Arsenic-containing Ribosides from the Brown Alga *Sargassum lacerifolium*: X-Ray Molecular Structure of 2-Amino-3-[5'-deoxy-5'-(dimethylarsinoyl)-ribosyloxy]propane-1-sulphonic Acid

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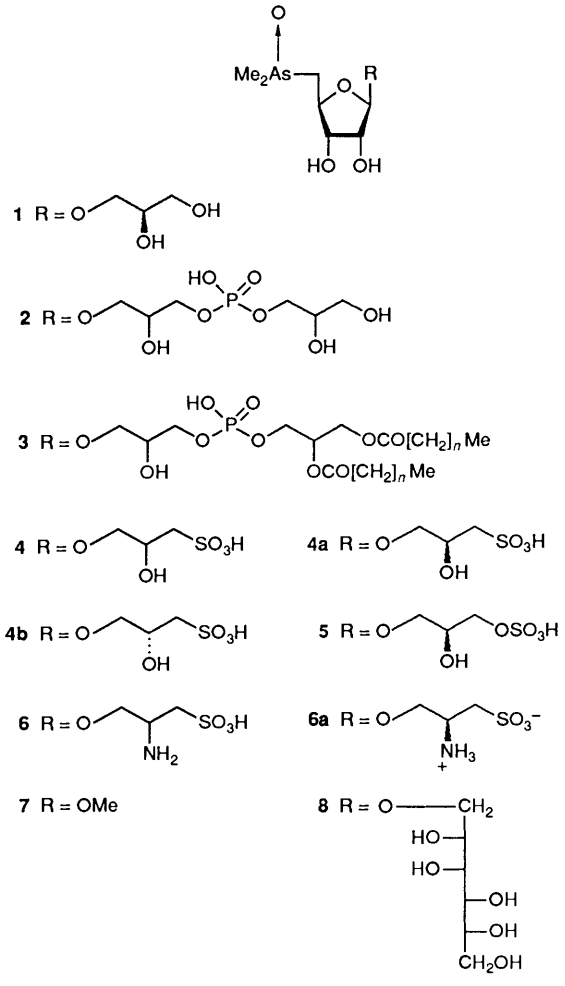
Three novel arsenic-containing ribosides, methyl 5-deoxy-5-(dimethylarsinoyl)-β-D-riboside, 1-*O*-[5'-deoxy-5'-(dimethylarsinoyl)-β-D-ribosyl]mannitol, and a dimethylarsonio-β-D-riboside have been isolated from extracts of the brown alga *Sargassum lacerifolium*. In addition, five previously reported arsenic-containing ribosides, and some arsenate, were isolated from *Sargassum*, and dimethylarsinic acid was also shown to be present. The compounds were identified chiefly by NMR spectroscopy, and an X-ray molecular structure is reported for one of them, 2-amino-3-[5'-deoxy-5'-(dimethylarsinoyl)ribosyloxy]propane-1-sulphonic acid. The stereochemistry of the aglycones in these arsenic-containing ribosides is discussed and the configuration of 3-[5'-deoxy-5'-(dimethylarsinoyl)-β-D-ribosyloxy]-2-hydroxypropane-1-sulphonic acid **4** was assigned as 2*S* on the basis of a comparison of NMR spectra with those of synthetic model compounds.

Jones[1] reported in 1922 that marine algae contained considerable quantities of naturally acquired arsenic. Subsequent work has shown that marine algae absorb oceanic arsenate and transform it into a number of arsenic-containing ribosides.[2] It is not known if these compounds serve any function within algae but any possible biochemical role may be revealed as additional related arsenicals are identified. The major arsenic compound in the brown algae *Ecklonia radiata*[3] and *Laminaria japonica*[4] (both of the order Laminariales) was shown to be the sulphonic acid **4**, whereas the sulphuric acid ester **5** was not detected. On the other hand, the two brown algae *Hizikia fusiforme*[5] and *Sargassum thunbergii*[6] (both of the order Fucales) contained the sulphuric acid ester **5** as their major arsenic-containing riboside. The occurrence of the various arsenic compounds may possibly be related to algal taxonomy. The brown alga *Sargassum lacerifolium* is abundant in the Marmion lagoon system (near Perth, Western Australia), and previous work[3] on the arsenic compounds of *Ecklonia* was carried out on a sample from this same area. We here report the examination of the arsenic constituents of the brown alga *Sargassum lacerifolium*.

Results and Discussion

Isolation of Arsenic Compounds from Sargassum.—Fresh *Sargassum lacerifolium* (4 kg, ~40 μg g^{-1} As) was extracted with methanol and the two major acidic arsenical constitutents were isolated from the extract by gel permeation chromatography (GPC) on Sephadex G-15, anion-exchange chromatography on DEAE Sephadex, and TLC on cellulose. They were identified by ^1H NMR spectroscopy as 3-[5-deoxy-5-(dimethylarsinoyl)-β-D-ribosyloxy]-2-hydroxypropyl 2,3-dihydroxypropyl hydrogen phosphate **2** and (2'*S*)-3'-[5-deoxy-5-(dimethylarsinoyl)-β-D-ribosyloxy]-2'-hydroxypropyl hydrogen sulphate **5**. The absolute configuration of compound **5** has been previously established by an X-ray crystal structure of material isolated from the giant clam *Tridacna maxima*.[7] Although the absolute configuration of compound **2** has not been established, the likely biosynthetic origin of arsenic-containing ribosides (from *S*-adenosylmethionine)[2] suggests that the ribose unit in compound **2** (and in related compounds) is D-ribose, and this will be assumed in the discussion that follows.



Compounds **2** and **5** constituted ~10% and 85%, respectively, of the total arsenic in the *Sargassum* extract. A minor acidic arsenical (which was retained on the DEAE Sephadex medium following elution with 0.05 mol dm^{-3} Tris buffer) in the extract was shown to be inorganic arsenate by

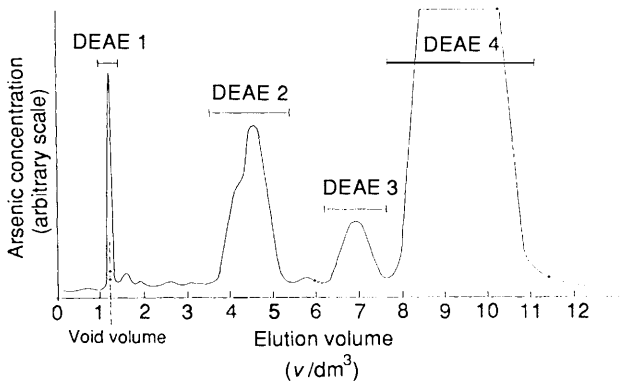


Fig. 1 Separation of the arsenic compounds in *Sargassum* extract on DEAE Sephadex (50 × 900 mm; 0.05 mol dm^{-3} Tris buffer, pH 8.0)

GPC and TLC, and by reduction to arsine without prior chemical degradation. Several minor arsenic compounds with basic properties were also present in the extract but the small quantities available precluded their identification.

A second, larger-scale, extraction was then carried out. Fresh *Sargassum* (40 kg) was steeped in methanol (45 dm^3); since water constitutes ~90% of the fresh alga, the resulting extract comprised aq. methanol (1:1). The large quantity of alga necessitated a fairly crude preliminary fractionation. Salt-like material was removed by pouring a conc. aq. solution of the original extract into methanol and discarding the resultant precipitate. Less polar material was removed by pouring a methanol solution of this salt-free extract into acetone and, after storage, separating the liquid from the desired precipitated material. Both of these steps resulted in increased concentrations of arsenic in the final extract but were accompanied by losses of arsenic. TLC of the two 'discarded' fractions indicated that they contained predominantly the same arsenic compounds as the extract, only at lower concentrations.

GPC separated the arsenic in the extract into three major broad bands, designated 'early', 'mid', and 'late'. Small quantities of arsenic trailed from the column, suggesting interaction with the medium; this material was collected but will not be examined in the present study.

Buffered anion-exchange chromatography (DEAE Sephadex) of the three bands from GPC showed that they contained similar arsenic compounds but in varying amounts. These fractions were combined where considered appropriate, depending upon the relative quantities of arsenic and total material. Thus, DEAE chromatography separated the arsenic into nonacidic material (elution at the void volume of the column, fraction DEAE 1) and three fractions (DEAE 2–4) containing arsenicals of increasing acidity (Fig. 1).

The nonacidic material (fraction DEAE 1) was further fractionated by buffered cation-exchange chromatography on a column of CM Sephadex whereupon most of the arsenic eluted with the weakly basic material. Traces of more strongly basic arsenic were also present but were not further examined. TLC of the weakly basic fraction separated three arsenic compounds. The major compound, isolated as a syrup (11 mg), was shown (^{1}H NMR) to be (2′*R*)-2′,3′-dihydroxypropyl 5-deoxy-5-(dimethylarsinoyl)-β-D-riboside **1**, previously isolated from algae.[2]

The second compound was very mobile on TLC (cellulose, system A,* R_f 0.66) and was readily obtained as a syrup (1.1 mg). It was identified as methyl 5-deoxy-5-(dimethylarsinoyl)-β-D-riboside **7** by comparison of its ^{1}H NMR spectrum and chromatographic properties with those of an authentic sample. Compound **7** was prepared by treatment of the bromide **12** with dimethylarsinosodium followed by oxidation with hydrogen peroxide, and deprotection of the arsinoyl-β-D-riboside **13** so formed. A methyl β-D-glycoside (methyl β-D-glucopyranoside) has been reported as a naturally occurring constituent of some terrestrial plants.[8] However, it is possible that the methyl β-D-riboside **7** reported here in *Sargassum* may be an artefact of the isolation procedure since methanol was used as the first solvent of extraction.

The third arsenical with basic properties was isolated as a syrup (0.5 mg). NMR spectroscopy showed it to be a new dimethylarsinoylriboside with eight non-exchangeable protons and six carbons (shown by DEPT experiments to comprise four methines and two methylenes) assignable to the aglycone portion. The ^{13}C NMR chemical shifts (δ_C 63–71) for the aglycone carbons were consistent with all six each being bound to an oxygen atom, and the chemical shifts (δ_C 63.4, 70.2) for the ^{13}C signals assigned to the two methylene carbons indicated that the glycosidic linkage was through one of these methylenes. The signals attributed to the four methines occurred in the narrow range δ_C 69.2–71.0.

These data suggested that the aglycone portion of the new compound was a hexitol attached to the ribose ring through a methylene group. Five of the ten possible hexitols occur naturally, three of which (D-mannitol, D-glucitol, and galactitol) are found in marine algae.[9] When a portion of the natural material from *Sargassum* was subjected to acid hydrolysis, mannitol was identified in the hydrolysate by liquid chromatography (LC). On the basis of these data the new compound was formulated as 1-*O*-[5′-deoxy-5′-(dimethylarsinoyl)-β-D-ribosyl]mannitol **8**. D-Mannitol is a common major constituent of algae and serves as an important osmolyte.[10]

Fraction DEAE 2 contained the phosphoric acid diester **2** (isolated in the first extraction) and a minor arsenical which separated from compound **2** on further DEAE chromatography and which was isolated as a solid (1.2 mg). NMR spectral data of this material were virtually identical with those reported for a minor acidic arsenical isolated from the brown alga *Hizikia fusiforme*.[5] On that occasion, ^{13}C NMR data and ^{1}H NMR data collected at various pH-values were used to assign the compound as being 2-amino-3-[5′-deoxy-5′-(dimethylarsinoyl)-β-D-ribosyloxy]propane-1-sulphonic acid **6**. The material from *Sargassum* was subsequently obtained crystalline, and a single-crystal X-ray structure determination confirmed the structure proposed on the basis of the NMR data. The stereochemistry of the molecule at C-2 is discussed further below.

Fraction DEAE 3 contained dimethylarsinic acid (identified by chromatography) as a minor component. Dimethylarsinic acid has been previously isolated from the green macroalga *Codium fragile*[11] and has also been reported in some unicellular algae;[12] its presence in algae may represent an intermediate step in the biosynthesis of dimethylarsinoylribosides, or a stage in their degradation. The major arsenic constituent (>90%) of this fraction was isolated as a syrup (2 mg) and was identified (^{1}H NMR) as 3-[5′-deoxy-5′-(dimethylarsinoyl)-β-D-ribosyloxy]-2-hydroxypropane-1-sulphonic acid **4**. The stereochemistry of the aglycone moiety (C-2) in compound **4** is discussed further below.

Fraction DEAE 4 contained the sulphuric acid ester **5** as the major arsenical. A minor arsenic constituent separated from compound **5** following repeated anion exchange chromatography and was isolated as a syrup (0.7 mg). Inspection of the NMR data suggested, by the duplication of some signals, that this new material was a mixture (1:1) of diastereoisomers of arsenic-containing ribosides. For ease of discussion, the mixture of proposed diastereoisomers will be referred to in the following interpretation of those data as if it were a single compound.

* TLC solvent systems are described in the Experimental section.

Table 1 ^{1}H NMR data for the dimethylarsonioriboside **10** from *Sargassum lacerifolium*

δ	Multiplicity	Integration
1.93, 1.97[a]	2 s	6 H
2.58–2.85[a]	m	4 H
2.95	m	1 H
3.46–3.65	m	5 H
3.74–3.82	m	3 H
3.88	m	1 H
4.05	m	3 H
4.12	d	1 H
4.23	m	2 H
5.00	s	1 H

[a] The other diastereoisomer registered clearly defined, separate signals in these regions only: singlets at δ 1.92 and δ 1.96, assigned to methyls on arsenic, and contributed to a multiplet assigned to methylene on arsenic at δ 2.6–2.8.

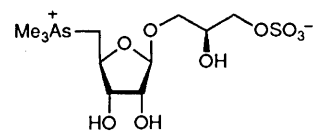
Table 2 ^{13}C NMR data for the dimethylarsonioriboside **10** from *Sargassum lacerifolium* and the trimethylarsonioriboside **9**

Dimethylarsonioriboside **10**		Compound **9**	
δ_C	Assignment	δ_C	Assignment
7.6, 8.1[a]	2 × Me	7.7	3 × Me
31.3	CH_2	30.4	CH_2
67.9	CH	67.7	CH
68.4	2 × CH_2	68.1	2 × CH_2
74.6	CH	74.2	CH
76.1	CH	76.0	CH
77.2	CH	77.0	CH
107.7	CH	107.6	CH
26.0[a]	CH_2		
43.5	CH		
63.0	CH_2		
70.8	CH		
72.2	CH_2		
73.0	CH_2		
178.8[b]	CO_2^-		

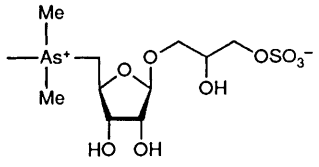
[a] The other diastereoisomer registered separate signals in these regions only: at δ_C 7.9 and 8.6 for methyls on arsenic, and at δ_C 26.1 for methylene on arsenic. [b] This signal was small and not well defined.

The NMR spectra showed that the new compound was structurally more complex than the dimethylarsinoylribosides described above. The ^{1}H NMR spectrum (Table 1) contained signals attributable to 27 non-exchangeable protons. Signals downfield of δ 4.1 were readily assigned to the four methine protons in a ribose ring, but the signals upfield of δ 2.9 indicated that the usual dimethylarsinoyl moiety was absent. For example, the two-proton system at δ ~2.45–2.65, characteristic of the methylene group attached to arsenic in dimethylarsinoylribosides, was replaced by a four-proton multiplet at δ 2.58–2.85. Further, the two methyl groups on arsenic gave resonances in the ^{1}H NMR spectrum at slightly lower field than had been observed for dimethylarsinoylribosides. These data were consistent with the presence of a dimethylarsonio moiety ($-CH_2\overset{+}{A}sMe_2CH_2-$).

Interpretation of the remaining signals in the ^{1}H NMR spectrum was aided by an analysis of the ^{13}C NMR data for the new compound (Table 2), and comparison of these data with those for a model, zwitterionic compound, (2′*S*)-3′-[5-deoxy-5-(trimethylarsonio)-β-D-ribosyloxy]-2′-hydroxypropyl sulphate **9**, prepared by reduction of the naturally occurring arsinoyl compound **5**, and treatment of the arsine so formed with methyl iodide. The correspondence of chemical shifts for all signals for the two compounds allowed assignment of part of the new compound as **I**.



9



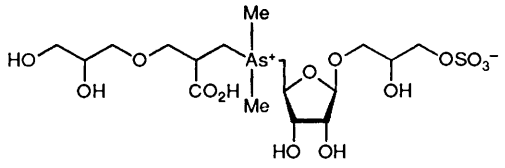
I

The remainder of the new compound contained seven carbons and ten non-exchangeable hydrogens. The resonance at δ_C 26.0 (^{13}C NMR spectrum) was consistent with a methylene group attached to a quaternary arsenic atom, and was in keeping with the ^{1}H NMR spectral data discussed above. The small signal at δ_C 178.8 suggested the possible presence of a carboxy group and support for this was provided by an absorbance in the IR spectrum at 1587 cm^{-1} (it was anticipated that, as a consequence of the isolation procedure involving anion-exchange chromatography at pH 8.0, an acidic compound would be isolated as a salt). Moreover, indirect evidence for the presence of a carboxy group came from the acidity of the new compound as indicated by its elution position on DEAE Sephadex. At pH 8.0 the model compound **9**, containing trimethylarsonio and sulphuric acid ester groupings, was only weakly acidic. Incorporation of an additional acidic moiety in the new compound was necessary to explain its relatively strongly acidic properties; a carboxy group best accommodated these properties.

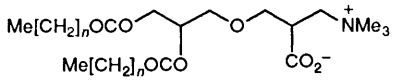
IR spectroscopy provided further structural information. The new compound displayed a strong absorbance at 1094 cm^{-1}, characteristic of the C–O stretch of an ether. This signal was absent from the IR spectrum of compound **9**. The presence of an ether linkage was supported by the ^{13}C NMR data which showed two methylene resonances at δ_C 72.2 and δ_C 73.0; in dipropyl ether, for example, the methylene carbons involved in the ether linkage give a ^{13}C signal at δ_C 73.2. Two of the remaining three signals were readily assigned to CHOH (δ_C 70.8) and CH_2OH (δ_C 63.0) in a terminal glycerol residue, and the third (δ_C 43.5, methine) was well accommodated by the presence of a $>CHCO_2H$ grouping. On the basis of the above data the new compound from *S. lacerifolium* was determined to be the dimethylarsonioriboside **10** (drawn as the carboxylic acid).

The ^{1}H NMR data, although too complex in the range δ 3.46–3.82 to allow ready interpretation, were in agreement with the above structural assignment. Homonuclear decoupling experiments also produced results consistent with the proposed structure but again, because of the complex nature of the spectra, results were not amenable to simple analysis. The exact nature of the diastereoisomerism of compound **10** is uncertain, but possibly the two compounds in the mixture differ from one another only in the configuration at the methine attached to the carboxy group, since duplication of signals (^{13}C NMR) was most noticeable for carbons close to this asymmetric centre.

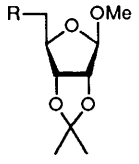
As formulated, compound **10** is analogous to a membrane lipid **11** isolated from a unicellular alga (*Ochromonas danica*)[13] and recently shown to be also present in *Sargassum lacerifolium*.[14] Compound **11** contains quaternary nitrogen attached to the same seven carbon moiety (in an acylated form) as that



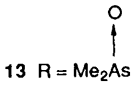
10



11



12 R = Br



13 R = Me_2As

bound to quaternary arsenic in compound **10** from *Sargassum*. The ^{13}C NMR data for this seven carbon moiety in compound **10** corresponded closely with those for the analogous carbons in compound **11**[14] except for the methylene carbon adjacent to arsenic in compound **10** (δ 26.0) or nitrogen in compound **11** (δ 68.3). For the methine carbon attached to the carboxy group (and β to the heteroatom), the values were quite similar: δ 43.5 in **10** and δ 46.2 in **11**.[14]

Lipid-type arsenic occurs in varying amounts in marine algae. It was proposed[2] that this arsenic is present as acylated derivatives of the phosphoric acid ester **2**, and indeed, one such compound, **3**, has been isolated from a brown alga.[15] The presence of the quaternary arsonio compound **10** in *Sargassum lacerifolium*, and its obvious capacity to form lipid-type compounds by acylation of the free hydroxy groups of the terminal glycerol residue, suggests the possibility that some lipid-type arsenic in algae may be accounted for by acylated derivatives of **10**. In view of the importance of the quaternary ammonium compound **11** as a membrane lipid in *Ochromonas*, and perhaps in other algae as well, it seems possible that an analogous arsenic compound may have some biochemical role in algae.

In the second extraction of *Sargassum* the approximate percentage contribution of the various arsenic compounds to the total amount of arsenic may be summarised as follows: compound **5** (70%), compound **2** (10%), compound **4a** (5%), compound **1** (5%), compound **6a** (0.5%), dimethylarsinic acid (0.2%), compound **7** (0.2%), compound **8** (0.1%), compound **10** (0.1%). Most of the arsenic unaccounted for in this *Sargassum* extract was retained on the DEAE Sephadex medium following elution with 0.05 mol dm^{-3} Tris buffer and, as shown in the first extract, was probably present as arsenate. The values for the percentage contribution of the compounds apply to the extract obtained following the initial fractionation employed to remove very polar (salt) and non-polar (lipid-type) materials.

The samples of *S. lacerifolium* (order Fucales) and *Ecklonia radiata* (order Laminariales), taken from the same lagoonal system, contained different arsinoylribosides as their major arsenic constituents. The major compound in *Sargassum* was the sulphuric acid ester **5**, whereas in *Ecklonia*[3] this arsenical was not found and the major compound was the sulphonic acid **4**. *S. lacerifolium* is thus consistent with the two species of brown algae from the order Fucales previously examined (*Hizikia fusiforme*[5] and *Sargassum thunbergii*[6]) which also contained compound **5** as the major arsenic constituent. The relative occurrence of these arsenic compounds may reflect the presence of different enzyme systems capable of carrying out the glycosidation reaction. The observation that compound **8** (containing the mannitol moiety) was present as only a minor constituent in *S. lacerifolium* suggests that the relative quantities of arsenic-containing ribosides in an alga does not merely reflect the availability of an alcohol able to form the glycosidic bond.

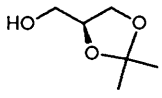
Stereochemistry of Arsenic-containing Ribosides.—This paper reports the isolation of seven dimethylarsinoylribosides and a mixture of two dimethylarsonioribosides from *S. lacerifolium*. NMR evidence demonstrated that all have a β-ribofuranose nucleus and, because of the biological origin of the compounds and their probable immediate derivation from *S*-adenosylmethionine,[2] all are likely to be β-D-ribosides. Although ^1H NMR spectral data were obtained for all compounds, information on the configuration of the aglycone moieties was not readily apparent. A single-crystal X-ray diffraction structure determination of the sulphuric acid ester **5**, when isolated from the giant clam *Tridacna maxima*,[7] established the absolute configuration as β-D-*ribo*- and 2′*S*. NMR data and the specific rotation of the sulphuric acid ester isolated from *S. lacerifolium* were identical with those of compound **5**. Total synthesis of compound **1**,[16] and a comparison of NMR data with those of the natural product from *S. lacerifolium*, indicated the absolute configuration of the natural compound as β-D-*ribo* and 2′*R*, and it is therefore of the same configuration as the sulphuric acid ester **5**.

A single-crystal X-ray diffraction structure determination was carried out on the amino sulphonic acid from *S. lacerifolium*. Although the stereochemistry at C-2 relative to that of the ribose ring was defined, the absolute configuration of the amino sulphonic acid could not be established. However, on the assumption that the algal arsinoylribosides contain a D-ribose ring system, this compound could be assigned the 2*S* configuration, namely compound **6a**, and therefore has the same configuration as compounds **1** and **5**.

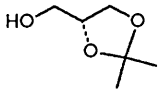
When compound **4** was isolated from the brown algae *Hizikia fusiforme*[5] and *Laminaria japonica*[4] it was obtained as a mixture (3:1) of C-2 diastereoisomers. The ^1H NMR spectra of the two diastereoisomers were virtually identical except for the chemical shifts and coupling constants of the C-3 methylene protons (adjacent to the anomeric centre). One only of the two possible C-2 diastereoisomers of compound **4** was isolated from the brown alga *Ecklonia radiata*, ^1H NMR data of which indicated it was identical with the major component of the mixture of C-2 diastereoisomers isolated from *Hizikia* and *Laminaria*. The sulphonic acid isolated from *S. lacerifolium* was shown (^1H NMR) also to be identical with the major component of the mixture of C-2 diastereoisomers. Signals characteristic of the minor diastereoisomer were not evident (< 15%) in the ^1H NMR spectrum.

To facilitate configurational assignment for the hydroxy sulphonic acid **4**, model compounds **20a** and **20b** were synthesized. The approach taken for the synthesis of these model compounds was the attachment of the chiral three-carbon aglycone at C-1 of the D-ribose moiety, followed by replacement of the terminal free hydroxy group in the aglycone by a sulphonic acid group. Compound **15a**, prepared from the substituted glycerine **14a** by the method of McAdam,[16] was treated with iodine in methanol[17] to give the diol **16a**. Monobromination of this diol proved difficult. Treatment with triphenylphosphine and carbon tetrabromide in pyridine solution gave the dibromide **17** as the major product, and the

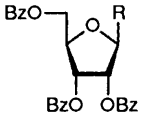
monobromide **18a** was obtained in only 24% yield. Presumably, bromination of the secondary hydroxy group of the diol **16a** would have occurred with inversion of configuration. However, the configuration of the aglycone could not be determined from an inspection of the NMR spectra; difficulties in interpretation of the ^{1}H NMR spectrum in sufficient detail were compounded by marked Van der Waals deshielding (0.4–0.5 ppm) of the aglycone protons caused by steric interactions following the introduction of the second bromine atom. Monobromination of diol **16a** in satisfactory yield (70%) was eventually achieved by addition of solid triphenylphosphine portionwise to a mixture of compound **16a** and carbon tetrabromide in dry methylene dichloride at room temperature, and careful monitoring of the reaction by TLC.

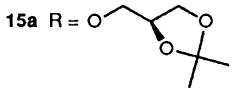


14a

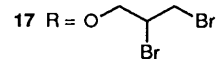


14b

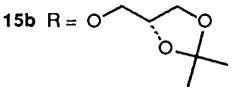




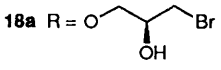
15a R =



17 R =



15b R =



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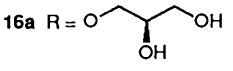
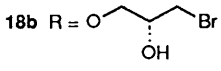
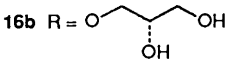


16a R =



18b R =



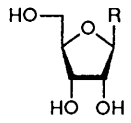
16b R =

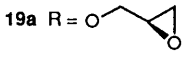
Treatment of the monobromide **18a** with a strongly basic anion-exchange resin in dry methanol removed the benzoyl groups and effected conversion of the bromohydrin into the epoxide **19a** in quantitative yield. The epoxide was not isolated but was instead stirred with aq. sodium sulphite to give the desired (2*S*)-sulphonate **20a**.

Beginning with the alcohol **14b**, the above reaction sequence was repeated leading to the (2*R*)-sulphonate **20b**.

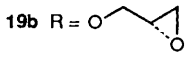
Although one diastereoisomer only of the hydroxy sulphonic acid **4** was found in *S. lacerifolium*, both diastereoisomers were isolated from *Hizikia*[5] and *Laminaria*.[4] ^{1}H NMR data were therefore available for both the naturally occurring hydroxy sulphonic acids for comparison with those for the synthetic model compounds **20a** and **20b**. From inspection of the chemical shifts and coupling constants of the methylene protons adjacent to the anomeric centre (Table 3), the sulphonic acid isolated from *S. lacerifolium* (and from *Ecklonia*) may be assigned the 2*S* configuration (compound **4a**) Similarly, the major compound of the mixture of naturally occurring diastereoisomers from *Hizikia* and *Laminaria* may now be assigned the 2*S* configuration (compound **4a**), and the minor component the 2*R* configuration (compound **4b**).

In the methylene group adjacent to the anomeric centre, the proton giving rise to the more downfield signal in the NMR spectrum of the 2*S*-compounds **4a** and **20a** exhibited the larger vicinal coupling constant (5.7 Hz *vs.* 3.5 Hz). This order was reversed for the 2*R*-compounds **4b** and **20b**. NMR data for

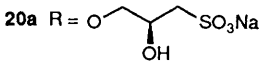




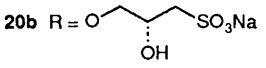
19a R =



19b R =



20a R =



20b R =

naturally occurring arsinoylribosides (compounds **1**,[3] **5**,[7] and **6a**) which have the same configuration at the asymmetric centre in the aglycone (*i.e.* at C-2 or C-2′) as that for compounds **4a** and **20a** show the same pattern of the more downfield proton having the larger vicinal coupling constant. ^{1}H NMR data for the phosphoric acid ester **2** (H_A, δ 3.65, J_{vic} 4.0 Hz; H_B, δ 3.81, J_{vic} 6.0 Hz) are consistent with this pattern and suggest that this compound has the same configuration at C-2′, as that found at the equivalent carbon (C-2 or C-2′) in compounds **1**, **4a**, **5**, and **6a**. The ^{1}H NMR spectrum of the mannitol derivative **8** was too complex in the area of interest to allow relevant information to be obtained on the configuration at C-2. Similarly, the configuration at C-2′ in the aglycone portion of the dimethylarsonioriboside **10** could not be established from the ^{1}H NMR spectrum, although it seems likely that the zwitterion **10** would have the same C-2′ configuration as that shown by the related compound **5**.

Experimental

Arsenic concentrations in samples were determined by atomic absorption spectrophotometry (AAS) with Varian instrumentation. Wet-tissue or crude extracts were digested in an oxidising acid mixture and the As^V species so formed were determined by vapour-generation AAS. Arsenic in chromatographic fractions was determined by graphite-furnace atomic absorption spectrophotometry (GFAAS) using nickel(II) nitrate as co-analyte.

GPC was carried out with Sephadex G-15 and Sephadex LH-20 media supplied by Pharmacia LKB Biotechnology. Unless otherwise stated, GPC refers to chromatography on Sephadex G-15 with water as eluent. Depending on the weight of material being chromatographed, one of three column sizes was used for GPC: 50 × 850 mm (up to 25 g), 26 × 900 mm (up to 0.5 g) or 16 × 600 mm (up to 20 mg). Anion-exchange chromatography refers to chromatography on DEAE A-25 Sephadex 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 equilibrated with 0.1 mol dm^{-3} ammonium formate buffer at pH 6.5. As a guide to the sizes of columns used, chromatography of material under ~1 g was carried out on 26 × 900 mm or 26 × 300 mm columns, and larger quantities of material (up to tens of grams) were chromatographed on 50 × 900 mm columns. In all cases, following ion-exchange chromatography, buffer was separated from the arsenic constituents in the fractions of interest by GPC.

TLC was performed on layers (1 mm thickness) of cellulose (Whatman CC41) or Silica 60 (Merck, Darmstadt) on glass

Table 3 ^{1}H NMR data for the 3-methylene protons (adjacent to the anomeric centre) in naturally occurring compounds **4a** and **4b**, and in synthetic model compounds **20a** and **20b**

Compound and source	^{1}H NMR data				
	3-H^A	3-H^B	$J_{3,3}$/Hz	$J_{2,3A}$/Hz	$J_{2,3B}$/Hz
4a *Sargassum lacerifolium*, *Ecklonia*[3]	3.64	3.80	10.6	3.5	5.6
4a *Hizikia*,[5] *Laminaria*[4] major constituent	3.66	3.81	10.4	3.5	5.7
4b *Hizikia*,[5] *Laminaria*[4] minor constituent	3.57	3.90	10.9	6.9	3.4
20a Synthesis	3.64	3.79	10.5	3.3	5.8
20b Synthesis	3.56	3.86	10.8	6.4	3.5

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. As a guide, the following quantities of material were applied to a single plate: 20–50 mg (1 mm thickness); 5–20 mg (0.5 mm); and < 5 mg (0.1 mm). TLC plates were developed in mixtures of solvents: system A, butan-1-ol–acetic acid–water (60:15:25); system B, propan-1-ol–aq. NH_3 (7:3); system C, propan-2-ol–ethyl acetate–water (7:1:2); system D, propan-1-ol–acetic acid–water (60:15:25); or system E, ethanol–water (4:1). Arsenic compounds were located by scribing of plates into 5 mm bands and analysis of each band for arsenic by GFAAS, following extraction with water.

NMR spectra were recorded on a Hitachi–Perkin-Elmer 12–24B spectrometer at 60 MHz (^{1}H), a Bruker WP-80 at 80 MHz (^{1}H) and 20.1 MHz (^{13}C), and a Bruker AM 300 at 300 MHz (^{1}H), and 75.5 MHz (^{13}C). For ^{1}H and ^{13}C NMR spectra recorded in $CDCl_3$, tetramethylsilane was an internal standard; ^{1}H NMR spectra in D_2O were recorded relative to external 2,2-dimethyl-2-silapentane-5-sulphonate (DSS) at δ 0.00; and for ^{13}C NMR spectra in D_2O, methanol (δ_C 49.00) served as an external standard. Assignment of ^{13}C signals was assisted by DEPT experiments. *J*-Values are given in Hz. IR absorbance spectra were obtained for samples as films on a diamond cell with a Biorad FTS-40 spectrophotometer. Light petroleum refers to that fraction boiling in the range 60–80 °C.

First Extraction of Sargassum lacerifolium.—*Preliminary work-up.* A quantity of fresh *S. lacerifolium* (Turn.) C. Agardh. was collected from Mullaloo Beach, Perth, Western Australia. After sorting and a brief rinse in tap water, a portion (4 kg, ~40 μg g^{-1} As wet wt) of the bulked *Sargassum* was extracted with methanol (7 dm^3), and the methanol was removed by rotary evaporation to yield a brown syrup (130 g, 49 mg As). This syrup was re-extracted with methanol (300 cm^3) to produce a dark solution and a pale, salty residue. Evaporation of the solution yielded a dark syrup (48 g, 32 mg As) which was again extracted with methanol (250 cm^3) to yield a dark solution and a second salty residue. The filtrate was evaporated to dryness, the residue was dissolved in water (200 cm^3), and the mixture was filtered to remove an oily precipitate. The red-brown filtrate (250 cm^3) was left overnight at 4 °C and some brown material precipitated. The solution was filtered again and stored (−18 °C) as five equal portions.

A portion (50 cm^3) of the above solution was subjected to GPC whereupon the major arsenic-containing fractions eluted as two bands at 700–850 cm^3 eluent ('early' material) and 850–1050 cm^3 eluent ('late' material). This operation was repeated four times to give the 'early' material (300 mg, 1.9 mg As) and the 'late' material (1.9 g, 26 mg As) as syrups.

Isolation of compound **2** *in the 'early' material.* The 'early' material from GPC was subjected to anion-exchange chromatography on DEAE Sephadex. A single arsenic compound was obtained (1300–1480 cm^3 eluent) which, after removal of Tris buffer (Sephadex LH-20–water–methanol column), was obtained as a syrup (20 mg, 1.1 mg As). TLC (cellulose, system A, R_f 0.22), followed by GPC yielded the arsenic compound as a syrup (3 mg, 600 μg As). It was shown by comparison of ^{1}H NMR spectra to be identical with 3-[5-deoxy-5-(dimethylarsinoyl)-β-D-ribosyloxy]-2-hydroxypropyl 2,3-dihydroxypropyl hydrogen phosphate **2** previously isolated from *Ecklonia radiata* and *Hizikia fusiforme*.

Isolation of compound **5** *in the 'late' material.* The 'late' material from GPC (1.9 g, 26 mg As) was subjected to anion-exchange chromatography on DEAE Sephadex. Some arsenical material eluted at the void volume (350–370 cm^3 eluent) and was obtained as a syrup (43 mg, 200 μg As). TLC of this material on cellulose produced a broad spread of arsenic (R_f 0.1–0.6), suggesting the presence of several arsenic compounds. One major acidic arsenic compound eluted (2175–2725 cm^3 eluent) from the DEAE column and was recovered as a syrup (700 mg, 21 mg As). This material was again subjected to anion-exchange chromatography and the arsenical material was obtained as a syrup (550 mg, 21 mg As). A portion (150 mg, 5.5 mg As) was then subjected to TLC (cellulose, system A, R_f 0.32) and GPC to give the arsenic compound as a syrup (7 mg, 1.4 mg As). It was shown by comparison of ^{1}H NMR spectra and specific rotations to be identical with (2′*S*)-3′[5-deoxy-5-(dimethylarsinoyl)-β-D-ribosyloxy]-2′-hydroxypropyl hydrogen sulphate **5**, previously isolated from *Tridacna maxima*.

After passage through the DEAE Sephadex column of 0.05 mol dm^{-3} Tris buffer (4 dm^3) at pH 8.0, the concentration of the buffer was increased to 0.5 mol dm^{-3}. A further single arsenic compound was eluted (580–600 cm^3 eluent after 0.5 mol dm^{-3} Tris was applied to the column), and shown to be inorganic arsenate by TLC co-ordinates and by its reduction ($NaBH_4$) to arsine without prior chemical destruction.

Second (Large-scale) Extraction of Sargassum lacerifolium.—*Preliminary work-up.* Fresh *S. lacerifolium* was collected by divers from inshore reefs in the Marmion Marine Park near Perth in March 1988. Extraneous material was removed and a quantity of the alga (40 kg) was packed into 15 5-dm^3 conical flasks and steeped in methanol (45 dm^3) for 2 days. The extract was filtered through cheesecloth; methanol was removed from the filtrate by evaporation and the resultant aq. extract was further concentrated to 1.5 dm^3 (containing 350 mg As). The alga was subjected to two additional extractions with aq. methanol (1:1) (20 and then 8 dm^3) which removed a further 160 and 70 mg of arsenic, respectively. These two extracts were not further examined. After storage overnight at 4 °C, the first extract was filtered through a small quantity of alumina (100 g) to remove a black gum, and the resultant clear black filtrate was poured into methanol (3 dm^3), and the mixture was kept at −18 °C for 1 h. Filtration yielded salty material (670 g, 50 mg As), which was not further examined, and a black filtrate, which was poured into acetone. The clear black liquid was decanted from the brown residue, and on evaporation gave a black oil (185 g, 70 mg As). The brown residue (340 g, 190 mg As) was made up to 1500 cm^3 in water and stored in polyethylene bottles at −18 °C in 30 50-cm^3 portions. These were separately

subjected to GPC. For each column, arsenic eluted mainly from 600 to 1200 cm^3 and was split into three major fractions. The appropriate arsenic bands were combined for the 30 columns to give 'early' (9.7 g, 30 mg As), 'mid' (17 g, 80 mg As), and 'late' (60 g, 50 mg As) arsenic fractions. A further small quantity of arsenic (2 mg) which eluted very late from the column as a trailing, ill defined band was retained (−18 °C) but was not further examined here.

The 'early' fraction from the Sephadex G-15 column was subjected to anion-exchange chromatography whereupon the arsenic eluted at the void volume (to give fraction DEAE 1), and then in three major bands of increasing acidity (to give fractions DEAE 2–4). Anion-exchange chromatography on the 'mid' and 'late' fractions (from GPC) produced similar bands in varying quantities, which were combined where appropriate. Fig. 1 shows a typical elution profile for this purification step.

Examination of non-acidic material (fraction DEAE 1); isolation of compounds **1**, **7** *and* **8**. The non-acidic material (fraction DEAE 1, 2.2 g, 5 mg As) was subjected to cation-exchange chromatography (CM Sephadex). A major arsenic band eluted with basic material at 200–250 cm^3 (void volume was 100 cm^3). This basic-arsenic material was subjected to TLC (cellulose, system A) whereupon the arsenic separated into three overlapping bands, with R_f 0.3 (0.15 mg As), R_f 0.5 (4 mg As), and R_f 0.65 (0.2 mg As).

The arsenical at R_f 0.5 was isolated by TLC (cellulose, system A, R_f 0.47 and system B, R_f 0.48) and GPC to give a syrup (11 mg), shown to be (2′*R*)-2′,3′-dihydroxypropyl 5-deoxy-5-(dimethylarsinoyl)-β-D-riboside **1** by ^1H NMR spectroscopy.

The most mobile arsenical (R_f 0.65) was purified by TLC (cellulose, system A, R_f 0.66) and GPC to give a syrup (1.1 mg), identified as methyl 5-deoxy-5-(dimethylarsinoyl)-β-D-riboside **7** by comparison of its ^1H NMR spectrum and TLC properties with those of an authentic specimen.

The least mobile band (R_f 0.3) was subjected to cation-exchange chromatography and the arsenic was recovered in material (60 mg) obtained from the eluate. This arsenical was purified by TLC (cellulose, system A, R_f 0.28; cellulose, system D, R_f 0.44; cellulose, system B, R_f 0.33), and GPC to give a syrup (0.5 mg); δ_H(300 MHz; D_2O) 1.84 and 1.86 (6 H, 2 s, Me_2As), 2.49 (1 H, dd, $J_{5',5''}$ 14.2, $J_{4',5''}$ 10.4, 5′-H), 2.63 (1 H, dd, $J_{5',5''}$ 14.2, $J_{4',5''}$ 3.3, 5′-H), 3.6–4.05 (8 H, m, 1-, 2-, 3-, 4-, 5- and 6-H), 4.15 (1 H, m, 2′-H), 4.25 (2 H, m, 3′- and 4′-H) and 5.03 (1 H, s, 1′-H); δ_C(75.5 MHz; D_2O) 14.3 and 14.6 (2 C, Me_2As), 36.2 (C-5′), 63.4 (C-6), 69.2, 69.3, 69.6 and 71.0 (C-2, -3, -4 and -5), 70.2 (C-1), 74.4 (C-2′), 75.9 (C-3′), 77.1 (C-4′) and 108.2 (C-1′).

A portion (200 μg) of this compound was heated (100 °C) with 0.1 mol dm^{-3} HCl (30 mm^3) for 2 h. The reaction mixture was evaporated and the residue was dissolved in water and subjected to LC analysis (column: Shodex Ionpak CH-801, ion-exchange resin of sulphonated styrene–divinylbenzene copolymer; eluent water). Mannitol was identified in the hydrolysate by comparison of its retention time with the retention times of the three hexitols [D-mannitol (6.8 min), galactitol (8.9 min), and D-glucitol (10.0 min)] known to occur in algae.[9] On the basis of these data, the new compound was formulated as 1-*O*-[5′-deoxy-5′-(dimethylarsinoyl)-β-D-ribosyl]mannitol **8**.

Examination of the weakly acidic material (fraction DEAE 2); isolation of compound **6a**. When fraction DEAE 2 was subjected to further anion-exchange chromatography the arsenic separated into two discrete bands, with retention volumes of 1310 cm^3 (0.4 mg As) and 1460 cm^3 (8.9 mg As). The major arsenical had identical chromatographic properties (DEAE Sephadex and Sephadex G-15) as the phosphoric acid diester **2** isolated in the first extraction of *Sargassum*.

The minor arsenical (with retention volume 1310 cm^3 on DEAE Sephadex) was subjected to TLC (cellulose, system A, R_f 0.18; cellulose, system B, R_f 0.33) and GPC, and was obtained as a solid (1.2 mg) which crystallised as needles, m.p. >300 °C (decomp.) (from water). The material was identified (^1H, ^{13}C NMR spectra) as compound **6**, previously isolated from *Hizikia fusiforme*.[5] A single-crystal X-ray diffraction structure determination showed the compound to have the (2*S*) configuration **6a**, assuming a β-D-ribose ring system; δ_H(300 MHz; D_2O) 1.85 and 1.87 (6 H, 2 s, Me_2As), 2.48 (1 H, dd, $J_{5',5''}$ 13.7, $J_{4',5''}$ 9.9, 5′-H), 2.65 (1 H, dd, $J_{5',5''}$ 13.7, $J_{4',5''}$ 3.0, 5′-H), 3.15 (1 H, dd, $J_{1,1}$ 14.6, $J_{1,2}$ 7.6, 1-H), 3.27 (1 H, dd, $J_{1,1}$ 14.6, $J_{1,2}$ 5.2, 1-H), 3.75 (1 H, dd, $J_{3,3}$ 10.6, $J_{2,3}$ 3.7, 3-H), 3.86 (1 H, m, 2-H), 3.99 (1 H, dd, $J_{3,3}$ 10.6, $J_{2,3}$ 5.1, 3-H), 4.16 (1 H, d, $J_{2',3'}$ 3.9, 2′-H), 4.27 (2 H, m, 3′- and 4′-H) and 5.05 (1 H, s, 1′-H); δ_C(75.5 MHz; D_2O) 14.3 and 14.5 (Me_2As), 36.0 (C-5′), 49.0 (C-1), 50.4 (C-2), 67.4 (C-3), 74.4 (C-2′), 75.8 (C-3′), 77.1 (C-4′) and 107.4 (C-1′).

Examination of the moderately acidic material (fraction DEAE 3); isolation of compound **4a**. This fraction, when subjected to GPC, produced two arsenic-containing bands. The minor constituent (100 μg As) was shown to be dimethylarsinic acid by a comparison of its chromatographic properties (Sephadex G-15; DEAE Sephadex; TLC, cellulose, system D, R_f 0.64) with those of synthetic material.

The major arsenical constituent was further concentrated (190 mg, 1.4 mg As) by GPC and subjected to TLC (cellulose, system A, R_f 0.3, system B, R_f 0.29; silica, system E, R_f 0.49) and GPC to give a syrup (2.0 mg), identified by its ^1H NMR spectrum and comparison with the ^1H NMR spectra of model compounds **20a** and **20b**, as (2*S*)-3-[5′-deoxy-5′-(dimethylarsinoyl)-β-D-ribosyloxy]-2-hydroxypropane-1-sulphonic acid **4a**, previously isolated from *Ecklonia radiata*.[3] The (2*R*)-diastereoisomer **4b** was not evident (<15%) from the ^1H NMR spectrum of compound **4a**.

Examination of the strongly acidic material (fraction DEAE 4); isolation of compound **10**. Further anion-exchange chromatography of fraction DEAE 4 separated the arsenic into two components. The major component (70 mg As) co-chromatographed (DEAE; G-15; TLC, cellulose, system D, R_f 0.47) with compound **5**, isolated in the first extraction of *Sargassum*, and was put aside.

The minor component (70 mg, 100 μg As) was purified by repeated anion-exchange chromatography followed by TLC (cellulose, system D, R_f 0.55, system B, R_f 0.29; silica, system E, R_f 0.64; cellulose, system A, R_f 0.24) then GPC, and was isolated as a syrup (0.7 mg) (see Results and Discussion section for NMR spectral data of this material, compound **10**); ν_{max}/cm^{-1} 1587, 1420, 1248, 1217, 1126, 1094, 1065 and 1003.

Synthesis of Compound **7**.—*Methyl 5-bromo-5-deoxy-2,3-O-isopropylidene-β-D-riboside* **12**. A solution of di-*O*-benzoyl-1,2-*O*-(1′-methoxybenzylidene)-α-D-ribofuranose, prepared by the method of McAdam,[16] (26 g, 54.6 mmol) in methylene dichloride–methanol (1:1; 400 cm^3) was refluxed with mercury(II) bromide (50 mg) and a catalytic amount of pyridinium toluene-*p*-sulphonate. Evaporation of the solvent followed by column chromatography [SiO_2; EtOAc–light petroleum (2:8)] gave methyl tri-*O*-benzoyl-β-D-ribofuranoside as an oil (24 g, 92%).

This material (22.8 g, 48 mmol) was stirred with methanol (500 cm^3) containing potassium hydroxide (10.7 g, 190 mmol) at room temperature for 30 min. The residue obtained on evaporation of the methanol was dissolved in water and passed through a column (60 × 160 mm) of mixed-bed ion-exchange resin (Permutit DMF resin). Elution with water, followed by evaporation, yielded methyl β-D-ribofuranoside (6.44 g, 82%), δ_H(60 MHz; D_2O) 3.56 (3 H, s, OMe), 3.90 (2 H, m, 5-H_2), 4.2 (3 H, m, 2-, 3- and 4-H) and 5.03 (1 H, s, 1-H).

Methyl β-D-ribofuranoside (6.33 g, 38 mmol) was stirred at room temperature with acetone (300 cm^3), 2,2-dimethoxy-

propane (10 cm^3) and camphorsulphonic acid (100 mg) for 22 h. Anhydrous sodium carbonate (1.5 g) was added, the mixture was stirred for 10 min, and the residue obtained on evaporation was subjected to column chromatography [SiO_2; EtOH–EtOAc–light petroleum (2:5:5)] to give methyl 2,3-*O*-isopropylidene-β-D-riboside as a free flowing oil (6.88 g, 87%).

A mixture of this protected methyl-β-D-riboside (2.88 g, 14 mmol) in methylene dichloride (100 cm^3) was treated with carbon tetrabromide (9.31 g, 28.0 mmol) and triphenylphosphine (7.34 g, 28.0 mmol), and the mixture was stirred at room temperature for 25 h. Following the addition of methanol (25 cm^3) the mixture was stirred (1 h), then concentrated by evaporation, and the resultant residue was subjected to column chromatography [EtOAc–light petroleum (2:8)] to give the bromide **12** as an oil (3.10 g, 82%), $[\alpha]_D$ −79° (*c* 6.4, MeOH; lit.,[18] −80°); δ_C(20.1 MHz; $CDCl_3$) 24.9 and 26.3 (*Me*$_2$C), 32.4 (C-5), 55.0 (OMe), 82.6, 85.1 and 86.6 (C-2, -3 and -4), 109.6 (C-1) and 112.6 (Me$_2$*C*).

Methyl 5-deoxy-5-(dimethylarsinoyl)-2,3-O-isopropylidene-β-D-riboside **13**. The bromide **12** (1.15 g, 4.3 mmol) was stirred at room temperature in dry tetrahydrofuran (THF) (10 cm^3) under nitrogen as a solution of dimethylarsinosodium (Me_2AsNa)[16] (5 mmol in THF) was added. The mixture was stirred at room temperature for 2.5 h, then filtered through a plug of silica. Evaporation of the filtrate gave the crude arsine (1.4 g), which was dissolved in THF (10 cm^3) and oxidised by the dropwise addition of H_2O_2 (0.7 cm^3; 30% w/v). Evaporation of the solvent followed by column chromatography [SiO_2; EtOAc–EtOH–water (45:30:25)] gave *methyl 5-deoxy-5-(dimethylarsinoyl)-2,3-O-isopropylidene-β-D-riboside* **13** as hygroscopic prisms (820 mg, 62%), m.p. 144–146 °C; $[\alpha]_D$ +0.4° (*c* 4.8, MeOH) (Found: C, 40.6; H, 6.8. $C_{11}H_{21}AsO_5 \cdot H_2O$ requires C, 40.5; H, 7.1%); 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), 2.52–2.64 (2 H, m, 5-H_2), 3.44 (3 H, s, OMe), 4.62 (1 H, m, 4-H), 4.88 (2 H, m, 2- and 3-H) and 5.12 (1 H, s, 1-H); δ_C(75.5 MHz; D_2O) 14.1 and 14.4 (Me_2As), 23.9 and 25.5 (*Me*$_2$C), 36.1 (C-5), 55.9 (OMe), 81.4 (2 C), 84.6 (C-2, -3 and -4), 110.1 (C-1) and 113.6 (Me$_2$*C*).

Methyl 5-deoxy-5-(dimethylarsinoyl)-β-D-riboside **7**. The protected arsinoyl-β-D-riboside **13** (516 mg, 1.7 mmol) was swirled with aq. trifluoroacetic acid [5 cm^3 of a mixture of $CF_3CO^-{}_2$–water (9:1)] for 6 min, and the mixture was then evaporated to dryness. The residue was dissolved in water (10 cm^3) and applied to a small column of Amberlite IR-45 (OH^-). Elution with water followed by evaporation of the eluate and GPC gave *methyl 5-deoxy-5-(dimethylarsinoyl)-β-D-riboside* **7** as a syrup (410 mg, 92%), $[\alpha]_D$ +3.9° (*c* 4.1, MeOH) (Found: C, 33.5; H, 6.5. $C_8H_{17}AsO_5 \cdot H_2O$ requires C, 33.6; H, 6.7%); δ_H(300 MHz; D_2O) 1.83 and 1.85 (6 H, 2 s, Me_2As), 2.46 (1 H, dd, $J_{5,5}$ 13.9, $J_{4,5}$ 10.5, 5-H), 2.61 (1 H, dd, $J_{5,5}$ 13.9, $J_{4,5}$ 3.5, 5-H), 3.41 (3 H, s, OMe), 4.07 (1 H, br d, $J_{2,3}$ 4.5, 2-H), 4.15 (1 H, m, 3-H), 4.26 (1 H, m, 4-H) and 4.90 (1 H, s, 1-H); δ_C(75.5 MHz; D_2O) 14.1 and 14.6 (Me_2As), 36.0 (C-5), 55.6 (OMe), 74.2 (C-2), 75.7 (C-3), 77.0 (C-4) and 108.5 (C-1).

Synthesis of Compound **9**.—The sulphuric acid ester **5** (0.50 mg As, 0.007 mmol) was stirred in methanol (1.5 cm^3) with 2,3-dimercaptopropanol (0.01 mmol) and methyl iodide (0.1 cm^3) for 16 h at room temp. The reaction mixture was then partitioned between diethyl ether and water, and the aq. layer was subjected to anion-exchange chromatography. The material eluting at 170 cm^3 was subjected to GPC to give (2′*S*)-3′-[5-deoxy-5-(trimethylarsonio)-β-D-ribosyloxy]-2′-hydroxypropyl hydrogen sulphate **9** as a solid (0.30 mg As, 60%); ν_{max}/cm^{-1} 1418, 1249, 1217, 1131, 1063 and 1002; δ_H(300 MHz; D_2O) 2.01 (9 H, s, Me_3As), 2.71 (1 H, dd, $J_{5,5}$ 13.8, $J_{4,5}$ 10.3, 5-H), 2.88 (1 H, dd, $J_{5,5}$ 13.8, $J_{4,5}$ 3.3, 5-H), 3.64 (1 H, dd, $J_{1',1'}$ 10.6, $J_{1',2'}$ 3.1, 1′-H), 3.86 (1 H, dd, $J_{1',1'}$ 10.6, $J_{1',2'}$ 4.0, 1′-H), 4.05–4.14 (3 H, m, 2′-H and 3′-H_2), 4.17 (1 H, dd, $J_{2,3}$ 4.0, $J_{2,4}$ 0.6, 2-H), 4.21–4.33 (2 H, m, 3- and 4-H) and 5.05 (1 H, s, 1-H); δ_C(75.5 MHz; D_2O) 7.7 (9 H, Me_3As), 30.4 (C-5), 67.7 (C-2′), 68.1 (2 C, C-1′ and -3′), 74.2 (C-2), 76.0 (C-3), 77.0 (C-4) and 107.6 (C-1).

Synthesis of Model Compounds **20a** *and* **20b**.—(2′R)-2′,3′-*Dihydroxypropyl tri-O-benzoyl-β-D-ribofuranoside* **16a**. The glycoside **15a** (1.8 g), prepared from the alcohol **14a** by the method of McAdam,[16] was stirred with methanol (40 cm^3) containing 1% w/v iodine[17] (room temp., 24 h). Saturated methanolic sodium thiosulphate was then added dropwise until the colour of the iodine was just discharged. After removal of solvent, the residue was repeatedly extracted with the solvent mixture EtOAc–MeOH–light petroleum (4:1:5), and column chromatography [SiO_2; EtOAc–MeOH–light petroleum (4:1:5)] yielded the *diol* **16a** as an oil which slowly crystallised (1.3 g, 76%), m.p. 85 °C (from EtOAc–light petroleum); $[\alpha]_D$ +31.1° (*c* 4.8, in CH_2Cl_2) (Found: C, 64.8; H, 5.3. $C_{29}H_{28}O_{10}$ requires C, 64.9; H, 5.3%); δ_H(300 MHz; $CDCl_3$) 3.56–3.90 (5 H, m, 1′- and 3′-H_2 and 2′-H), 4.58–4.78 (3 H, m, 4-H and 5-H_2), 5.32 (1 H, s, 1-H), 5.70 (1 H, m, 2-H), 5.83 (1 H, m, 3-H), 7.27–7.61 (9 H, m, Ph) and 7.88–8.08 (6 H, m, Ph); δ_C(75.5 MHz; $CDCl_3$) 63.5 (C-3′), 64.9 (C-5), 70.6 (C-1′), 70.7 (C-2′), 72.3 (C-3), 75.5 (C-2), 79.4 (C-4), 106.3 (C-1), 128.4–133.6 (18 C, Ph) and 165.3, 165.4 and 166.3 (3 C, CO).

2′,3′-*Dibromopropyl tri-O-benzoyl-β-D-ribofuranoside* **17**. The diol **16a** (240 mg, 0.45 mmol), carbon tetrabromide (420 mg, 1.27 mmol) and triphenylphosphine (325 mg, 1.24 mmol) were dissolved in dry pyridine (10 cm^3) and the solution was stirred at room temperature for 1 h before the reaction was quenched by the addition of methanol. After removal of solvent, column chromatography [SiO_2; EtOAc–light petroleum (4:6)] yielded the *dibromide* **17** (21.0 mg, 71%) as an oil (270 mg, 87%), $[\alpha]_D$ +30.0° (*c* 7.9, CH_2Cl_2) (Found: C, 52.4; H, 3.9. $C_{29}H_{26}Br_2O_8$ requires C, 52.6; H, 4.0%); δ_H(300 MHz; $CDCl_3$) 3.73–3.76 (2 H, m, 3′-H_2), 3.91–3.96 (1 H, m, 2′-H), 4.18–4.26 (2 H, m, 1′-H_2), 4.58–4.64 (1 H, m, 4-H), 4.72–4.81 (2 H, m, 5-H_2), 5.36 (1 H, s, 1-H), 5.76 (1 H, d, $J_{2,3}$ 5.0, 2-H), 5.85–5.89 (1 H, m, 3-H), 7.26–7.61 (9 H, m, Ph) and 7.87–8.08 (6 H, m, Ph); δ_C(75.5 MHz; $CDCl_3$) 32.4 (C-3′), 48.1 (C-2′), 65.0 (C-5), 69.2 (C-1′), 72.4 (C-3), 75.3 (C-2), 79.2 (C-4), 105.6 (C-1), 128.3–133.5 (18 C, Ph) and 165.1, 165.3 and 166.1 (3 C, CO).

The monobromide **18a** was obtained as a minor product from this reaction.

(2′S)-3′-*Bromo-2′-hydroxypropyl tri-O-benzoyl-β-D-ribofuranoside* **18a**. The diol **16a** (1.15 g, 2.15 mmol) and carbon tetrabromide (1.4 g, 4.3 mmol) were dissolved in dry methylene dichloride and triphenylphosphine (770 mg, 2.9 mmol) was added portionwise to the stirred solution during 1 h (room temp.). The reaction was constantly monitored by analytical TLC [SiO_2; EtOAc–light petroleum (1:2)], and when the quantity of the dibromide formed was judged to be equal to the quantity of unchanged diol [the monobromide being the major component of the reaction mixture at that time (80 min)] the reaction was quenched by the addition of methanol. Column chromatography [SiO_2; EtOAc–light petroleum (4:6)] yielded the *monobromide* **18a** as prisms (890 mg, 70%), m.p. 115 °C (from EtOAc–light petroleum); $[\alpha]_D$ +28.2° (*c* 4.2, CH_2Cl_2) (Found: C, 58.0; H, 4.7. $C_{29}H_{27}BrO_9$ requires C, 58.1; H, 4.5%); δ_H(300 MHz; $CDCl_3$) 3.47 (2 H, m, 3′-H_2), 3.71 (1 H, dd, $J_{1',1'}$ 10.1, $J_{1',2'}$ 4.4, 1′-H), 3.88 (1 H, dd, $J_{1',1'}$ 10.1, $J_{1',2'}$ 5.6, 1′-H), 3.94 (1 H, m, 2′-H), 4.56–4.78 (3 H, m, 4-H and 5-H_2), 5.32 (1 H, s, $J_{1,2}$ 0.0, 1-H), 5.70 (1 H, m, 2-H), 5.82 (1 H, m, 3-H), 7.26–7.61 (9 H, m, Ph) and 7.88–8.09 (6 H, m, Ph); δ_C(75.5 MHz; $CDCl_3$) 35.0 (C-3′), 64.8 (C-5), 69.6 (C-2′), 70.6 (C-1′), 72.2 (C-3), 75.4 (C-2), 79.4 (C-4), 106.1 (C-1), 128.4–133.6 (18 C, Ph) and 165.3, 165.4 and 166.2 (3 C, CO).

Table 4 Non-hydrogen co-ordinates for compound **6a**, with estimated standard deviations in parentheses

Atom	*x*	*y*	*z*
C(1)	0.834 9(3)	0.070 5(7)	0.428(1)
S(1)	0.796 11(8)	0.173 3(2)	0.258 1(3)
O(11)	0.832 8(3)	0.287 1(6)	0.224(1)
O(12)	0.738 3(2)	0.202 6(6)	0.344 1(8)
O(13)	0.788 4(2)	0.091 5(6)	0.088 2(8)
C(2)	0.823 4(2)	0.110 2(7)	0.635 1(9)
N(2)	0.844 5(2)	0.003 1(6)	0.763 1(9)
C(3)	0.852 3(3)	0.237 7(7)	0.698(1)
O(3)	0.915 6(2)	0.224 1(5)	0.678 1(8)
O	0.933 2(2)	0.451 3(4)	0.674 7(7)
C(1′)	0.947 3(3)	0.328 4(7)	0.766(1)
C(2′)	1.013 0(3)	0.307 7(7)	0.728(1)
O(2′)	1.047 7(2)	0.385 1(5)	0.853 2(7)
C(3′)	1.017 9(3)	0.356 0(6)	0.521(1)
O(3′)	1.075 6(2)	0.393 9(6)	0.466 9(7)
C(4′)	0.973 2(3)	0.470 0(6)	0.517(1)
C(5′)	0.940 1(3)	0.480 4(7)	0.328(1)
As(5′)	0.879 85(3)	0.611 33(7)	0.309 8(1)
O(51′)	0.909 3(2)	0.758 6(5)	0.351 7(8)
C(52′)	0.814 0(3)	0.585 1(9)	0.477(1)
C(53′)	0.850 9(3)	0.595(1)	0.053(1)

(2S)-2-*Hydroxy*-3-(β-D-*ribofuranosyloxy*)*propane*-1-*sulphonic acid, sodium salt* **20a**. The bromide **18a** (120 mg, 0.20 mmol) was dissolved in methanol (4 cm^3), and Amberlite IRA 400 (OH^-) chromatographic-grade anion-exchange resin (400 mg) was added (the resin had been cycled to constant titre, washed, and dried). After the mixture had been stirred for 45 min (room temp.) the resin was removed by filtration and the filtrate was evaporated to dryness. ^{13}C NMR spectroscopic examination of the residue (syrup) indicated that the bromide **18a** had been quantitatively converted into the epoxide **19a**, δ_C(20.1 MHz; D_2O) 46.7 (C-3′), 52.8 (C-2′), 64.6 (C-5), 69.7 (C-1′), 72.4 (C-3), 76.1 (C-2), 84.7 (C-4) and 108.9 (C-1). This epoxide was used in the next reaction step without purification.

The epoxide **19a** (45 mg) was dissolved in water (4 cm^3), and aq. sodium sulphite (75 mg, 0.6 mmol in 1 cm^3) was added. The clear solution was stirred (room temp.) for 24 h, then was evaporated to dryness, and the white residue was examined by ^1H NMR spectroscopy which indicated, by the absence of signals at δ 2.6–2.8 (epoxide CH_2) and the presence of a characteristic multiplet at δ 3.0 (CH_2SO_3Na), that a quantitative conversion of the epoxide **19a** into the sulphonate **20a** had been achieved. GPC of the residue (2 ×) afforded the pure *sulphonate* **20a** as a syrup [55 mg, 89% (based on the bromide)], $[\alpha]_D$ −39.2° (*c* 4.2, water) (Found: C, 31.1; H, 4.8. $C_8H_{15}NaO_9S$ requires C, 31.0; H, 4.9%); δ_H(300 MHz; D_2O) 3.05 (1 H, dd, $J_{1,1}$ 14.5, $J_{1,2}$ 7.2, 1-H), 3.14 (1 H, dd, $J_{1,1}$ 14.5, $J_{1,2}$ 4.8, 1-H), 3.64 (1 H, dd, $J_{5',5'}$ 12.0, $J_{4',5'}$ 6.5, 5′-H), 3.64 (1 H, dd, $J_{3,3}$ 10.5, $J_{2,3}$ 3.3, 3-H), 3.79 (1 H, dd, $J_{3,3}$ 10.5, $J_{2,3}$ 5.8, 3-H), 3.82 (1 H, dd, $J_{5',5'}$ 12.0, $J_{4',5'}$ 3.1, 5′-H), 4.02 (1 H, m, 4′-H), 4.11 (1 H, d, $J_{2',3'}$ 4.6, 2′-H), 4.23–4.28 (2 H, m, 2′- and 3′-H) and 5.03 (1 H, s, 1′-H); δ_C(75.5 MHz; D_2O) 53.8 (C-1), 62.8 (C-5′), 66.5 (C-2), 70.7 (C-3), 70.8 (C-3′), 74.5 (C-2′), 82.8 (C-4′) and 107.1 (C-1′).

(2′R)-2′,3′-*Isopropylidenedioxypropyl tri*-O-*benzoyl*-β-D-*ribofuranoside* **15b**. Di-*O*-benzoyl-1,2-*O*-(1′-methoxybenzylidene)-α-D-ribofuranose (7.0 g, 14.5 mmol) and the alcohol **14b** (ex. Sigma Chemical Co., St. Louis, USA; 2.0 g, 14.5 mmol) in dry toluene (100 cm^3) were treated as in the synthesis of the glycoside **15a**. Column chromatography [SiO_2; EtOAc–light petroleum (3:7)] of the reaction mixture gave the *glycoside* **15b** as an oil (4.4 g, 51%), $[\alpha]_D$ +28.8° (*c* 6.3, CH_2Cl_2) (Found: C, 66.8; H, 5.7. $C_{32}H_{32}O_{10}$ requires C, 66.7; H, 5.6%); δ_H(300 MHz; $CDCl_3$) 1.34 and 1.41 (6 H, 2 s, Me), 3.56 (1 H, dd, $J_{3',3'}$ 10.3, $J_{2',3'}$ 6.6, 3′-H), 3.62 (1 H, dd, $J_{1',1'}$ 8.4, $J_{1',2'}$ 6.6, 1′-H), 3.78 (1 H, dd, $J_{3',3'}$ 10.3, $J_{2',3'}$ 4.5, 3′-H), 3.95 (1 H, dd, $J_{1',1'}$ 8.4, $J_{1',2'}$ 6.5, 1′-H), 4.17–4.24 (1 H, m, 2′-H), 4.50–4.56 (1 H, m, 4-H), 4.70–4.78 (2 H, m, 5-H_2), 5.32 (1 H, s, 1-H), 5.76 (1 H, d, $J_{2,3}$ 4.9, 2-H), 5.87–5.91 (1 H, m, 3-H), 7.27–7.61 (9 H, m, Ph) and 7.86–8.09 (6 H, m, Ph); δ_C(75.5 MHz; $CDCl_3$) 25.3 and 26.7 (2 C, Me), 64.7 (C-3′), 66.4 (C-5), 69.2 (C-1′), 72.2 (C-2′), 74.4 and 75.4 (C-2 and -3), 79.1 (C-4), 105.6 (C-1), 109.6 (Me_2C), 128.3–133.4 (18 C, Ph) and 165.1, 165.3 and 166.0 (3 C, CO).

(2′S)-2′,3′-*Dihydroxypropyl tri*-O-*benzoyl*-β-D-*ribofuranoside* **16b**. The glycoside **15b** (2.3 g) was converted into the diol **16b** by the same procedures used to synthesize the diol **16a**. Column chromatography [SiO_2; EtOAc–MeOH–light petroleum (4:1:5)] of the product yielded the *diol* **16b** as an oil which readily crystallised (1.36 g, 65%), m.p. 81 °C (from EtOAc–light petroleum); $[\alpha]_D$ +38.4° (*c* 5.5, CH_2Cl_2) (Found: C, 64.9; H, 5.4. $C_{29}H_{28}O_{10}$ requires C, 64.9; H, 5.3%); δ_H(300 MHz; $CDCl_3$) 3.52–3.88 (5 H, m, 1′- and 3′-H_2 and 2′-H), 4.56–4.77 (3 H, m, 4-H and 5-H_2), 5.30 (1 H, s, 1-H), 5.71 (1 H, m, 2-H), 5.84 (1 H, m, 3-H), 7.26–7.60 (9 H, m, Ph) and 7.88–8.08 (6 H, m, Ph); δ_C(75.5 MHz; $CDCl_3$) 63.2 (C-3′), 64.7 (C-5), 70.1 (C-1′), 70.6 (C-2′), 72.2 (C-3), 75.5 (C-2), 79.3 (C-4), 106.2 (C-1), 128.35–133.5 (18 C, Ph) and 165.3, 165.4 and 166.2 (3 C, CO).

(2′R)-3′-*Bromo*-2′-*hydroxypropyl tri*-O-*benzoyl*-β-D-*ribofuranoside* **18b**. The diol **16b** (1.15 g, 2.15 mmol) and carbon tetrabromide (1.43 g, 4.3 mmol) were dissolved in dry methylene dichloride, and triphenylphosphine (780 mg, 3.0 mmol) was added portionwise to the stirred mixture during 1 h (room temp.). As for the synthesis of the bromide **18a**, the reaction was monitored by analytical TLC, and when the yield of desired product was judged to be at a maximum (105 min) the reaction was quenched by the addition of methanol. After removal of solvent by evaporation, the residue was subjected to column chromatography [SiO_2; EtOAc–light petroleum (4:6)] to yield the *monobromide* **18b** as an oil which crystallised readily on storage (797 mg, 62%), m.p. 96 °C (from EtOAc–light petroleum); $[\alpha]_D$ +33.7° (*c* 4.5, CH_2Cl_2) (Found: C, 58.4; H, 4.7. $C_{29}H_{27}BrO_9$ requires C, 58.1; H, 4.5%); δ_H(300 MHz; $CDCl_3$) 3.36 (1 H, dd, $J_{3',3'}$ 10.4, $J_{2',3'}$ 5.7, 3′-H), 3.44 (1 H, dd, $J_{3',3'}$ 10.4, $J_{2',3'}$ 5.3, 3′-H), 3.67–3.72 (1 H, m) and 3.85–3.93 (2 H, m, 1′-, 1′- and 2′-H), 4.54–4.61 (1 H, m) and 4.73–4.78 (2 H, m, 4-, 5- and 5-H), 5.32 (1 H, s, 1-H), 5.71 (1 H, m, 2-H), 5.84 (1 H, m, 3-H), 7.26–7.61 (9 H, m, Ph) and 7.88–8.10 (6 H, m, Ph); δ_C(75.5 MHz; $CDCl_3$) 34.3 (C-3′), 64.5 (C-5), 69.7 (C-2′), 70.6 (C-1′), 72.1 (C-3), 75.4 (C-2), 79.4 (C-4), 106.2 (C-1), 128.4–133.6 (18 C, Ph) and 165.2, 165.4 and 166.1 (3 C, CO).

(2R)-2-*Hydroxy*-3-(β-D-*ribofuranosyloxy*)*propane*-1-*sulphonic acid, sodium salt* **20b**. The bromide **18b** (207 mg, 0.35 mmol) was treated with Amberlite IRA 400 (OH^-) as in the synthesis of the sulphonate **20a**. The epoxide **19b** thus formed [δ_H(80 MHz; D_2O) 2.75–2.81 (1 H, m) and 2.84–2.99 (1 H, m, 3′-H_2), 3.04–4.27 (8 H, m) and 5.07 (1 H, s, 1-H)] was used in the next reaction stage without further purification.

The epoxide **19b** (70 mg) was dissolved in water (5 cm^3) and aq. sodium sulphite (105 mg, 0.83 mmol) in 1 cm^3) was added. The solution was stirred (24 h, room temp.) and then evaporated to dryness. As for the synthesis of the sulphonate **20a**, ^1H NMR examination of the residue indicated complete conversion of the epoxide into the sulphonate **20b**. GPC of the residue (2 ×) yielded the pure *sulphonate* **20b** as a syrup [91 mg, 85% (based on the bromide)], $[\alpha]_D$ −41.0° (*c* 6.1, water) (Found: C, 31.0; H, 5.1. $C_8H_{15}NaO_9S$ requires C, 31.0; H, 4.9%); δ_H(300 MHz; D_2O) 3.04 (1 H, dd, $J_{1,1}$ 14.5, $J_{1,2}$ 7.1, 1-H), 3.14 (1 H, dd, $J_{1,1}$ 14.5, $J_{1,2}$ 5.0, 1-H), 3.56 (1 H, dd, $J_{3,3}$ 10.8, $J_{2,3}$ 6.4, 3-H), 3.64 (1 H, dd, $J_{5',5'}$ 12.6, $J_{4',5'}$ 6.2, 5′-H), 3.82 (1 H, dd, $J_{5',5'}$ 12.6, $J_{4',5'}$ 3.2, 5′-H), 3.86 (1 H, dd, $J_{3,3}$ 10.8, $J_{2,3}$ 3.5, 3-H), 3.99–4.04 (1 H, m, 4′-H), 4.10–4.12 (1 H, m, 2′-H), 4.21–4.29 (2 H, m, 2- and 3′-H) and 5.02 (1 H, s, 1′-H); δ_C(75.5 MHz; D_2O) 53.8

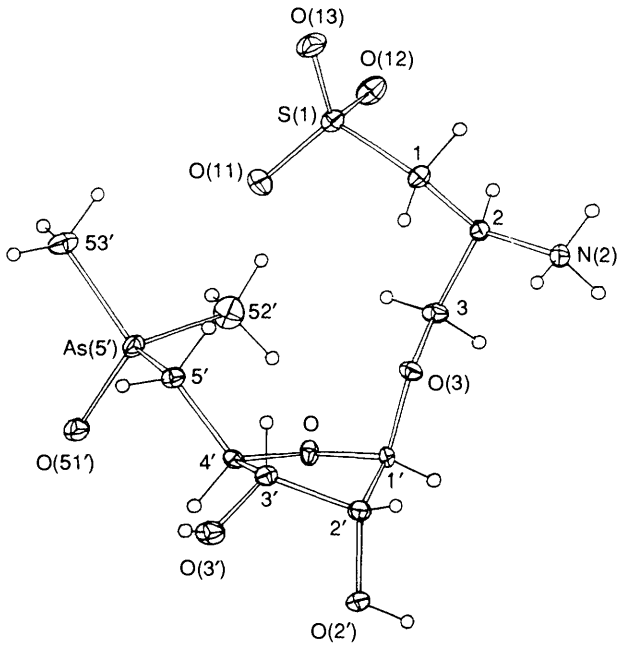


Fig. 2 Projection of the molecule **6a** showing non-hydrogen-atom labelling and 20% thermal ellipsoids. Hydrogen atoms have arbitrary radii of 0.1 Å.

(C-3), 62.7 (C-5'), 66.7 (C-2), 70.8 (C-3'), 71.1 (C-1), 74.4 (C-2'), 82.9 (C-4') and 107.6 (C-1').

Structure Determination of Compound **6a**.—A unique data set was measured at ≈295 K within the limit $2\theta_{max} = 60°$ by using an ENRAF–Nonius CAD-4 diffractometer in conventional $2\theta/\theta$ scan mode (monochromatic Mo-$K\alpha$ radiation, $\lambda = 0.7107_3$ Å). 2567 Independent reflections were measured, 2167 with $I > 3\sigma(I)$ being considered 'observed' and used in the full-matrix least-squares refinement after gaussian absorption correction. Anisotropic thermal parameters were refined for the non-hydrogen atoms; $(x, y, z, U_{iso})_H$ were included at estimated/idealised values after all hydrogen atoms had been located in difference maps. Residuals at convergence were $R = 0.048$, $R_w = 0.058$ for both chiralities; the molecular configuration presented is based on the assumption of a D-ribose moiety. The ring conformation is an envelope, with torsion angles around the ring from O being 28.2(6), −39.7(6), 35.6(6), −20.2(6), −4.6(2)°. Bond lengths and angles are substantially as expected. Statistical weights derivative of $\sigma^2(I) = \sigma^2(I_{diff}) + 0.0004\ \sigma^4(I_{diff})$ were employed. Neutral-atom complex scattering factors were used;[19] computation used the XTAL 2.6 program system[20] implemented by S. R. Hall. Pertinent results are given in Fig. 2 and Table 4.*

Crystal data for compound **6a**. $C_{10}H_{22}AsNO_8S$, M = 391.3. Orthorhombic, space group $P2_12_12_1$ (D_2^4, No. 19), a = 22.396(5), b = 10.226(7), c = 6.952(2) Å, V = 1592 Å^3, D_c (Z = 4) = 1.63 g cm^{-3}, $F(000)$ = 808. μ_{Mo} = 22.0 cm^{-1}, specimen 0.45 × 0.30 × 0.23 mm; $A^*_{min,max}$ = 1.39, 1.81.

* *Supplementary data* (see section 5.6.3 of Instructions for Authors, January issue). H-Atom co-ordinates, thermal parameters, and molecular non-H geometry tables have been deposited at the Cambridge Crystallographic Data Centre.

Acknowledgements

We thank Dr. L. Byrne for NMR spectra and Mrs. J. Moore for technical support. Dr. W. Eichenberger kindly provided ^{13}C NMR data on compound **11**. Financial support from the Australian Research Council is gratefully acknowledged.

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Paper 1/02934G
Received 17*th June* 1991
Accepted 3*rd July* 1991