Arsenic-containing Ribofuranosides: Isolation from Brown Kelp *Ecklonia radiata* and Nuclear Magnetic Resonance Spectra

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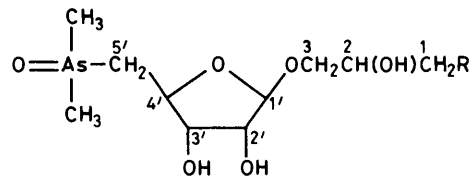
2-Hydroxy-3-sulphopropyl 5-deoxy-5-(dimethylarsinoyl)-β-ribofuranoside (1a) and 2,3-dihydroxypropyl 5-deoxy-5-(dimethylarsinoyl)-β-ribofuranoside (1b) have been isolated from the brown kelp *Ecklonia radiata*. Extraction of a second batch of *Ecklonia* yielded 3-' glycerophosphoryl '-2-hydroxy-1-[5-deoxy-5-(dimethylarsinoyl)-β-ribofuranosyloxy]propane (1c) together with compound (1a). These compounds were identified chiefly by n.m.r. spectroscopy. N.m.r. data for the related (2*S*)-3-[5-deoxy-5-(dimethylarsinoyl)-β-D-ribofuranosyloxy]-2-hydroxypropyl hydrogen sulphate (1d), extracted [together with compound (1b)] from the giant clam *Tridacna maxima*, are presented also.

Discussion

It has been known since the early studies of Jones [1] that marine algae accumulate substantial concentrations of arsenic. In studies to determine the source of arsenic in fauna [2,3] from the unpolluted coastal waters of Western Australia we have examined the arsenical constituents of the brown kelp *Ecklonia radiata* which contains arsenic at a concentration of *ca.* 10 mg kg^{-1} (wet weight).

In a preliminary communication [4] we reported the isolation of 2-hydroxy-3-sulphopropyl 5-deoxy-5-(dimethylarsinoyl)-β-ribofuranoside (1a) † and 2,3-dihydroxypropyl 5-deoxy-5-(dimethylarsinoyl)-β-ribofuranoside (1b) ‡ from *Ecklonia radiata*. We now report that a second extraction of *Ecklonia*, undertaken chiefly to refine and streamline the isolation procedures, yielded the novel 1-[5-deoxy-5-(dimethylarsinoyl)-β-ribofuranosyloxy]-3-' glycerophosphoryl '-2-hydroxypropane-(1c) § together with compound (1a). Compound (1b) was absent from the second extraction.

Freshly collected *Ecklonia* was extracted with methanol and compounds (1a) and (1b) (first extraction) and (1a) and (1c) (second extraction) were isolated from the extracts by chromatography on Sephadex LH-20 and ion-exchange Sephadex resins and by preparative layer chromatography (p.l.c.). Buffered ion-exchange chromatography, close to neutrality, was necessary as decomposition of the compounds to dimethylarsinic acid occurred at extremes of pH. The presence of compound (1c) rather than compound (1b) in the second extraction requires some discussion. Gel-permeation chromatography (g.p.c.) of the crude extract of the first batch separated the arsenical materials into two bands. The faster running band on being re-chromatographed under identical conditions again produced two bands with the same elution positions as before. We assumed, therefore, that the faster running arsenical was slowly decomposing to form the slower running material. This hypothesis was difficult to sustain when the identification of the arsenic compounds in each band suggested that such a conversion was unlikely. The problem was clarified by the extraction of a second batch of *Ecklonia*. In this case the slow-running arsenic material was absent but splitting of the broad arsenic-containing band into two showed, eventually, a substantial quantity of compound (1c) in the faster running portion and only compound (1a) in the slower. In the first extraction any residual compound



(1)

a; R = SO_3H
b; R = OH
c; R = $OP(O^-)OCH_2CH(OH)CH_2OH$
d; R = OSO_3H

(1c) must have been eliminated from compound (1a) during ion-exchange chromatography on Sephadex DEAE. It is evident that hydrolysis of compound (1c) could give rise to compound (1b) and it is possible that a precipitation of polar materials by the addition of acetone to the preliminary methanol extract (carried out for the second extraction only) could have deactivated hydrolytic enzymes that remained active during the early work-up of the first batch. However, it is not easy to see how such enzymes could have remained active when they were presumably separated from their substrate by g.p.c. on Sephadex LH-20. Consequently the reasons for the difference in behaviour of the two batches of *Ecklonia* remains unclear.

Compound (1c) has some analogy to, for example, glycerophosphoryl choline and esterification by long-chain fatty acids of the two free hydroxy groups of the terminal glycerol residue would produce a phospholipid analogous to lecithin. Although lipid-soluble arsenic is present in low levels in *Ecklonia*, other macroalgae (notably *Fucus* spp.) have been shown [5] to contain the bulk of their arsenic as lipid-soluble compounds. Such algae have also been shown [5] to contain water-soluble arsenicals, the reported chromatographic properties of which suggest their possible identity with the ribofuranoside derivatives reported here. It is possible therefore than compound (1c) is an intermediate in the biosynthesis of arsenic-containing phospholipids.

We have previously reported [6] the isolation of the arsenic-containing ribofuranosides (1b) and (1d) from the kidney of the giant clam, *Tridacna maxima*, taken from Shark Bay, Western Australia. As compounds (1b) and (1d) are likely to be present in the clam kidney as metabolic waste products of the unicellular algae (zooxanthellae) present in clam

† 3-[5-Deoxy-5-(dimethylarsinoyl)-β-ribofuranosyloxy]-2-hydroxypropanesulphonic acid.
‡ 3-[5-Deoxy-5-(dimethylarsinoyl)-β-ribofuranosyloxy]-propylene glycol.
§ 3-[5-Deoxy-5-(dimethylarsinoyl)-β-ribofuranosyloxy]-2-hydroxypropyl 2,3-dihydroxypropyl phosphate.

Table 1. ^{1}H N.m.r. data * for compounds (1a—d)

Compound		2 × CH_3	H_A–C–H_B 5′	CH 4′	CH 3′	CH 2′	CH 1′
(1a)	δ	1.75,	2.38,	4.11m	4.11m	3.99	4.88
270 MHz; D_2O		1.72	2.51				
	J_{gem}		13.6				
	J_{vic}		10.1,			4.0	
			3.5				
(1b)	δ	1.70,	2.33,	4.12m	4.12m	3.98	4.86
270 MHz; D_2O		1.68	2.48				
	J_{gem}		13.6				
	J_{vic}		9.9,			3.8	
			3.2				
(1c)	δ	1.67,	2.29,	4.06m	4.06m	3.93	4.89
400 MHz; D_2O		1.64	2.43				
	J_{gem}		14.4				
	J_{vic}		10.0,			4.5,	
			3.4			0.7	
(1d)	δ	1.70,	2.32,	4.05m	4.05m	3.91	4.80
400 MHz; D_2O		1.66	2.46				
	J_{gem}		14.7				
	J_{vic}		10.0,			3.8	
			2.7				

Compound		H_A–C–H_B 1	CH 2	H_A–C–H_B 3	H_A–C–H_B 1″	CH 2″	H_A–C–H_B 3″
(1a)	δ	2.89,	4.11m	3.49,			
270 MHz; D_2O		3.00		3.65			
	J_{gem}	14.6		10.4			
	J_{vic}	6.8,		3.5,			
		5.7		5.5			
(1b)	δ	3.43,	3.75m	3.46,			
270 MHz; D_2O		3.50		3.62			
	J_{gem}	11.6		10.5			
	J_{vic}	6.2,		2.7,			
		4.9		6.2			
(1c)	δ	3.68m	3.82m	3.42,	3.68m	3.68m	3.39,
400 MHz; D_2O				3.58			3.47
	J_{gem}			10.4			11.2
	J_{vic}			4.0,			6.0,
				5.6			4.4
(1d)	δ	3.84m	3.84m	3.38,			
400 MHz; D_2O				3.60			
	J_{gem}			10.5			
	J_{vic}			3.5,			
				4.5			

* Chemical shifts are reported relative to internal HOD which is taken as δ 4.60 relative to $SiMe_4$. Some correction of data already published [4] for compounds (1a) and (1b) was necessary. Integrations of the signals were in accord with their assignments. *J* in Hz.

tissues,[7] we suggest that the biosynthesis of arsenic-containing ribofuranosides and possibly phospholipids based upon them is a general response of algae to oceanic arsenate.

Compounds (1a—d) are likely to be the results of detoxification processes but, because of their low concentrations, are unlikely to have other metabolic significance. However, algae provide a substantial contribution to human nutrition, particularly in Japan for example, and consequently the toxicological effects of arsenic-containing ribofuranosides on human consumers will be of interest. The mechanism of the biosynthesis by algae of the arsenic-containing ribofuranosides from absorbed oceanic arsenate remains to be elucidated. Presumably the pathway involves methylation by *S*-adenosylmethionine following reduction of arsenate to arsenite.[8] *S*-Adenosylmethionine is also possibly responsible for the transfer of an adenosyl group to arsenic and is thus the likely source of the β-ribo system in the arsenic-containing sugars.

The structures of compounds (1a—d) were determined chiefly by ^{1}H and ^{13}C n.m.r. spectroscopy. Although information on compounds (1a), (1b), and (1d) has previously been briefly reported it is convenient to consider here n.m.r. data for the group of compounds as a whole.

Initial structural assignments were made for compounds (1a) and (1b) only, and were based upon comparison of the ^{1}H n.m.r. spectra of both compounds together with the ^{13}C n.m.r. spectrum of compound (1a). The 270 MHz ^{1}H n.m.r. spectra (Table 1) indicated a $C_{10}H_{17}$ skeleton (two CH_3 groups, three CH_2, five CH) for each compound and showed that they were closely related. Both contained signals [δ 1.75 (3 H) and 1.72 (3 H) for compound (1a); δ 1.70 (3 H) and 1.68 (3 H) for compound (1b)] readily assigned to methyl groups on arsenic (see data on model compounds in Table 4 and discussion below), and an eight-line system centred at δ 2.45 [compound (1a)] and δ 2.41 [compound (1b)] which could be attributed to a methylene group attached to arsenic, and both contained four low-field signals, due to methine protons, indicating the presence of a sugar system (ref. 9 and

Table 2. ^{1}H N.m.r. decoupling experiments *

Compound	Position of irradiation (δ)	Results of irradiation
(1a)	4.11	AB qs centred at δ 2.44 (J_{gem} 13.6 Hz, $\Delta\delta_{AB}$ 0.13 p.p.m.)
		δ 3.57 (J_{gem} 10.4 Hz, $\Delta\delta_{AB}$ 0.16 p.p.m.)
		δ 2.94 (J_{gem} 14.6 Hz, $\Delta\delta_{AB}$ 0.11 p.p.m.)
(1b)	4.12	AB q centred at δ 2.40 (J_{gem} 13.6 Hz, $\Delta\delta_{AB}$ 0.15 p.p.m.)
	3.75	AB qs centred at δ 3.54 (J_{gem} 10.5 Hz, $\Delta\delta_{AB}$ 0.16 p.p.m.)
		δ 3.46 (J_{gem} 11.6 Hz, $\Delta\delta_{AB}$ 0.07 p.p.m.)
(1c)	3.82	AB q centred at δ 3.50 (J_{gem} 10.4 Hz, $\Delta\delta_{AB}$ 0.16 p.p.m.)
(1d)	4.05	AB q centred at δ 2.39 (J_{gem} 14.7 Hz, $\Delta\delta_{AB}$ 0.14 p.p.m.)
	3.84	AB q centred at δ 3.49 (J_{gem} 10.5 Hz, $\Delta\delta_{AB}$ 0.22 p.p.m.)

* Only results of the most informative decoupling experiments are shown. In each case no other part of the spectrum was altered. Subsequent irradiation at the chemical shifts of the simplified signals in these experiments resulted in alterations to the spectra at the original positions of irradiation, but such changes were not amenable to simple analysis. Irradiation at δ 4.06 and δ 3.68 for compound (1c) might have been expected to produce structural information but such irradiations were not carried out.

Table 3. ^{13}C N.m.r. data * for compounds (1a—d)

Compound	2 × CH_3	C-5′	C-4′	C-3′	C-2′	C-1′
(1a) 20.1 MHz; D_2O	14.7, 15.1	36.5	77.3	76.2	74.8	108.2
(1b) 20.1 MHz; CD_3OD	15.0, 15.7	38.0	78.4	77.6	76.2	109.4
(1c) 20.1 MHz; D_2O	14.2, 14.5	36.1	76.8	75.7	74.3	107.6
(1d) 90 MHz; D_2O	15.0, 15.2	36.7	77.5	76.6	75.1	108.3

Compound	C-1	C-2	C-3	C-1″	C-2″	C-3″
(1a) 20.1 MHz; D_2O	54.3	66.9	71.3			
(1b) 20.1 MHz; CD_3OD	64.1	72.1	70.4			
(1c) 20.1 MHz; D_2O	66.5d [a] $J_{(P-C)}$ 5.6 Hz	69.0d $J_{(P-C)}$ 8 Hz	68.6	66.3d [a] $J_{(P-C)}$ 5.6 Hz	70.8d $J_{(P-C)}$ 8 Hz	62.3
(1d) 90 MHz; D_2O	68.5 [b]	68.8 [b]	69.2 [b]			

* Chemical shifts are reported relative to external methanol at δ_C 49.00 p.p.m. [compound (1a)], internal methanol at δ_C 49.00 p.p.m. [compounds (1b) and (1c)], and internal dioxane at δ_C 67.40 p.p.m. [compound (1d)]. CH_3, CH_2, and CH groups were identified by off-resonance decoupling [compounds (1a), (1b), (1d)] and by gated spin-echo techniques [compound (1c)].

[a] The assignments of these signals are uncertain and may be reversed. [b] The assignments of these signals are uncertain in relation to each other.

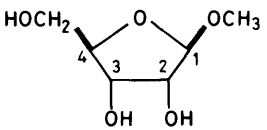
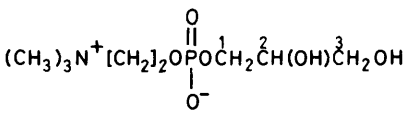
Table 4). One methine signal was, in each case, appreciably more deshielded than the others [δ 4.88, compound (1a); δ 4.86, compound (1b)] and was attributable to the sugar anomeric proton.[9] The observation that this signal was a singlet for both compounds was of diagnostic value [10] in determining the stereochemistry of the sugar system when considered together with the ^{13}C n.m.r. spectrum of compound (1a).

The ^{1}H n.m.r. spectra of compounds (1a) and (1b) differed substantially in only two respects. First, the eight-line (AB part of an ABX system) centred at δ 2.95 in the spectrum of compound (1a) was shifted downfield and centred at δ 3.47 in the spectrum of compound (1b); second, the three-proton multiplet centred at δ 4.11 in the spectrum of compound (1a) was replaced by a two-proton multiplet centred at δ 4.12 and a one-proton multiplet at δ 3.75 in the spectrum of compound (1b). Irradiation (Table 2) at δ 4.11 [compound (1a)] caused collapse of all three eight-line systems (centred at δ 2.45, 2.95, and 3.57) to AB quartets. Structural information obtained was limited, we assumed by coincidence of methine signals at δ 4.11. However, irradiation of the one-proton multiplet at δ 3.75 for compound (1b) resulted in the collapse to AB quartets of the two eight-line systems centred at δ 3.47 and 3.54. The eight-line system centred at δ 2.41 and attributed to a methylene group attached to arsenic (see above) was unaffected. From these observations we inferred that the differences between compounds (1a) and (1b) lay in the substituents of a three-carbon ($^-CH_2CHCH_2^-$) moiety giving rise to signals centred at δ 2.95 (2 H), 4.11 (1 H), and 3.57 (2 H) for compound (1a), and δ 3.47 (2 H), 3.75 (1 H), and 3.54 (2 H) for compound (1b).

The ^{13}C n.m.r. data for compound (1a) (Table 3) confirmed the $C_{10}H_{17}$ skeleton (two CH_3 groups, three CH_2, five CH). Shieldings of the two methyl groups and one methylene were consistent with their being bound to arsenic. The four downfield methine signals demonstrated the presence of a furanoside sugar system.[11]

The lowest-field methine (δ_C 108.2 p.p.m.) was attributed to the anomeric carbon and was accommodated by a furanoside system with a transoid substitution arrangement at C-1 and C-2.[11] This conclusion was supported by the absence of splitting in the signal attributed to the anomeric proton in the

Table 4. ^1H and ^{13}C N.m.r. data for model compounds

Compound	^1H N.m.r. chemical shifts relative to $SiMe_4$: Methyl groups	Methylene or methine 1	2	3	^{13}C N.m.r. chemical shifts relative to $SiMe_4$: Methyl groups	Methylene, methine, or carbonyl 1	2	3	4	Reference to n.m.r. data or synthetic method
$O{=}As(CH_3)_2{}^1CH_2{}^2CH_2OH$ *	1.72	2.36	3.90		14.2	33.7	55.5			(^1H and synthesis) [a]
$O{=}As(CH_3)_3$	1.73									*b*
$(CH_3)_3As^+{}^1CH_2{}^2CO_2$ *	1.87	3.28			7.6	33.8	171.6			Ref. 2 (^1H and synthesis)
$(CH_3)_2AsO_2H$ *	1.90				16.8					(^1H) [b]
$(CH_3)_3As$	0.91				11.4					(^1H),[b] (^{13}C) [c]
$O{=}As(OH)_2{}^1CH_2{}^2CH_2OH$ *·†		2.79	3.95			36.3	55.9			(Synthesis) [d]
		Anomeric protons in sugar systems 4.5—5.5	Methines in sugar systems 3.0—4.5			108.0	74.3	70.9	83.0	Ref. 9 (^1H), ref. 11 (^{13}C)
$HO^3CH_2{}^2CH(OH)^1CH_2SO_3H$ *·†		3.02	4.09	3.58		53.7	68.1	64.7		Ref. 16 (Synthesis)
$HO^1CH_2{}^2CH(OH)^1CH_2OH$		3.61	3.79			64.5	73.7			(^1H),[e] (^{13}C) [f]
		3.90	3.90	3.65		67.6 $J_{(P-C)}$ 6 Hz	71.7 $J_{(P-C)}$ 8 Hz	63.2		Ref. 14 (^1H and ^{13}C)

* ^{13}C N.m.r. spectra were recorded in D_2O with methanol (δ_C 49.00 p.p.m.) as internal standard. † ^1H N.m.r. spectra were recorded in D_2O with $SiMe_4$ as internal standard. Conditions for recording of other spectra are given in the original references.

[a] J. S. Edmonds, K. A. Francesconi, and J. A. Hansen, *Experientia*, 1982, **38**, 643. [b] G. E. Parris and F. E. Brinckman, *Environ. Sci. Technol.*, 1976, **10**, 1128. [c] G. Balimann and P. S. Pregosin, *Helv. Chim. Acta*, 1975, **58**, 1913. [d] R. H. Edee, *J. Am. Chem. Soc.*, 1928, **50**, 1394. [e] Spectrum 395 in *Varian NMR Spectra Catalog*, N. S. Bhacca *et al.*, Varian Associates, 1963. [f] J. B. Stothers, ' Carbon-13 NMR Spectroscopy,' Academic Press, New York and London, 1972, p. 143.

^{1}H n.m.r. spectrum of compound (1a).[10] The observation that the eight-line system (^{1}H n.m.r.) assigned to a methylene on arsenic [δ 2.45, compound (1a); δ 2.41, compound (1b)] collapsed to an AB quartet upon irradiation of a methine included in the sugar system [δ 4.11 for compound (1a); δ 4.12 for compound (1b)] indicated the presence of a 5-deoxyfuranoside with arsenic bonded to C-5 of the sugar residue.

Taken together the four low-field methines in the ^{13}C n.m.r. spectrum of compound (1a) were best accommodated by a β-ribo system when the effects on C-4 and C-3 of the postulated 5-deoxyfuranoside structure had been taken into account. It was assumed that the less electronegative substituent ($^-CH_2As$) at the 5 position would produce effects on the shieldings of C-3 and C-4 analogous to (for example) the differences between the shielding of C-4 and C-5 in glucose and 6-deoxyglucose.[12] Thus, differences in shieldings of *ca.* 5 p.p.m. for C-3 and C-4 (in opposite directions) when compared with methyl β-ribofuranoside were accommodated.

The chemical shifts (^{1}H and ^{13}C n.m.r.) of the methyl and methylene substituents at the arsenic atom were consistent with a trialkylated arsine oxide, *i.e.* with arsenic in the pentavalent state, and were sufficiently different from those expected for an arsine (arsenic in trivalent state) for the latter to be disregarded (see data for model compounds in Table 4). The expected instability of an arsine in aqueous media would also render its presence unlikely. The formulation of compound (1a) as an arsine oxide was reinforced by the presence of a strong absorbance at 845 cm^{-1} in the i.r. spectrum.[13]

Compound (1a) gave a positive test for sulphur but a negative test for sulphate. Analysis revealed one atom of sulphur per molecule. Sulphur was absent from compound (1b). The identity of the three-carbon side-chain as a 1-sulpho-2-hydroxypropoxy unit in compound (1a) and a 2,3-dihydroxypropoxy moiety in compound (1b) was inferred from n.m.r. data and supported by t.l.c. [compound (1a)] and high-pressure liquid chromatography (h.p.l.c.) [compound (1b)] of acid hydrolysates.

On the basis of the above, compounds (1a) and (1b) were formulated as shown.

Sulphur was absent from compound (1c) but one atom of phosphorus per molecule was present. The ^{1}H n.m.r. spectrum of compound (1c) also contained signals attributable to a 5-deoxy-5-(dimethylarsinoyl)-β-ribofuranosyl system but it was apparent that five non-exchangeable additional protons were present when compared with compounds (1a), (1b), and (1d). The ^{13}C n.m.r. spectrum (Figure) revealed thirteen carbons in the molecule and splitting due to carbon–phosphorus coupling was evident for four signals. The coupling constants indicated that two carbons (shown by gated spin-echo techniques to be methylene carbons) were α to a phosphate group and two (methines) β to phosphate.[14] Shieldings of seven carbons were consistent with the presence of a 5-deoxy-5-(dimethylarsinoyl)-β-ribofuranosyl system and those of the remaining six carbons with their formulation as a 3-' glycerophosphoryl '-2-hydroxypropyl moiety. Low-resolution field desorption mass spectrometric examination of compound (1c) provided confirmation of the $C_{13}H_{27}AsO_{12}P$ formulation.

Compounds (1b) and (1d) were subsequently extracted from the kidneys of the giant clam *Tridacna maxima* as has been previously described.[6] Compound (1b) from this source was identified by comparison of its ^{1}H n.m.r. spectrum and t.l.c. properties with those of a sample isolated from *Ecklonia*. Compound (1d) was identified by inspection of its ^{1}H and ^{13}C n.m.r. spectra and the structure was confirmed by *X*-ray diffraction which also revealed the absolute configuration of the molecule.[6] Although the stereochemistry of the ribose system in compounds (1a—c) remains undetermined, it is likely, by analogy, to be the same as in compound (1d), *i.e.* D-ribose.

Experimental

N.m.r. spectra were recorded on Bruker instruments at 270 and 400 MHz (^{1}H) and 20.1 and 90 MHz (^{13}C). I.r. spectra were recorded as films on a thallium selenide plate using a Pye Unicam SP2000 spectrophotometer. Arsenic analyses were carried out and arsenic located in chromatographic fractions by vapour generation or electrothermal atomic absorption spectrophotometry using Varian instrumentation. C and H Microanalyses were performed by the Australian Microanalytical service. ' Evaporation ' refers to removal of solvent under reduced pressure at 40 °C on a rotary evaporator. Tris refers to aminotris(hydroxymethyl)methane. After column chromatography on Sephadex DEAE, Tris buffer was removed from eluted material in each case by passage through a Sephadex LH-20–water column. All cellulose p.l.c. and t.l.c. plates were developed with butan-1-ol–acetic acid–water (60 : 15 : 25) unless otherwise stated.

Two separate batches of *Ecklonia radiata*, collected at different times, were extracted. The second extraction was carried out to refine isolation procedures and provide additional quantities of arsenic-containing compounds. The first extraction had, therefore, more of a trial nature. However, as the results of each extraction differed we have reported experimental details of both extractions.

First Extraction and Isolation of Arsenic Compounds from Ecklonia radiata.—*Preliminary work-up.* A quantity (11 kg; *ca.* 0.001% As) of fresh *Ecklonia radiata* was collected from Whitford Beach, 20 km north of Perth, Western Australia in August 1979. [The kelp had been dislodged from underwater reefs and deposited on the beach during rough weather the previous day.] After being sorted it was extracted with methanol (3 × 7 l). The extract, on evaporation, yielded a solid black gum (750 g; 70 mg As). This material was re-extracted with methanol (3 × 1 l), the mixture was filtered, the filtrate evaporated, and the resulting residue partitioned between water and chloroform. The dark green organic layer (0.8 mg As) was not further examined. The aqueous layer was evaporated to yield a solid black gum (297 g; 62.0 mg As) which was dissolved in methanol (1 l) and 800 ml (in 16 equal portions of 50 ml) was subjected to g.p.c. on Sephadex LH-20 (540 × 60 mm column; methanol as eluant). The arsenic-containing fractions separated into two distinct bands after elution with 750—875 ml and 900—1 050 ml of eluant. Re-chromatography, under the same conditions, of the latter band showed no further separation of arsenic-containing material. However, re-chromatography of the first band separated the arsenic-containing material into two bands (750—875 ml and 900—1 050 ml of eluant). Some decomposition of at least one arsenical was therefore apparent (see Discussion section).

Isolation of compound (1b). The fractions constituting the slower band (900—1 050 ml) were bulked and the methanol was removed by evaporation. The resulting syrup was partitioned between phenol and water which removed most of the more polar impurities. After recovery the phenol-soluble material was applied to a Sephadex DEAE A25 column (equilibrated with 0.05M Tris buffer at pH 7.6) and eluted with Tris at the same concentration and pH (isocratic elution). All of the arsenic was recovered at the void volume (1 g; 7.9 mg As). P.l.c. on cellulose produced one major arsenic-containing fraction (200 mg; 5.6 mg As; R_F 0.5) and two minor ones (110 mg; 0.2 mg As; R_F 0.25 and 60 mg; 0.1 mg As;

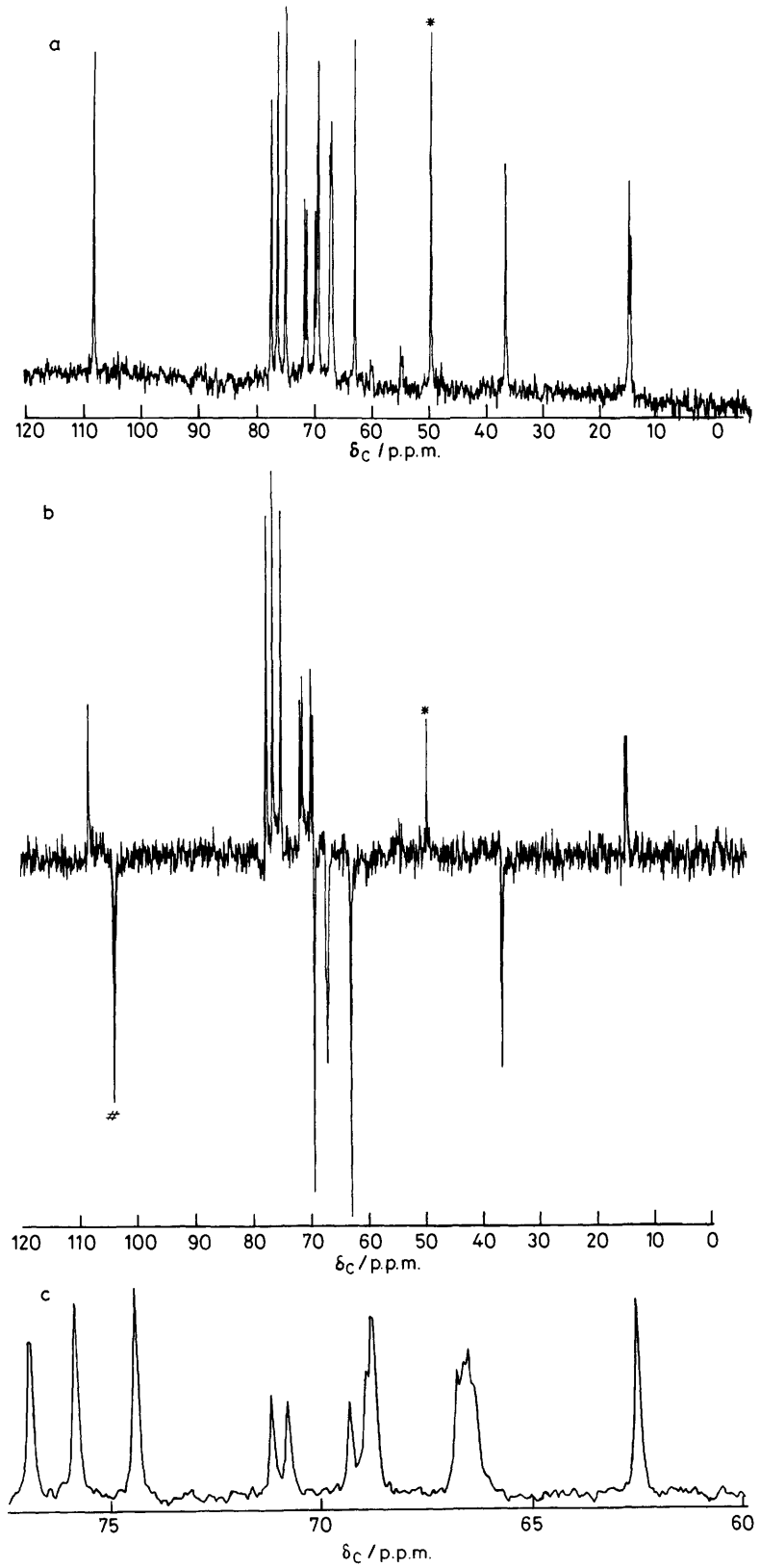


Figure. 20.1 MHz ^{13}C N.m.r. spectrum of compound (1c). * Indicates signals due to internal methanol standard. a, Broad-band decoupled spectrum. b, Gated spin-echo spectrum with methyl and methine signals displayed upwards and methylene signals downwards. # Indicates a spurious instrument-produced signal. c, Expansion of part of spectrum displayed in a

R_F 0.6). The major fraction was subjected to ion-exchange chromatography (Sephadex CM-25; phosphate buffer pH 6; isocratic elution) and p.l.c. on cellulose (R_F 0.5) and silica with propan-2-ol–ethyl acetate–water (7 : 1 : 2) as developer (R_F 0.14) which concentrated the arsenical to a syrup (16.9 mg; 2.4 mg As; 14.2% As). H.p.l.c. (C_{18} reverse-phase column; water–methanol 4 : 1) removed minor impurities from this material to yield a syrup (5.0 mg; 1.0 mg As; 20% As), δ_H (270 MHz; D_2O) see Table 1. From these data we assign structure (1b) to this compound. The identification of the side-chain as a 2,3-dihydroxypropoxy group was confirmed by acid hydrolysis

Acid hydrolysis of compound (1b). A small sample (400 μg) of compound (1b) together with 2M HCl (100 μl) were maintained at 100 °C for 20 h in a sealed tube. The HCl was then removed by evaporation and the residue was subjected to analytical h.p.l.c. (C_{18} reverse-phase column; water–methanol 4 : 1). 1,2,3-Trihydroxypropane (*ca.* 40 μg) was identified by retention time and by co-chromatography with standard material.

Isolation of compound (1a). Fractions constituting the faster running band from the Sephadex LH-20–methanol column (750—875 ml of eluant) were bulked and, after removal of solvent by evaporation, were subjected to repeated ion-exchange chromatography (Sephadex DEAE A25; 0.05M Tris; pH 7.6; isocratic elution). The arsenic-containing fractions were recovered as a syrup (232.6 mg; 8.7 mg As) after removal of Tris. P.l.c. on cellulose followed by h.p.l.c. (C_{18} reverse-phase column; water–methanol 4 : 1) yielded a syrup (30 mg; 5.25 mg As; 17.5% As). Analysis of this material for sulphate by the method of Dodgson [15] after perchloric acid oxidation revealed the presence of 7.5% sulphur as sulphate. Without perchloric acid oxidation no sulphate was detected (Found: C, 26.9; H, 5.3; As, 17.5; S, 7.5. $C_{10}H_{21}AsO_9S.3H_2O$ requires C, 26.9; H, 6.0; As, 16.8; S, 7.2%); δ_H (270 MHz; D_2O) see Table 1; $\nu_{max.}$ (film) 3 400, 2 940, 1 650, 1 420, 1 165, 1 135, 1 040, 967, and 845 cm^{-1}. From these data we assign structure (1a) to this compound. The identification of the side-chain as a 2-hydroxy-3-sulphopropoxy group was confirmed by acid hydrolysis.

Acid hydrolysis of compound (1a). As for compound (1b) a small sample (400 μg) of compound (1a) was hydrolysed with HCl. 2,3-Dihydroxypropanesulphonic acid was identified in the hydrolysate by analytical t.l.c. and co-chromatography with standard material synthesized by the method of Friese.[16]

Second Extraction and Isolation of Arsenic Compounds from Ecklonia radiata.—*Preliminary work-up.* A quantity (32.7 kg) of fresh *Ecklonia radiata* was collected from the shoreline north of Waterman Bay, Western Australia in March 1981. [Again the kelp had been dislodged from reefs and deposited on the shore during rough weather the previous day.] After being sorted it was packed into a large PVC cylinder (1.8 m × 230 mm) and methanol (in 10 l batches) was allowed to percolate slowly through it, the flow of methanol being controlled by a stopcock at the base of the cylinder. The eluate was recycled through the column ten times for each of four separate 10 l batches of methanol. The fourth extract contained less than 5% of the total arsenic extracted and consequently a fifth extraction was not carried out. Initial work-up procedures for the extracts were designed to provide a crude separation of soluble material on the basis of their polarities. The first fractions contained much water and the more polar solutes predominated; the latter fractions had a higher content of lipid materials. Portions (*ca.* 3 l) of the methanol eluate from the large extraction column were subjected to the following general procedure. Solvent was removed by evaporation to give a solid brown gum (typically *ca.* 190 g; 18 mg As). This material was refluxed with methanol (1 l) and the resulting mixture was filtered to yield a buff solid [typically *ca.* 100 g; 2.5 mg As; fraction (i)], and a dark filtrate which was concentrated to *ca.* 300 ml and poured into acetone (1.5 l). After 2 h the mixture was filtered to yield a sticky solid [typically *ca.* 45 g; 15 mg As; fraction (ii)] and a filtrate which, on evaporation, gave a black syrup [typically *ca.* 50 g; 3 mg As; fraction (iii)]. T.l.c. examination of this syrup showed that the arsenic was present in the polar lipid fraction. Although the above figures are for a typical batch, the nature of the arsenic distribution in the three fractions changed as more methanol was taken from the large extraction column, with lipid-type arsenic (not precipitated by acetone) providing a larger contribution to later fractions. After all the extract had been processed in this way the three fractions contained (i) 960 g (33.3 mg As); (ii) 613 g (187 mg As); (iii) 500 g (36 mg As). Fraction (iii) (lipid-type arsenic) was not further examined in this study. Fraction (i) was extracted again with methanol and t.l.c. examination of the extract indicated the presence of the same arsenic compounds as in fraction (ii). It was not considered productive to extract exhaustively fraction (i) and consequently it was discarded. The isolation of arsenical materials from fraction (ii) is described below.

Chromatographic isolation of arsenic compounds. Fraction (ii) (613 g; 187 mg As) was dissolved in water and the volume adjusted to 2.5 l. Portions (100 ml) were subjected to g.p.c. on a Sephadex LH-20–H_2O column (860 × 55 mm). The arsenic-containing fractions from all column runs were bulked and evaporated to yield a brown syrup (175 g). This material was dissolved in water and the volume adjusted to 500 ml. Portions (30 ml) were again subjected to g.p.c. on Sephadex LH-20 (860 × 55 mm column; eluant H_2O). The arsenic-containing fractions (eluted at 800—1 200 ml eluant) were divided into two groups (800—1 050 and 1 050—1 200 ml eluant), bulked, and evaporated to dryness to yield pale brown syrups. The first of these (800—1 050 ml eluant; 73 g; 55 mg As) was dissolved in Tris buffer (pH 8.8; 0.05M; 500 ml) and isocratically chromatographed on a Sephadex DEAE A25 column (500 × 60 mm; equilibrated with 0.05M Tris buffer at pH 8.8). Arsenic-containing material was eluted at 5—20.5 l eluant and the arsenic-containing fractions were split into two groups, 5—14 l eluant designated fraction A1, and 14—20.5 l, fraction A2 and, after removal of Tris, yielded syrups (A1, 3.8 g; 50 mg As; A2, 200 mg; 5 mg As). The second batch of arsenic-containing material from the Sephadex LH-20/H_2O column (eluted at 1 050—1 200 ml, see above; 50.6 g; 37 mg As) was dissolved in Tris buffer (pH 8.8; 0.05M; 350 ml) and chromatographed on Sephadex DEAE A25 as described above; the arsenic-containing fractions were eluted at 7.5—12.5 l eluant. After removal of Tris from the arsenic-containing fractions, a syrup was obtained (4.6 g; 37 mg As; fraction B).

Fraction A1 was dissolved in Tris buffer (0.05M; pH 8.8; 40 ml) and isocratically chromatographed on Sephadex DEAE A25 (500 × 60 mm column) as before. The arsenical material was eluted in two distinct bands (at 5.5—7.1 l and 7.1—9.8 l eluant) apparently corresponding to two separate arsenic compounds. Tris was removed from each of these to yield syrups (460 mg; 15 mg As; 5.5—7.1 l fraction, and 2.3 g; 32 mg As; 7.1—9.8 l fraction). Fractions A2 and B were combined, dissolved in Tris buffer (0.05M; pH 8.8; 30 ml), and isocratically chromatographed on Sephadex DEAE A25 (500 × 60 mm column) as above. Arsenical material was eluted as a single band (7.8—10 l eluant). Tris was removed to yield a syrup (2.0 g; 42 mg As). At this stage each of the three arsenic-containing syrups were examined by analytical t.l.c. on 0.1 mm cellulose layers. A single arsenic-

containing band was revealed in each case: 5.5—7.1 l fraction (460 mg; 15 mg As), R_F 0.19; 7.1—9.8 l fraction (2.36 g; 32 mg As), R_F 0.22; 7.8—10 l fraction (2.0 g; 42 mg As), R_F 0.22. It was evident that the latter two fractions contained the same arsenic compound and only that containing the higher concentration was worked up further in this study.

Isolation of compound (1c). The first of these fractions (460 mg; 15 mg As) was dissolved in Tris buffer (0.05M; pH 7.2; 6 ml) and isocratically chromatographed on Sephadex DEAE A25 (830 × 28 mm column equilibrated at pH 7.2 with 0.05M Tris buffer). The arsenic-containing fractions (eluted as a single band at 1 010—1 250 ml eluant) were combined and Tris was removed to yield a syrup (170 mg; 15 mg As). This material was dissolved in water and subjected to p.l.c. on seven 200 × 200 × 0.75 mm cellulose layers (Whatman CC41). The most concentrated region of the single arsenic-containing band (R_F 0.18—0.21) was scraped off, extracted with water, and recovered by filtration. After concentration the aqueous solution was subjected to g.p.c. (small Sephadex LH-20–H_2O column) to yield, after evaporation, a syrup (55 mg; 7.2 mg As). The analytical method of Chen *et al.*[17] revealed the presence of 4.6% phosphorus; δ_H (400 MHz; D_2O) see Table 1. δ_C (20.1 MHz; D_2O) see Table 3 and the Figure. Field-desorption (f.d.) m.s. (emitter current 14 mA) produced a molecular ion at *m/z* 527 ($C_{13}H_{27}AsO_{12}$ P + 2Na),$^+$ and a doubly charged ion at *m/z* 275 ($C_{13}H_{27}AsO_{12}P$ + 3Na)$^{2+}$. From these data we assign structure (1c) to this compound.

Isolation of compound (1a). The arsenic-containing material eluted from the Sephadex DEAE column at 7.8—10 l (2.0 g; 42 mg As; R_F 0.22) was dissolved in water (10 ml) and two separate portions (5 ml each) were chromatographed on a Sephadex DEAE column (830 × 28 mm; 0.05M Tris; pH 7.2; isocratic elution). The peak arsenic-containing fractions were bulked and, after removal of Tris, evaporated to yield a syrup (500 mg; 25 mg As). A portion of this syrup (250 mg; 12 mg As) was dissolved in water and subjected to p.l.c. (seven 200 × 200 × 1.0 mm cellulose layers). The band corresponding to the arsenic compound (R_F 0.18—0.36) was scraped off, extracted with water, and the solution was filtered. After evaporation and final clean-up by passage through a small Sephadex LH-20–H_2O column the arsenic compound was obtained as a syrup (84.7 mg; 11.5 mg As). It was shown, by co-t.l.c. on cellulose and comparison of ^{1}H n.m.r. spectra, to be identical with the acidic arsenic-containing sugar (1a) isolated from the first batch of *Ecklonia radiata*.

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