Without further purification, compound **8** was treated with trifluoroacetic acid (TFA) to give (2*S*)-3-(5′-deoxy-5′-dimethylarsinoyl-β-D-ribosyloxy)-2-hydroxypropanoic acid **4a** in 40% overall yield from the fully protected compound **6**. On evaporation with aqueous ammonia, compound **4a**, as the ammonium salt, recorded NMR spectra identical with those of the major component of the mixture of natural diastereoisomers isolated from *Tridacna*, and allowed assignment of this major component as the 2*S* diastereoisomer to be made.

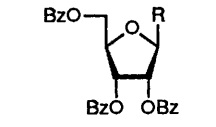
In the synthesis of compound **4b**, selective oxidation was carried out on the tetraol **1b**. Treatment of the protected tetraol **13** (prepared essentially by the method used by McAdam *et al.*[5] in the preparation of compound **6**) with TFA gave the tetraol **1b**, which was oxidised (oxygen/platinum) to (2*R*)-3-(5′-deoxy-5′-dimethylarsinoyl-β-D-ribosyloxy)-2-hydroxypropanoic acid **4b**. In the ^{1}H NMR spectrum of acid **4b** the methyl groups on arsenic gave signals markedly downfield from the position expected for an arsine oxide, indicating that the acid **4b** existed in solution as the arsonium zwitterion.[5] Evaporation with aq. ammonia yielded the ammonium salt of acid **4b**, which had NMR spectra identical with those of the minor constituent of the natural mixture of diastereoisomers isolated from *Tridacna*.



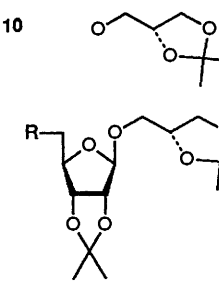
The remaining arsenic band (of intermediate acidity) from DEAE-Sephadex chromatography was shown, by TLC, to consist of one major and three minor arsenic compounds; the minor arsenicals were not further examined. Further purification of the major arsenic component by repeated anion-exchange chromatography and TLC gave a new compound as a syrup (1.7 mg). The ^{1}H and ^{13}C NMR spectra of this material showed that it also contained a dimethylarsinoylribosyl moiety. However, signals assigned to the anomeric carbon and hydrogen showed clear differences from those of related compounds isolated hitherto and indicated that the new compound did not contain the usual glycosidic linkage. For the dimethylarsinoylribosides previously encountered, the C-1 signal occurred consistently in the range δ_C 107–109, and that of 1-H at δ 5.0, whereas for the new compound C-1 and 1-H gave resonances at δ_C 101.3 and δ_H 5.9, respectively. Both of these differences were consistent with replacement of -OR by -OCOR at the anomeric centre, resulting in shielding of the anomeric carbon (C-1) which is *gamma* to the carbonyl oxygen, and deshielding of the anomeric proton (1-H) because of anisotropic effects. Similar differences in the chemical shifts of C-1 and 1-H are observed upon comparison of the NMR data for the alkyl β-D-ribofuranoside **10** ($\delta_{C\text{-}1}$ 105.8; $\delta_{1\text{-}H}$ 5.33) with those for the acetyl-β-D-ribofuranose **9** ($\delta_{C\text{-}1}$ 98.4; $\delta_{1\text{-}H}$ 6.44).

The NMR spectra of the new compond displayed another unusual feature. There was a duplication of some signals (2:1 ratio) which appeared to be inexplicable in terms of a mixture of diastereoisomers since no asymmetric centre was apparent in the aglycone. When ^{1}H NMR spectra were run at elevated temperatures (D_2O; 20–60 °C in 10 °C increments) this duplicity of signals disappeared, which suggested that, at room temperature, the new compound existed in solution as two, slowly interconverting isomers. This behaviour is similar to that shown by amides which display partial double-bond character and restricted rotation around the C(O)–N bond, and has also been observed[10,11] in naturally occurring formamides. These observations suggested that the compound contained a C(O)–N bond, and existed in solution as a pair of geometric isomers (2:1 ratio).

When resonances for the dimethylarsinoylribosyl moiety were assigned, the NMR spectra for the new compound showed



	R
9	OAc



	R
11	OH
12	Cl

13	Me_2As

the presence of three additional carbons and two additional hydrogens. For the major isomer the ^{13}C NMR signals occurred at δ_C 44.0 (CH_2), 156.4 and 177.1, and the ^1H NMR signals occurred at δ 3.67 (1 H, d, J 17.6 Hz) and δ 3.74 (1 H, d, J 17.6 Hz). The signal at δ_C 177.1 suggested the presence of a carboxylate group and this was supported by the IR spectrum which showed a strong absorbance at 1602 cm^{-1}. The large geminal coupling constant (J 17.6 Hz) for the methylene protons was in accord with their being adjacent to the carboxylate group, and the ^{13}C and ^1H chemical shifts for this methylene group were also consistent with its being bound to nitrogen. Finally, the ^{13}C signal at δ_C 156.4 was that expected for a carbamate, and the absorbance at 1716 cm^{-1} in the IR spectrum was also in agreement with that assignment. These data enabled the three-carbon aglycone to be formulated as $OCONHCH_2CO_2^-$. The ammonium salt of benzyloxycarbonylglycine ($PhCH_2OCONHCH_2CO_2^-$ NH_4^+) served as a useful model for this portion of the molecule: δ_H 3.66 (s, NCH_2); δ_C 44.3, 45.0 (6:1) (NCH_2), 158.6 (OCONH) and 177.5 (CO_2^-).

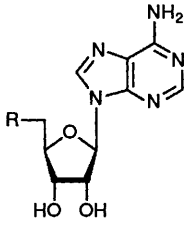
On the basis of this evidence, the new compound from *Tridacna* was identified as *N*-(5′-deoxy-5′-dimethylarsinoyl-β-D-ribosyloxycarbonyl)glycine **5**. Again, as for compounds **4a** and **4b**, compound **5** was isolated as a salt but is shown here as the free acid. The data on which structure **5** was assigned allow for the possibility of its being a β-L-riboside. However, in view of the likely biogenetic origin of arsinoylribosides (see below), it has been assumed that naturally occurring arsinoylribosides have the β-D-configuration.

In summary, from the results reported here and those from our earlier study,[4] the arsenic compounds identified in the first extraction of *Tridacna* kidneys were compounds **2** (constituting 50% of the total arsenic), **1a** (30%), **4a** (3%), **4b** (2%), **3a** (2%), **5** (2%) and **3b** (0.5%). The presence of small amounts of other arsenic compounds was also noted, and these were examined in a second study as reported below.

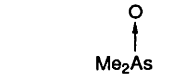
Isolation of Arsenic Compounds from the Second Extraction of Tridacna *Kidneys.*—A further sample of giant clams was collected near Exmouth, Western Australia in 1988. Clam kidneys were extracted with aq. methanol and the extract was subjected to GPC. Chromatography on DEAE-Sephadex was then used to separate the arsenic into two fractions, one containing non-acidic arsenicals and the second containing acidic arsenicals.

The fraction of non-acidic arsenicals contained one major arsenic compound, previously shown to be the dimethylarsinoylriboside **1a**, and several minor arsenicals which were separated by GPC. One of these compounds, constituting about 0.4% of the total arsenic in *Tridacna* kidney, was purified by buffered cation-exchange chromatography, TLC and GPC on Sephadex G-15/water. Further GPC on Sephadex LH-20 with methanol as eluent (see Experimental section) yielded a glass (1.5 mg), the NMR spectra of which indicated that it was another dimethylarsinoylriboside. However, signals for the (usually) three-carbon aglycone portion typical of dimethylarsinoylribosides previously isolated were absent. Instead, the NMR spectra showed the presence of a purine ring system [δ_H 8.24 (1 H, s) and 8.30 (1 H, s). δ_C 119.3, 140.7, 149.0, 153.1, 155.9 (5 C)], and indicated that this compound from *Tridacna* was the novel compound 9-(5′-deoxy-5′-dimethylarsinoyl)-9*H*-adenosine **16**—an assignment confirmed by synthesis.

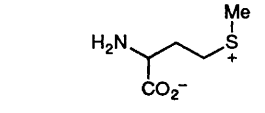
The arsenic-containing nucleoside **16** was prepared from 5′-chloro-5′-deoxyadenosine **15**. Although we initially prepared the chloride **15** by treatment of adenosine **14** with triphenylphosphine and carbon tetrachloride in pyridine,[12] the method of Kikugawa and Ichino[13] using thionyl dichloride in hexamethylphosphoric triamide (HMPA) was simpler, higher yielding (85%) and more suitable for larger scale preparations. Previously, the dimethylarsino moiety has been introduced into a protected riboside by addition of dimethylarsinosodium (Me_2AsNa) in tetrahydrofuran (THF) to a solution of the appropriate halide in THF.[5,9] However, initial attempts at displacement of the chlorine atom of compound **15** with the arsenic nucleophile (Me_2AsNa) proved unsuccessful. Probably the dimethylarsinosodium reagent was sufficiently basic to react with the hydroxy groups of substrate **15**, thereby consuming the reagent before the desired displacement of chloride had occurred. This problem was overcome by adding the chloride **15** to a solution containing the arsenic nucleophile (5 mol equiv.). Oxidation of the resultant arsine with hydrogen peroxide then gave the desired product, 9-(5′-deoxy-5′-dimethylarsinoyl)-9*H*-adenosine **16**, as a glass.



	R
14	OH
15	Cl



16	Me_2As



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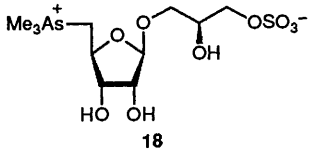
The fraction of acidic arsenicals (from DEAE-Sephadex) was subjected to further anion-exchange chromatography. The elution positions of the major arsenic peaks indicated the presence of the arsenicals previously isolated from *Tridacna* kidney (first extraction), and these were not further examined. In addition, there were also several minor, acidic arsenic compounds, two of which were further investigated.

The first minor acidic arsenical (representing 0.4% of the total arsenic in *Tridacna* kidney) eluted close to the void volume on DEAE-Sephadex chromatography. Removal of Tris* buffer yielded a crystalline material (86 mg), shown to be predominantly trigonelline (1-methylpyridinium-3-carboxylate inner salt) by ^{1}H NMR spectroscopy. Under the elution conditions, trigonelline chromatographed as a weak acid (its aromatic nature may have contributed to its retardation) and it proved difficult to remove from the desired arsenical. Purification was finally effected by applying the mixture to a column of DEAE-Sephadex equilibrated with 0.01 mol dm^{-3} Tris/0.02 mol dm^{-3} boric acid. The arsenical was strongly retained on this column before being removed with 0.5 mol dm^{-3} Tris. TLC and GPC yielded a syrup (0.7 mg), identified as (2*S*)-3-[(5′-deoxy-5′-trimethylarsonio-β-D-ribosyloxy)-2-hydroxypropyl]sulfate **18** by comparison of ^{1}H and ^{13}C NMR data with those of synthetic material.[9] This quaternary arsonio compound has previously been reported as a trace constituent of the brown alga *Sargassum thunbergii*.[14]



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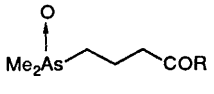
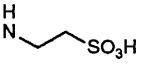
The second minor acidic arsenical, representing 0.2% of the total arsenic in *Tridacna* kidney, was shown to be strongly acidic by DEAE chromatography. This compound was isolated as a syrup (0.8 mg), the NMR data of which clearly indicated that it was **not** a riboside. The ^{1}H NMR spectrum comprised a six-proton singlet (δ 1.7) and five two-proton multiplets or triplets (centred at δ 1.92, 2.15, 2.4, 3.08 and 3.58), and the ^{13}C NMR spectrum contained signals assigned to two methyl groups (δ_C 12.8), five methylene groups (δ_C 18.3, 29.3, 35.3, 36.3, 49.9), and a carbonyl group (δ_C 175.2). These data, together with the IR spectrum which displayed two strong absorbances at 1648 and 1560 cm^{-1}, characteristic of a secondary amide, suggested that the compound was the novel *N*-[4-(dimethylarsinoyl)butanoyl]taurine **21** (presumably isolated as the sulfonate), and this was confirmed by synthesis.



	R
19	OEt
20	OH
21	

The approach taken for the preparation of the amide **21** was to prepare a suitable dimethylarsinoylcarboxylic acid derivative, followed by aminolysis with 2-aminoethanesulfonic acid (taurine). The ester **19** was prepared by treatment of ethyl 4-bromobutanoate with dimethylarsinosodium, then hydrogen peroxide, in the usual way.[5,9] Attempted aminolysis of the ester **19** with a salt derived from taurine was unsuccessful. Amides of taurine have been prepared[15] by the addition of taurine in aqueous base to the mixed anhydride formed on treatment of the *N*-protected amino acid with ethyl chloroformate. Thus, 4-(dimethylarsinoyl)butanoic acid **20** was treated with ethyl chloroformate to give the mixed anhydride as an active intermediate. Attempted aminolysis of this intermediate with taurine in aqueous base at room temperature was not successful, but when the reactants were heated (120 °C) in acetonitrile the desired product **21** was obtained in low (25%) yield.

Biogenesis of Arsenic Compounds in Marine Algae.—The identification of the arsenic-containing nucleoside **16** in *Tridacna* kidney (resulting from unicellular algal metabolism) supports the proposed role of *S*-adenosylmethionine (AdoMet) **17** in the biogenesis of arsenic-containing ribosides in marine algae (Scheme 1).[1b] The pathway requires reduction and oxidative methylation of absorbed oceanic arsenate by algae in two stages, giving dimethylarsinic acid, a known algal metabolite.[16] Reduction followed by oxidative adenosylation would give the nucleoside **16**, which, on glycosidation, would yield the range of dimethylarsinoylribosides that have been identified from algal sources. Although the order of alkylation in the biogenesis of dimethylarsinoylribosides as shown in Scheme 1 is considered the most likely, adenosylation may precede one, or both, of the methylation stages. Trimethylarsonioribosides (of which the zwitterion **18** is, so far, the only known naturally occurring example) may also be formed by this pathway, although the stage at which the third methyl group is transferred to arsenic (shown in Scheme 1 as the final stage) is less certain. The possibility also exists for the methylation of arsenic to proceed in algae without adenosylation, yielding a tetramethylarsonium salt. Such an arsenic species has yet to be identified in algae although it is a common constituent of bivalve molluscs which feed on unicellular algae.[17]

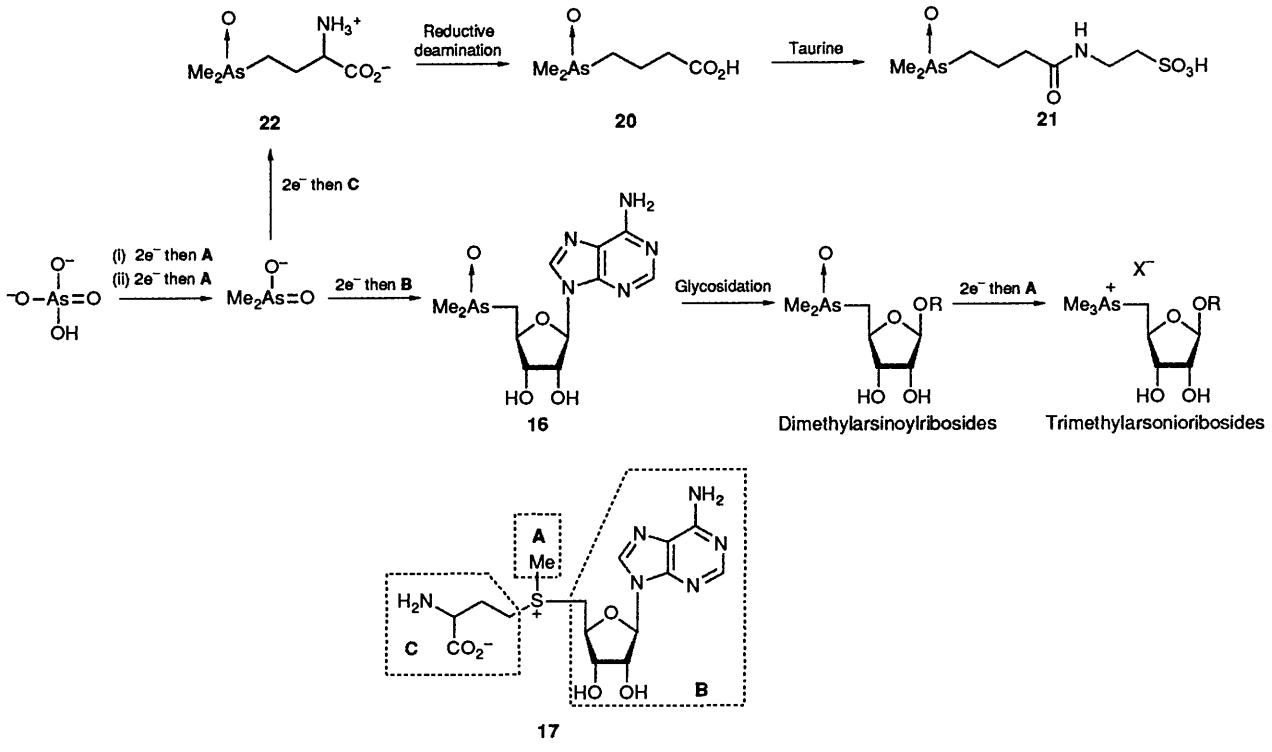
The biogenesis of *N*-[4-(dimethylarsinoyl)butanoyl]taurine **21** may also involve methylation and alkylation by AdoMet **17**. In addition to its primary role as a methyl donor, AdoMet has been reported to transfer its 3-amino-3-carboxypropyl group, for example to the nucleophilic nitrogen of uridine.[18] Possibly a similar transfer may occur to a dimethylated arsenic species, leading to the intermediate **22** as outlined in Scheme 1. Reductive deamination[19] to give compound **20**, followed by aminolysis with taurine,[20] would yield the observed natural product **21**. Intermediates **22** and **20** in this proposed biogenetic pathway have not yet been detected in *Tridacna* kidney.

It is of interest that tripolyphosphate is the only previously recorded acceptor of an adenosyl group from AdoMet. Cantoni predicted[21] that AdoMet would be found to function as an adenosyl donor to a variety of other suitable acceptors; the arsenic-containing nucleoside **16** described here represents the first, fully characterised compound resulting from likely adenosyl donation by AdoMet. In addition, the presence of the nucleoside **16** and the amide **21** in *Tridacna* may represent the first example of donation by AdoMet **17** of all three of its alkyl groups to a single acceptor within one organism.

Experimental

Throughout the isolation procedures arsenic compounds were monitored by determination of the arsenic concentrations in chromatographic fractions by graphite-furnace atomic absorption spectrophotometry (GFAAS). GPC was carried out with

* Tris = 2 amino-2-(hydroxymethyl)propane-1,3-diol.



Scheme 1 Proposed pathways for the biogenesis of arsenic compounds in marine algae

Sephadex G-15 or Sephadex LH-20 media supplied by Pharmacia LKB Biotechnology. Unless otherwise stated, GPC refers to chromatography on Sephadex G-15 (26 × 900 mm, void volume 150 cm^3) with water as eluent; anion-exchange chromatography refers to chromatography on DEAE A-25 Sephadex (26 × 900 mm, void volume 350 cm^3) equilibrated with 0.05 mol dm^{-3} Tris buffer at pH 8.0; and cation-exchange chromatography refers to chromatography on CM C-25 Sephadex (26 × 300 mm, void volume 100 cm^3) equilibrated with 0.1 mol dm^{-3} ammonium formate buffer at pH 6.5. In all cases following ion-exchange chromatography, buffer was separated from the arsenic constituents by GPC.

TLC was performed on layers (1 mm thickness) of cellulose (Whatman CC 41) or Silica 60 (Merck, Darmstadt) on glass plates (200 × 200 mm); or on glass plates precoated with layers of cellulose (0.5 mm or 0.1 mm) or silica (0.2 mm) available from Merck, Darmstadt. Arsenic compounds were located by scribing plates into 5 mm bands and analysing each band for arsenic by GFAAS, following extraction with water.

NMR spectra were recorded on Bruker and Varian spectrometers at 300 MHz (^1H), or 75.5 MHz (^{13}C). Spectra recorded in $CDCl_3$ had tetramethylsilane as an internal standard; ^1H NMR spectra in D_2O were recorded relative to external sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS) at δ 0.00, with J-values given in Hz; and for ^{13}C NMR spectra in D_2O, methanol (δ_C 49.00) served as an external standard. DEPT experiments were used to assist in assignment of ^{13}C NMR signals. IR absorbance spectra were obtained as films on a diamond cell with a Biorad FTS-40 spectrophotometer, and optical rotations were determined in a micro cell on a Perkin-Elmer 141 polarimeter at ambient temperature; $[\alpha]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}.

Light petroleum refers to the fraction boiling in the range 60–80 °C.

First Extraction of Tridacna.—A sample of giant clams (*Tridacna maxima*) was collected from Shark Bay, Western Australia in 1981. Details of the extraction of the clam kidneys and the isolation of the two major arsenic compounds, **1** and **2**, have been reported.[4]

Arsenic-containing material (880 mg, 4 mg As) which had eluted earlier than compound **2** on DEAE-Sephadex was subjected to further anion-exchange chromatography on DEAE-Sephadex. Arsenic eluted from the column in three overlapping bands with peaks at 1620 cm^3, 1820 cm^3 and 1980 cm^3. Repeated anion-exchange chromatography yielded three arsenic fractions of increasing acidity designated A/1 (85 mg, 1.5 mg As), A/2 (240 mg, 1.1 mg As) and A/3 (169 mg, 1.2 mg As), respectively.

Isolation of epimers **4a** *and* **4b**. The material in the least acidic fraction, A/1, was subjected to TLC [cellulose; butan-1-ol–acetic acid–water (60:15:25)], and one major arsenic band was located at R_f 0.46. Further TLC [cellulose; propan-1-ol–NH_4OH (7:3); R_f 0.33] followed by GPC yielded a syrup (5.3 mg), shown by NMR spectroscopy to be a mixture of C-2 diastereoisomers of 3-(5′-deoxy-5′-dimethylarsinoyl-β-D-ribosyloxy)-2-hydroxypropanoic acid, compounds **4a** and **4b** (see below for NMR data of individual compounds).

Isolation of compound **5**. The material in fraction A/2 (240 mg, 1.1 mg As) was subjected to TLC [cellulose; butan-1-ol–acetic acid–water (60:15:25); R_f 0.44 and propan-1-ol–NH_4OH (7:3); R_f 0.29] to give one major arsenic fraction (8.3 mg, 0.8 mg As); three minor arsenicals also present were not further investigated. Further chromatography on DEAE-Sephadex and TLC [cellulose; propan-2-ol–NH_4OH (7:3); R_f 0.26] followed by GPC gave the arsenic compound **5** as a syrup (1.7 mg). In D_2O at room temperature, compound **5** existed as a pair of slowly interconverting isomers, as shown by a duplication of some signals in the NMR spectra; δ_H(300 MHz; D_2O) 1.82 and 1.84 (6 H, 2 s, Me_2As; minor isomer 1.84 and 1.86), 2.6 (2 H, m, 5′-H_2), 3.67 (1 H, d, $J_{2,2}$ 17.6, 2-H), 3.74 (1 H, d, $J_{2,2}$ 17.6, 2-H; minor isomer 3.59 and 3.68), 4.27–4.38 (3 H, m, 2′-, 3′- and 4′-H) and 5.92 (1 H, s, 1′-H; minor isomer 5.91); δ_C(75.5 MHz; D_2O) 14.0 and 14.7 (Me_2As; minor isomer 14.2 and 14.7), 35.9 (C-5′), 44.0 (C-2; minor isomer 44.9), 74.0 and 75.5 (C-2′, -3′; minor isomer 74.0 and 75.7), 78.0 (C-4′), 101.3 (C-1′), 156.4 (OCON) and 177.1 (CO_2);

ν_{max}/cm^{-1} 1716s, 1602s, 1400, 1274, 1260, 1135, 966, 853 and 778.

From these data compound **5** was designated as *N*-(5′-deoxy-5′-dimethylarsinoyl-β-D-ribosyloxycarbonyl)glycine.

Isolation of epimers **3a** *and* **3b**. The arsenical material in fraction A/3 was purified by TLC [cellulose; butan-1-ol–acetic acid–water (60:15:25); R_f 0.27; cellulose; propan-1-ol–NH_4OH (7:3); R_f 0.45; silica; ethanol–water (4:1), R_f 0.53] followed by GPC and was obtained as a syrup (3.9 mg). ^{1}H NMR spectroscopy indicated the presence of two diastereoisomers in the ratio 4:1. The ^{1}H and ^{13}C NMR spectra of the major component in this mixture were identical with those of (2*S*)-3-(5′-deoxy-5′-dimethylarsinoyl-β-D-ribosyloxy)-2-hydroxypropanesulfonic acid **3a** previously isolated from *Ecklonia radiata*.[6] The ^{1}H NMR spectrum of the minor component of this mixture was consistent with that reported for the 2*R*-isomer **3b**.[7]

Second Extraction of Tridacna.—Giant clams (*Tridacna maxima*) were collected near Exmouth, Western Australia in June and August 1988. The kidneys (700 g total wt) were removed from 74 individuals and homogenised with aq. methanol (1:1; 1 × 4 dm^3, 1 × 3 dm^3, 2 × 2 dm^3). The combined extracts were filtered to yield a dark brown powder (171 g, 3 mg As), which was not further examined, and a black filtrate, which was evaporated to dryness and extracted with acetone. Evaporation of the acetone gave a brown oil (3 g, 0.3 mg As) which was discarded. The acetone-insoluble black gum (200 g, 150 mg As) was taken up in water and subjected to GPC (Sephadex G-15/water; 50 × 850 mm, void volume 500 cm^3) in eight equal portions, whereupon the arsenic separated into two broad overlapping fractions designated 'early' (85 g, 110 mg As) and 'late' (73 g, 20 mg As).

The 'early' fraction from Sephadex G-15 was subjected to buffered anion-exchange chromatography (DEAE-Sephadex 50 × 900 mm; 0.05 mol dm^{-3} Tris, pH 8.0; void volume 1.2 dm^3) and the arsenic was separated into a non-acidic fraction (void volume, 50 mg As, predominantly compound **1** by TLC) and an acidic fraction (45 mg As). On further anion-exchange chromatography of the acidic fraction, under the same conditions as above, the majority of the arsenic eluted at 5–7 dm^3 (15 mg As) and at 8–10 dm^3 (30 mg As), the elution positions expected for the major arsenic constituents (**4a**, **4b**, **5**, **3a** and **3b**), and **2**, of *Tridacna* kidney identified in the previous extraction. Smaller quantities of acidic arsenicals were also collected; two of these (weakly acidic, elution volume 1.4 dm^3; and strongly acidic, elution volume 7.6 dm^3) were further examined as described below.

Isolation of compound **18**. Further anion-exchange chromatography separated the weakly acidic arsenic fraction into a homogeneous band eluting close to the void volume. Removal of Tris buffer yielded material (86 mg) shown to be predominantly trigonelline by ^{1}H NMR spectroscopy. This material was then applied to DEAE-Sephadex (26 × 635 mm) equilibrated with 0.01 mol dm^{-3} Tris/0.02 mol dm^{-3} boric acid, pH 8.0. The arsenic constituent was strongly retained and remained on the column after elution with 4 dm^3 of starting buffer and then 4 dm^3 of 0.05 mol dm^{-3} Tris. It was finally eluted with 0.5 mol dm^{-3} Tris and, on GPC, gave a solid (6.5 mg, 200 μg As) free of trigonelline. TLC [cellulose; butan-1-ol–acetic acid–water (60:15:25); R_f 0.36] followed by buffered cation-exchange chromatography and GPC (Sephadex LH-20/MeOH) yielded a syrup (0.7 mg), identified as (2*S*)-[3-(5′-deoxy-5′-trimethylarsonio-β-D-ribosyloxy)-2-hydroxypropyl]-sulfate **18** by comparison of ^{1}H and ^{13}C NMR spectra and chromatographic properties (G-15, DEAE, TLC) with those of material prepared[9] by methylation of the naturally occurring dimethylarsinoylriboside **2**.

Isolation of taurine derivative **21**. Anion-exchange chromatography of the small quantity of strongly acidic arsenic, followed by TLC [cellolose; butan-1-ol–acetic acid–water (60:15:25); R_f 0.30; cellulose; propan-1-ol–NH_4OH (7:3); R_f 0.4] gave a syrup (2.4 mg, 0.11 mg As) shown to be a mixture of three arsenic compounds [TLC; silica; ethanol–water (4:1); R_f 0.37, 0.52 and 0.65]. The major compound (R_f 0.52)) was recovered, following GPC, as a syrup (0.8 mg) and was shown to be *N*-[4-(dimethylarsinoyl)butanoyl]taurine **21** by comparison of ^{1}H, ^{13}C NMR and IR spectra, with those of a synthetic specimen.

Isolation of the arsenic-containing nucleoside **16**. The 'late' fraction (73 g, 20 mg As), from the initial Sephadex G-15 chromatography, was subjected to chromatography on DEAE-Sephadex (50 × 900 mm; 0.05 mol dm^{-3} Tris, pH 8.0; void volume 1.2 dm^3) whereupon virtually all of the arsenic eluted at the void volume (non-acidic fraction). On GPC (G-15/water; 50 × 850 mm, void volume 500 cm^3) of this non-acidic fraction some major and several minor arsenic bands were separated. One of the minor bands eluted very slowly (3 × void volume) from the column. Repeated GPC separated this arsenical into a discrete homogeneous peak (100 mg, 360 μg As). Buffered cation-exchange chromatography (CM-Sephadex; 26 × 300 mm, void volume 100 cm^3), yielded a single arsenic compound with elution volume of 150 cm^3, which was further purified by TLC [cellulose; butan-1-ol–acetic acid–water (60:15:25); R_f 0.47 and propan-1-ol–NH_4OH (7:3), R_f 0.30] to give a syrup. This syrup was applied to a column of Sephadex G-15 which had been freshly washed with 0.05 mol dm^{-3} acetic acid followed by water. Elution with water, however, failed to elute any arsenic from the column. Presumably, in a clean matrix and on a 'recharged' column, the arsenical was sufficiently basic to be retained by the CO_2^- residues on the G-15 medium. Subsequent elution with 0.1 mol dm^{-3} NH_4OH removed the arsenical, and further GPC (Sephadex LH-20/MeOH) then yielded a glass (1.5 mg), identified as 9-(5′-deoxy-5′-dimethylarsinoyl)-9*H*-adenosine **16** by a comparison of the ^{1}H, ^{13}C NMR and IR spectra, and chromatographic properties, with those of an authentic specimen.

(2′R)-2′,3′-*Dihydroxypropyl* 5-*Deoxy*-5-*dimethylarsinoyl*-2,3-O-*isopropylidene*-β-D-*riboside* **7**.—The di-*O*-isopropylidene compound **6** (87 mg, 0.21 mmol), prepared by the method of McAdam *et al.*,[5] was stirred in methanol with PPTS at room temperature for 17 days. The products of this reaction were submitted to TLC [silica; ethyl acetate–ethanol–water (45:30:25); R_f 0.40] followed by GPC to give a syrup which slowly formed *hygroscopic needles* (65 mg, 80%), $[\alpha]_D$ −0.2 (*c* 5.5, MeOH) (Found: C, 40.3; H, 6.7. $C_{13}H_{25}AsO_7 \cdot H_2O$ requires C, 40.4; H, 7.0%); δ_H(300 MHz; D_2O) 1.38 and 1.52 (6 H, 2 s, Me_2C), 1.84 and 1.86 (6 H, 2 s, Me_2As), 2.58 (1 H, dd, $J_{5,5}$ 14.1, $J_{4,5}$ 5.0, 5-H), 2.66 (1 H, dd, $J_{5,5}$ 14.1, $J_{4,5}$ 10.2, 5-H), 3.53–3.66 (3 H, m, 1′-H and 3′-H_2), 3.78 (1 H, dd, $J_{1',1'}$ 10.5, $J_{1',2'}$ 6.5, 1′-H), 3.88 (1 H, m, 2′-H), 4.63 (1 H, dd, $J_{4,5}$ 5.0 and 10.2, 4-H), 4.92 (2 H, s, 2- and 3-H) and 5.24 (1 H, s, 1-H); δ_C(75.5 MHz; D_2O) 14.2 (Me_2As), 24.0 and 25.5 (Me_2C), 36.2 (C-5), 62.5 (C-3′), 69.3 (C-1′), 70.4 (C-2′), 81.6, 84.7 and 84.8 (C-2, -3 and -4), 109.2 (C-1) and 113.6 (Me_2C).

(2S)-3-(5′-*Deoxy*-5′-*dimethylarsinoyl*-β-D-*ribosyloxy*)-2-*hydroxypropanoic Acid* **4a** (*and as its Ammonium Salt*).—The partially protected β-D-riboside **7** (14.8 mg, 0.04 mmol) was stirred with sodium hydrogen carbonate (30 mg) and freshly reduced Adam's catalyst (20 mg) in water (10 cm^3; 60 °C). A gentle stream of oxygen was bubbled through the solution for 4 days. The reaction mixture was centrifuged and the supernatant was applied to a column of DEAE-Sephadex (16 × 320 mm; 0.05 mol dm^{-3} Tris, pH 8.0; void volume 50 cm^3). Some arsenic (5%) eluted at the void volume (expected position for unchanged starting material) and the rest of the arsenic eluted as

a broad band peaking at 250 cm^3. GPC separated the arsenic into a minor component, shown to be dimethylarsinic acid by ^1H NMR spectroscopy, and a major component (11 mg) which was not further purified but which was treated with (9:1) aq. TFA (2 cm^3; 7 min). After removal of excess of TFA under reduced pressure, the residue was submitted to buffered anion-exchange chromatography (DEAE-Sephadex; 26 × 340 mm; 0.05 mol dm^{-3} Tris, pH 8.0; void volume 150 cm^3) followed by GPC to give the title compound **4a** as a syrup (6.5 mg, 50%). Evaporation with aq. ammonia gave the ammonium salt of acid **4a**; spectral data were recorded on this material; δ_H(300 MHz; D_2O) 1.89 and 1.93 (6 H, 2 s, Me_2As), 2.54 (1 H, dd, $J_{5',5'}$ 13.9, $J_{4',5'}$ 10.1, 5'-H), 2.66 (1 H, dd, $J_{5',5'}$ 13.9, $J_{4',5'}$ 3.2, 5'-H), 3.70 (1 H, dd, $J_{3,3}$ 10.3, $J_{2,3}$ 2.6, 3-H), 3.99 (1 H, dd, $J_{3,3}$ 10.3, $J_{2,3}$ 3.9, 3-H), 4.12 (1 H, br d, $J_{2',3'}$ 3.5, 2'-H), 4.21–4.30 (2 H, m, 3'- and 4'-H), 4.19 (1 H, dd, $J_{2,3}$ 2.6 and 3.9, 2-H) and 5.01 (1 H, s, 1'-H); δ_C(75.5 MHz; D_2O) 14.3 and 14.6 (Me_2As), 36.2 (C-5'), 70.0 (C-3), 71.6 (C-2), 74.4 (C-2'), 76.1 and 76.8 (C-3' and -4'), 107.3 (C-1') and 178.1 (CO_2^-); ν_{max}/cm^{-1} 1600s, 1418, 1316, 1133s, 1046, 965 and 850s.

(2'R)-2',3'-*Isopropylidenedioxypropyl* 2,3-O-*Isopropylidene*-β-D-*ribofuranoside* **11**.—A solution of (2'*R*)-2',3'-isopropylidenedioxypropyl tri-*O*-benzoyl-β-D-ribofuranoside **10**, prepared as previously reported[9] (6.8 g, 12 mmol), in MeOH (50 cm^3) containing Amberlite IRA-410 resin (OH^-; 12 cm^3) was stirred overnight. The mixture was filtered and work-up of the filtrate gave the 2,3,5-triol (2.8 g), which on treatment in acetone with HBF_4·Et_2O (8 drops) gave the alcohol **11** as an oil (2.6 g, 72%); δ_H(300 MHz; $CDCl_3$) 1.32, 1.37, 1.43 and 1.49 (12 H, 4 s, Me_2C), 3.31 (1 H, dd, $J_{5,5}$ 9.8, $J_{4,5}$ 3.9, 5-H), 3.58 (1 H, dd, $J_{1',1'}$ 11.2, $J_{1',2'}$ 5.7, 1'-H), 3.65 (1 H, dd, $J_{5,5}$ 9.8, $J_{4,5}$ 3.9, 5-H), 3.73 (1 H, dd, $J_{3',3'}$ 8.2, $J_{2',3'}$ 6.9, 3'-H), 3.85 (1 H, dd, $J_{1',1'}$ 11.2, $J_{1',2'}$ 3.4, 1'-H), 4.04 (1 H, dd, $J_{3',3'}$ 8.2, $J_{2',3'}$ 6.6, 3'-H), 4.32–4.39 (1 H, m, 2'-H), 4.41 (1 H, m, 4-H), 4.66 (1 H, d, $J_{2,3}$ 6.1, 2- or 3-H), 4.83 (1 H, d, $J_{2,3}$ 6.1, 3- or 2-H) and 5.11 (1 H, s, 1-H); δ_C(75.5 MHz; $CDCl_3$) 24.6, 25.3, 26.3 and 26.4 (4 C, Me_2C), 63.9 (C-5), 65.8 (C-3'), 68.5 (C-1'), 74.2 (C-2'), 81.4, 86.0 and 88.6 (C-2, -3 and -4), 109.4 (C-1) and 109.7 and 112.1 (2 C, Me_2C).

(2'R)-2',3'-*Isopropylidenedioxypropyl* 5-*Chloro*-5-*deoxy*-2,3-O-*isopropylidene*-β-D-*riboside* **12**.—A solution of the alcohol **11** (2.5 g, 8.2 mmol) in a mixture of pyridine (25 cm^3) and CCl_4 (25 cm^3) containing triphenylphosphine (6.5 g, 25 mmol) was heated (60 °C, 4.5 h), then MeOH was added and the mixture was reheated (4 h), diluted with EtOAc, washed with cold 4 mol dm^{-3} HCl, dried ($MgSO_4$), and evaporated. Flash chromatography [SiO_2; EtOAc–light petroleum (1:3)] gave the chloride **12** as an oil (2.4 g, 91%); δ_H(300 MHz; $CDCl_3$) 1.32, 1.37, 1.42 and 1.49 (12 H, 4 s, Me_2C), 3.48 (1 H, dd, $J_{5,5}$ 10.8, $J_{4,5}$ 6.2, 5-H), 3.51 (1 H, dd, $J_{1',1'}$ 10.4, $J_{1',2'}$ 9.3, 1'-H), 3.58 (1 H, dd, $J_{5,5}$ 10.8, $J_{4,5}$ 6.2, 5-H), 3.67 (1 H, dd, $J_{3',3'}$ 8.1, $J_{2',3'}$ 6.4, 3'-H), 3.74 (1 H, dd, $J_{1',1'}$ 10.4, $J_{1',2'}$ 4.3, 1'-H), 4.05 (1 H, dd, $J_{3',3'}$ 8.1, $J_{2',3'}$ 6.5, 3'-H), 4.20–4.29 (1 H, m, 2'-H), 4.31–4.38 (1 H, m, 4-H), 4.69–4.77 (2 H, m, 2- and 3-H) and 5.13 (1 H, s, 1-H); δ_C(75.5 MHz; $CDCl_3$) 24.9, 25.4, 26.4 and 26.8 (4 C, Me_2C), 44.3 (C-5), 66.4 (C-3'), 68.8 (C-1'), 74.5 (C-2'), 82.2, 85.1 and 86.8 (C-2, -3 and -4), 108.7 (C-1) and 109.8 and 112.8 (2 C, Me_2C).

(2'R)-2',3'-*Isopropylidenedioxypropyl* 5-*Deoxy*-5-*dimethylarsinoyl*-2,3-O-*isopropylidene*-β-D-*riboside* **13**.—To a suspension of Na metal (0.7 g, 30 mmol) in THF (15 cm^3) was added a solution of Me_2AsI (1.0 cm^3, 2.3 g, 10 mmol) in THF (5 cm^3), initially as 1 cm^3, then the remaining 5 cm^3 during 0.5 h. The mixture was stirred (0.5 h), then refluxed (0.5 h) and allowed to settle. Half of the supernatant was then added to a solution of the chloride **12** (0.32 g, 1 mmol) in THF (2 cm^3) and the mixture was stirred (1 h). Filtration through SiO_2 [EtOAc–light petroleum (1:2)], evaporation, and then flash chromatography [SiO_2; EtOAc–light petroleum (1:4)] gave the arsine (200 mg), which was treated in THF (2 cm^3) with 30% H_2O_2 (0.15 cm^3). After 3.5 h, evaporation and flash chromatography [SiO_2; EtOAc–MeOH (2:3)], followed by passage through CM-Sephadex and GPC, gave (2'R)-2',3'-*isopropylidenedioxypropyl* 5-*deoxy*-5-*dimethylarsinoyl*-2,3-O-*isopropylidene*-β-D-*riboside* **13** as a syrup (190 mg, 46% from **12**), $[\alpha]_D$ +5.3 (*c* 4.5, water) (Found: C, 46.9; H, 7.2. $C_{16}H_{29}AsO_7$ requires C, 47.1; H, 7.1%); δ_H(300 MHz; D_2O) 1.38, 1.40, 1.47 and 1.52 (12 H, 4 s, Me_2C), 1.89 and 1.91 (6 H, 2 s, Me_2As), 2.58–2.73 (2 H, m, 5-H_2), 3.59 (1 H, dd, $J_{1',1'}$ 11.0, $J_{1',2'}$ 6.5, 1'-H), 3.82 (1 H, dd, $J_{3',3'}$ 8.7, $J_{2',3'}$ 6.4, 3'-H), 3.91 (1 H, dd, $J_{1',1'}$ 11.0, $J_{1',2'}$ 3.1, 1'-H), 4.17 (1 H, dd, $J_{3',3'}$ 8.7, $J_{2',3'}$ 6.9, 3'-H), 4.44 (1 H, m, 2'-H), 4.64 (1 H, dd, $J_{4,5}$ 5.8 and 9.3, 4-H), 4.93 (2 H, s, 2- and 3-H) and 5.26 (1 H, s, 1-H); δ_C(75.5 MHz; D_2O) 14.2 and 14.4 (Me_2As), 24.0, 24.4, 25.5 and 25.8 (4 C, Me_2C), 36.1 (C-5), 65.3 (C-3'), 68.8 (C-1'), 74.7 (C-2'), 81.6, 84.5 and 84.7 (C-2, -3 and -4), 109.3 (C-1) and 110.4 and 113.7 (2 C, Me_2C).

(2'S)-2',3'-*Dihydroxypropyl* 5-*Deoxy*-5-*dimethylarsinoyl*-β-D-*riboside* **1b**.—The protected riboside **13** (57 mg, 0.14 mmol) was swirled with aq. TFA (5 cm^3 of 9:1 mixture) for 10 min. The syrup obtained on evaporation was subjected to chromatography on CM-Sephadex, G-15 and finally LH-20/MeOH to give the *tetraol* **1b** as a syrup (39 mg, 85%), $[\alpha]_D$ +9.6 (*c* 2.9, water) (Found: C, 36.4; H, 6.6. $C_{10}H_{21}AsO_7$ requires C, 36.6; H, 6.5%); δ_H(300 MHz; D_2O) 1.86 and 1.88 (6 H, 2 s, Me_2As), 2.50 (1 H, dd, $J_{5,5}$ 13.9, $J_{4,5}$ 10.1, 5-H), 2.65 (1 H, dd, $J_{5,5}$ 13.9, $J_{4,5}$ 3.2, 5-H), 3.50 (1 H, dd, $J_{1',1'}$ 10.3, $J_{1',2'}$ 6.3, 1'-H), 3.56 (1 H, dd, $J_{3',3'}$ 11.7, $J_{2',3'}$ 6.1, 3'-H), 3.63 (1 H, dd, $J_{3',3'}$ 11.7, $J_{2',3'}$ 4.8, 3'-H), 3.83 (1 H, dd, $J_{1',1'}$ 10.3, $J_{1',2'}$ 3.6, 1'-H), 3.88 (1 H, m, 2'-H), 4.13 (1 H, dd, $J_{2,3}$ 4.1, $J_{1,2}$ 0.9, 2-H), 4.20–4.30 (2 H, m, 3- and 4-H) and 5.02 (1 H, d, $J_{1,2}$ 0.9, 1-H); δ_C(75.5 MHz; D_2O) 14.2 and 14.6 (Me_2As), 36.2 (C-5), 62.5 (C-3'), 69.5 (C-1'), 70.8 (C-2'), 74.4 (C-2), 75.8 (C-3), 77.0 (C-4) and 108.9 (C-1).

(2R)-3-(5'-*Deoxy*-5'-*dimethylarsinoyl*-β-D-*ribosyloxy*)-2-*hydroxypropanoic Acid* **4b** (*and as its Ammonium Salt*).—A solution of the β-D-riboside **1b** (93 mg, 0.28 mmol) and sodium hydrogen carbonate (30 mg) in water (15 cm^3) was heated (70 °C) with freshly reduced Adam's catalyst (25 mg). Oxygen was bubbled through the solution and the reaction was monitored by CM-Sephadex chromatography. After 14 h the reaction products were subjected to chromatography on DEAE-Sephadex and G-15; passage through Amberlite CG-50 H^+ followed by GPC gave the *free acid* **4b** as a syrup (40 mg, 40%) (Found: C, 34.9; H, 5.7. $C_{10}H_{19}AsO_8$ requires C, 35.1; H, 5.6%). The ^1H NMR spectrum of this material was broadened and the signals assigned to groups on arsenic were downfield of those expected for a normal arsine oxide, indicating that it was present as the arsonium zwitterion.[5] Evaporation with aq. ammonia gave the ammonium salt of the acid **4b**; spectral data were recorded on this material; $[\alpha]_D$ +6.2 (*c* 4.0, water); δ_H(300 MHz; D_2O) 1.89 and 1.91 (6 H, 2 s, Me_2As), 2.53–2.70 (2 H, m, 5'-H_2), 3.70 (1 H, dd, $J_{3,3}$ 10.4, $J_{2,3}$ 4.9, 3-H), 3.96 (1 H, dd, $J_{3,3}$ 10.4, $J_{2,3}$ 2.3, 3-H), 4.11 (1 H, d, $J_{2',3'}$ 3.5, 2'-H), 4.16 (1 H, m, 2-H), 4.26 (2 H, m, 3'-and 4'-H) and 4.99 (1 H, s, 1-H); δ_C(75.5 MHz; D_2O) 14.3 and 14.7 (Me_2As), 36.2 (C-5'), 70.9 (C-3), 71.9 (C-2), 74.3 (C-2'), 75.9 (C-3'), 76.9 (C-4'), 108.1 (C-1') and 178.2 (CO_2^-).

Ethyl 4-(*Dimethylarsinoyl*)*butanoate* **19**.—Ethyl 4-bromobutanoate (0.62 g, 3.2 mmol) was stirred in dry THF (10 cm^3) under nitrogen as a solution (10 cm^3) of Me_2AsNa (6 mmol) in THF was added dropwise. After being stirred for 1 h the mixture was filtered through a plug of silica gel. The filtrate was evaporated and the resulting oil was redissolved in THF (10

cm^3) and stirred with 30% (w/v) hydrogen peroxide (0.5 cm^3) for 15 min. Evaporation gave a syrup which, when subjected to column chromatography [SiO_2; EtOAc–MeOH (1:1)], yielded ethyl 4-(dimethylarsinoyl)butanoate **19** as a syrup (270 mg, 36%); δ_H(300 MHz; D_2O) 1.24 (3 H, t, *J* 7.2, CH_2Me), 1.75 (6 H, s, Me_2As), 1.90–2.00 (2 H, m, $CH_2CH_2CH_2$), 2.16–2.22 (2 H, m, CH_2CO_2), 2.53 (2 H, t, *J* 7.1, $AsCH_2$) and 4.16 (2 H, q, *J* 7.2, OCH_2); δ_C(75.5 MHz; D_2O) 12.8 (Me_2As), 13.5 (Me), 17.4 ($CH_2CH_2CH_2$), 29.3 (CH_2CO_2), 34.7 ($AsCH_2$), 62.0 (OCH_2) and 175.5 (CO_2^-).

4-(*Dimethylarsinoyl*)*butanoic Acid* **20**.—The ester **19** (136 mg, 0.58 mmol) was stirred with 0.5 mol dm^{-3} NaOH (6 cm^3) for 2 h at room temperature. The solution was then made up to 0.05 mol dm^{-3} in Tris buffer and applied to a column (26 × 340 mm) of DEAE-Sephadex. Isocratic elution (0.05 mol dm^{-3} Tris, pH 8.0) provided arsenical material after elution of 800 cm^3 of eluate. On GPC, sodium 4-(dimethylarsinoyl)butanoate was obtained as a syrup (108 mg, 80%); δ_H(300 MHz; D_2O) 1.75 (6 H, s, Me_2As), 1.83–1.94 (2 H, m, $CH_2CH_2CH_2$), 2.13–2.19 (2 H, m, CH_2CO_2) and 2.31 (2 H, t, *J* 7.2, $AsCH_2$); δ_C(75.5 MHz; D_2O) 12.8 (Me_2As), 18.9 ($CH_2CH_2CH_2$), 29.9 (CH_2CO_2), 39.2 ($AsCH_2$) and 181.9 (CO_2^-). This material was passed through Amberlite CG-50 H^+ to give the *free acid* **20** as a syrup which formed needles, m.p. 135–137 °C (Found: C, 34.8; H, 6.2. $C_6H_{13}AsO_3$ requires C, 34.6; H, 6.3%); δ_H(300 MHz; D_2O) 1.92 (6 H, s, Me_2As), 1.94–2.02 (2 H, m, $CH_2CH_2CH_2$), 2.34–2.40 (2 H, m, CH_2CO_2) and 2.46 (2 H, t, *J* 7.1, $AsCH_2$); δ_C(75.5 MHz; D_2O) 13.3 (Me_2As), 18.0 ($CH_2CH_2CH_2$), 30.1 (CH_2CO_2), 35.8 ($AsCH_2$) and 179.3 (CO_2^-).

N-[4-(*Dimethylarsinoyl*)*butanoyl*]*taurine* **21**.—To a cold (0 °C) solution of the carboxylic acid **20** (16 mg, 0.08 mmol) and triethylamine (16 mm^3) in acetonitrile (200 mm^3) was added a cold solution of ethyl chloroformate (12 mm^3) in acetonitrile (200 mm^3). The mixture was left at 0 °C (with occasional swirling) for 30 min, then taurine (20 mg, 0.16 mmol) and triethylamine (20 mm^3) were added. This mixture was heated (sealed ampoule) for 4 h at 120 °C, and the residue obtained on evaporation was subjected to chromatography on DEAE-Sephadex (26 × 340 mm; 0.05 mol dm^{-3} Tris, pH 8.0; void volume 150 cm^3). The majority of arsenic eluted in two overlapping bands peaking at 840 cm^3 and at 1020 cm^3, the first of which corresponded to unchanged starting material. Material in the second band was subjected to GPC and passed through Amberlite CG-50 H^+ to give N-[4-(*dimethylarsinoyl*)*butanoyl*]*taurine* **21** as a syrup (6 mg, 25%) which slowly formed needles, m.p. 215–216 °C (Found: C, 30.4; H, 5.8. $C_8H_{18}AsNO_5S$ requires C, 30.5; H, 5.8%); δ_H(300 MHz; D_2O) 1.99–2.13 (2 H, m, $CH_2CH_2CH_2$), 2.10 (6 H, s, Me_2As), 2.45 (2 H, t, *J* 7.0, CH_2As), 2.54 (2 H, m, CH_2CO), 3.11 (2 H, t, *J* 6.6, CH_2S) and 3.61 (2 H, t, *J* 6.6, CH_2N); δ_C(75.5 MHz; D_2O) 13.0 (Me_2As), 18.0 ($CH_2CH_2CH_2$), 29.7 (CH_2CO), 35.3 and 35.6 ($AsCH_2$, NCH_2), 49.8 (CH_2S) and 174.9 (CO).

On evaporation of the taurine derivative **21** with aq. ammonia, the ammonium sulfonate, so formed, returned the following spectral data: δ_H(300 MHz; D_2O) 1.75 (6 H, s, Me_2As), 1.88–1.98 (2 H, m, $CH_2CH_2CH_2$), 2.12–2.19 (2 H, m, CH_2CO), 2.40 (2 H, t, *J* 7, $AsCH_2$), 3.08 (2 H, t, *J* 6.7, CH_2S) and 3.58 (2 H, t, *J* 6.7, NCH_2); δ_C(75.5 MHz; D_2O) 12.8 (Me_2As), 18.3 ($CH_2CH_2CH_2$), 29.3 (CH_2CO), 35.3 and 36.3 ($AsCH_2$, NCH_2), 49.8 (CH_2S) and 175.2 (CO); ν_{max}/cm^{-1} 1648s, 1560s, 1446, 1419, 1215s, 1175, 1046s and 852s. The spectral data for this salt of compound **21** were identical with those for the natural material isolated from *Tridacna*.

5′-*Chloro*-5′-*deoxyadenosine* **15**.—The chloride **15** was prepared by two procedures.

Method (*a*). After the method of Whistler and Anisuzzaman,[12] adenosine **14** (534 mg, 2 mmol) was taken up in hot, dry pyridine (35 cm^3) and the solution was then cooled to room temperature before the addition of triphenylphosphine (1.0 g, 4 mmol) and carbon tetrachloride (3.2 g, 20 mmol). After being stirred at room temperature for 2 h the reaction mixture was quenched by the addition of methanol (10 cm^3). The reaction mixture was partitioned between $CHCl_3$ and water, and the solid obtained on evaporation of the aqueous layer was subjected to column chromatography [silica; EtOAc–EtOH–water (42:5:3)] to give 5′-chloro-5′-deoxyadenosine **15** as needles (340 mg, 60%), m.p. 104–105 °C (from water) [lit.,[13] 190 °C (decomp.); lit.,[22] 75–85 °C; lit.,[23] 140–165 °C]; $[\alpha]_D$ −27.5 (*c* 2.6, 0.1 mol dm^{-3} HCl) (lit.,[13] −27.7).

Method (*b*). Following the method of Kikugawa and Ichino,[13] adenosine **14** (8.4 g, 0.03 mol) was added portionwise to a cooled (10 °C) solution of thionyl dichloride (12 cm^3) in HMPA (80 cm^3). The resultant orange-red solution was stirred (16 h; room temp.), then poured into water (1 dm^3). This aqueous solution was passed through a column of Dowex 50 H^+ resin (400 g dry resin) which was washed with water (to remove HMPA) until the eluate was neutral. The resin was removed from the column and swirled with warm, aq. NH_3 (1 mol dm^{-3}; 2 × 1 dm^3) and then MeOH (2 × 500 cm^3). On concentration of the combined washings, the product crystallised; recrystallisation from water gave the chloride **15** as needles, m.p. 104–106 °C (7.7 g, 85%).

9-(5′-*Deoxy*-5′-(*dimethylarsinoyl*)-9H-*adenosine* **16**.—A solution of the chloride **15** (570 mg, 2 mmol) in dry THF (20 cm^3) was added to a solution (20 cm^3) of Me_2AsNa (10 mmol) in THF prepared in the usual way,[5] and the mixture was stirred under nitrogen (16 h; room temp.). Addition of MeOH (40 cm^3) dissolved the solid material which had separated from the mixture, to give a clear solution. This reaction mixture was oxidised by the addition of 30% (w/v) H_2O_2 (2 cm^3) and, after 30 min, the resultant dark-brown, clear solution was poured into water (200 cm^3). The aqueous layer was washed with $CHCl_3$ (3 × 200 cm^3) and the remaining traces of I_2 and H_2O_2 removed by the addition of aq. $Na_2S_2O_3$ (2%; 20 cm^3). The aqueous layer was then concentrated (40 cm^3) and applied to DEAE-Sephadex (26 × 300 mm; 0.05 mol dm^{-3} Tris, pH 8.0; void volume 140 cm^3). The arsenic-containing material eluting at the void volume was subjected to GPC (Sephadex G-15/water; 50 × 850 mm, void volume 500 cm^3) to give, after 1600 cm^3, 9-(5′-*deoxy*-5′-*dimethylarsinoyl*)-9H-*adenosine* **16** as a glass (380 mg, 51%), $[\alpha]_D$ +54.2 (*c* 3.0, MeOH) (Found: C, 39.0; H, 5.1. $C_{12}H_{18}AsN_5O_4$ requires C, 38.8; H, 4.9%); δ_H(300 MHz; D_2O) 1.69 and 1.70 (6 H, 2 s, Me_2As), 2.69 (1 H, dd, $J_{5',5'}$ 13.9, $J_{4',5'}$ 3.8, 5′-H), 2.79 (1 H, dd, $J_{5',5'}$ 13.9, $J_{4',5'}$ 10.7, 5′-H), 4.38 (1 H, dd, $J_{2',3'} = J_{3',4'}$ = 5.0, 3′-H), 4.47 (1 H, m, 4′-H), 4.95 (1 H, dd, $J_{1',2'} = J_{2',3'}$ = 5.0, 2′-H), 6.06 (1 H, d, $J_{1',2'}$ 5.0, 1′-H) and 8.23 and 8.29 (2 H, 2 s, 2- and 8-H); δ_C(75.5 MHz; D_2O) 14.2 and 14.7 (Me_2As), 34.4 (C-5′), 73.0 and 74.4 (C-2′ and -3′), 78.8 (C-4′), 88.4 (C-1′), 118.8 (C-5), 140.2 (C-8), 148.5 (C-4), 152.7 (C-2) and 155.3 (C-6); ν_{max}/cm^{-1} 1649s, 1577, 1479, 1420, 1333, 1302, 1252, 1132, 1051 and 852s.

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