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THE CARBOHYDRATES OF THE GREEN SEAWEEDS *Urospora wormskioldii* AND *Codiolum pusillum**†

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ABSTRACT

Urospora wormskioldii and *Codiolum pusillum* are different life forms of this arctic alga. They both metabolise D-glucose, D-fructose, sucrose, *myo*-inositol, glyceric acid, and malto-oligosaccharides. In *Codiolum*, 1,3-linked D-glucose and L-rhamnose oligosaccharides were also present. The major polysaccharide extracted by water from both forms is a polydisperse, sulphated glucuronoxylorhamnan. Polysaccharides containing 1,3-, 1,4-, and triply linked D-glucose residues were also isolated from the aqueous extracts. Pure amylopectin-type polysaccharides were isolated from acid extracts of both forms of the weed. The major difference between the two forms was the presence in *Codiolum* of a sulphated (1→4)-linked β-D-mannan branched at C-6 and sulphated at C-2. The similarities and differences of the carbohydrates with those of *Urospora penicilliformis* and other green seaweeds are discussed.

INTRODUCTION

The artic species *Urospora wormskioldii* has cells (normally up to 15 cm × 4 mm) that are much larger than those of the two British species *U. bangioides* and *U. penicilliformis*. The last species is the only one for which any chemical studies of carbohydrates have been reported[1]. *Codiolum pusillum* is a unicellular stage in the life history of *U. wormskioldii* (hereinafter discussed as two algae). Cultivation has shown that temperature has a striking influence on the production of the different forms[2], and this results in a seasonal rhythm in the natural environment. Filaments of *U. wormskioldii* appear in March–May, and fertile *Codiolum* in late summer–autumn.

The samples of *U. wormskioldii* and of *Codiolum* investigated in this communication were collected by Mr. R. Hooper of the Memorial University, Newfoundland, the former in April 1973, and the latter from Bulls Bay, South of St. John's, New-

*Dedicated to the memory of Sir Edmund Hirst, C.B.E., F.R.S.

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foundland on 22nd August, 1974. Parallel investigations on the two materials were carried out.

RESULTS AND DISCUSSION

Table I gives the respective yields from the two algae on sequential extraction. The relative weights of carbohydrate in the ethanolic extracts and of polysaccharides in the aqueous extracts are approximately the same for both algae. In contrast, the ethanol-soluble material in the aqueous extract of *Codiolum* is two and a half times greater than that of *Urospora*, and the acidic extract five times as large.

TABLE I

YIELD OF CARBOHYDRATES ON SEQUENTIAL EXTRACTION OF THE ALGAE

Extract:	*Ethanolic*	*Aqueous*		*Acid*
		Ethanol soluble	*Ethanol insoluble*	
Urospora wormskioldii				
Weight (mg)	87.8[a]	10.2	97.6	29.2
Percentage of dry-weight of weed	10	1.1	11.2	3.3
Percentage of carbohydrate	54	54	70[b]	72[b]
Codiolum pusillum				
Weight (mg)	352[a]	120	550	770
Percentage of dry-weight of weed	7.4	2.5	11.6	16
Percentage of carbohydrate	42	45	80[b]	82[b]

[a]After removal of chlorophyll by water–toluene partition. [b]Measured on a glucose graph.

Carbohydrates of low molecular weight. — The ethanolic extracts from the two algae contained the same carbohydrates. D-Glucose and fructose were the major components, and smaller amounts of sucrose, *myo*-inositol, glyceric acid, and D-glucose-containing oligosaccharides were separated and characterised. These carbohydrates were the sole constituents of the alcohol-soluble material in the aqueous extract from *Urospora*, but that from *Codiolum* was devoid of monosaccharides. It contained 45% of carbohydrate, 10% of sulphate, and 4.8% of nitrogen, and amino acids were detected in a hydrolysate. The solubility in ethanol indicates that oligosaccharides, rather than polysaccharides, were present. After hydrolysis, D-glucose and L-rhamnose were present in equal amounts. After methylation and hydrolysis, 2,4-di- and 4-*O*-methylrhamnoses, together with smaller amounts of 2,3,4-tri- and 3,4-di-*O*-methylrhamnoses, were characterised. 2,3,4,6-Tetra- and 2,4,6-tri-*O*-methylglucoses, together with trace amounts of 4,6-di-*O*-methylglucose, were also present in this hydrolysate. In view of their similarity to the polysaccharides present in subsequent extracts, it is considered that these oligosaccharides are probably precursors of the larger molecules.

Polysaccharides from the aqueous extracts. — The polysaccharide material

separated from the aqueous extracts of the weeds by precipitation with ethanol (Table I) proved to be complex mixtures. Fractionation on DEAE cellulose separated the neutral from the acidic polymers (Table II); from the *Codiolum* extract, a sulphated mannan was separated from a sulphated glucuronoxylorhamnan by elution of the cellulose column with a KCl gradient (Table III).

TABLE II

FRACTIONATION OF THE WATER-SOLUBLE, ALCOHOL-INSOLUBLE EXTRACTS[a]

Fraction	*Recovery (mg)*		*Carbohydrate content (%)*		*Uronic acid content (%)*		*Sulphate content (%)*	
	a[a]	*b*	*a*	*b*	*a*	*b*	*a*	*b*
Aqueous	5.6	110	70	70	—	—	—	—
0.1M KCl	—	42	—	25		1		15
0.2M KCl	—	53	—	36		2		15
0.3M KCl	51.0	67	70	69	17	13	11	22
0.5MKCl	12.5	50	75	68	13	10		20
M KCl	3.0	10	70	48	[b]	6	[b]	[b]
Total	72.1	332	(= 54 mg)	(= 200 mg)				

[a]a, *Urospora wormskioldii* extract; b, *Codiolum pusillum* extract. [b] Not measured.

TABLE III

SUGARS PRESENT IN THE HYDROLYSATES OF THE FRACTIONS OF THE WATER-SOLUBLE, ALCOHOL-INSOLUBLE EXTRACTS

Fraction		*Glc*	*Man*	*Rha*	*Xyl*	*GlcA*
Aqueous	*U. wormskioldii*	++++	trace	a[b]	++	a[b]
	C. pusillum	++++	+	+	+	a
0.1M KCl[a]	*C. pusillum*	a[b]	++++	trace	trace	trace
0.2M KCl[a]	*C. pusillum*	a	++++	trace	trace	trace
0.3M KCl	*U. wormskioldii*	a	trace	++++	++	+
	C. pusillum	a	trace	++++	+	+
0.5M KCl	*U. wormskioldii*	a	trace	++++	++	+
	C. pusillum	a	trace	++++	+	+
M KCl	*U. wormskioldii*	a	trace	++	++	+
	C. pusillum	a	+++	++++	trace	+

[a]These concentrations were not used in fractionating the *Urospora* extract. [b]a, Absent.

(*a*) *The neutral polysaccharides.* The neutral polysaccharides from *Urospora* contained D-glucose, D-xylose, and D-mannose in the proportions of ~5:2:trace, whereas those from *Codiolum* contained mainly D-glucose, together with approximately equal amounts of D-xylose, D-mannose, and L-rhamnose. After methylation and hydrolysis of this fraction from both algae, the major products were 2,4,6- and 2,3,6-tri-*O*-methylglucose, together with smaller amounts of tetra-, 2,6-, and 4,6-di-*O*-

methylglucoses. The proportion of tetra-*O*-methylglucose was larger in the hydrolysate of the *Urospora* polysaccharide, and some 2,3-di-*O*-methylglucose was also present.

The methylated rhamnoses and xyloses were the same as those obtained from the methylated acidic glucuronoxylorhamnans described later, and the neutral polysaccharides are thought to be smaller molecular precursors of the acidic polysaccharides.

Both 2,3,6- and 2,4,6-tri-*O*-methylmannoses were present in the hydrolysates of the methylated polysaccharide from *Codiolum*, and this will be discussed later.

(*b*) *The acidic polysaccharides.* These were present in much greater amount than the neutral polysaccharides (Table II) in both forms of the alga. For *Urospora*, the ratio was 12:1, and for *Codiolum*, 2:1. The major acidic polysaccharide in both algae was a sulphated glucuronoxylorhamnan (Tables II and III) with $[\alpha]_D$ values of $-47°$ and $-89°$, respectively. The proportions of L-rhamnose, D-xylose, and D-glucuronic acid were 7.5:5.5:3 and 8.3:0.3:1.2, respectively. The sulphate content (11%) in *Urospora* corresponds to sulphation of about every 4th unit in the polysaccharide, and that (22%) in *Codiolum* to sulphation of every 2nd or 3rd unit. The i.r. spectra of these polysaccharides show a broad band at 1220–1270 cm^{-1} (S = O stretching), with bands at 850 (axial sulphate group) and 835 cm^{-1} (equatorial sulphate). The last band is virtually absent from the spectrum of *Codiolum* rhamnan.

Attempted removal of sulphate from the *Urospora* rhamnan with alkali was unsuccessful, and no new sugars were detected in the recovered polysaccharide. This indicates that any 1,2-linked rhamnose or any 1,4-linked xylose or glucuronic acid residues in the polysaccharide are devoid of sulphate[3].

Both rhamnans were methylated and hydrolysed, and a different pattern of methylated xyloses and rhamnoses was obtained from the two polysaccharides. However, this was due mainly to the incomplete methylation of the *Urospora* polysaccharide which, after a single methylation, contained a large proportion of unmethylated xylose and rhamnose residues. The uronic acid in both polysaccharides was partly reduced, the uronic acid content being decreased from 17 to 4.5% for *Urospora*, and to 2.5% for *Codiolum*. Both reduced polysaccharides were recovered in 90% yield. The reduced polysaccharide from *Urospora* was then desulphated with methanolic hydrogen chloride, and the product, recovered in 80% yield, gave an i.r. spectrum devoid of all the absorption bands previously attributed to sulphate.

The methylated sugars derived from the *Codiolum* rhamnan before and after reduction, and those from the reduced, desulphated *Urospora* rhamnan, are given in Table IV. The peaks for di- and mono-*O*-methylrhamnoses (as alditol acetates) were by far the largest. The di-*O*-methylrhamnoses have similar retention times and are measured as single peaks, but m.s. suggests that 2,4-di-*O*-methylrhamnoses were present in the largest amount. The formation of a small proportion of 2,3-di-*O*-methylrhamnose from the desulphated polysaccharide is the first evidence of the presence of 1,4-linked units, probably sulphated at C-2/3, in the original polysaccharide. The amount of mono-*O*-methylrhamnoses in the two *Codiolum* samples is about equal to

that of the di-*O*-methyl derivatives, but, apart from some 2- and 3-*O*-methylrhamnoses, mono-*O*-methyirhamnoses were not formed from the desulphated *Urospora*. This result suggests that the lower degree of methylation of the *Codiolum* rhamnan is due to sulphate groups.

TABLE IV

ANALYSIS OF THE ALDITOL ACETATES FROM THE METHYLATED RHAMNANS

Position of O-*methyl*	*Alditol acetates of*	*Peak areas in order of size*[a]		
		Codiolum	Codiolum *after reduction*	Urospora *reduced, desulphated*
2,4; 3,4; and 2,3[b]	Rhamnose	1[c]	1[c]	1
4 and 2[b]	Rhamnose	2	2	4[d]
Unmethylated	Rhamnose	3	4	9
2,3,4	Rhamnose	4	6	10
2,3,6	Mannose	4	7	[e]
2,3	Xylose	6	9	2
2,3,4	Xylose	7	8	7
2,3,6	Glucose	[e]	3	5
2,3,4,6[b]	Glucose	[e]	5	6[f]
2,4	Xylose			
3	Rhamnose	[e]	[e]	3
3	Xylose	[e]	[e]	8

[a]The largest peak is designated 1. [b]The alditol acetates of the three di-*O*-methylrhamnoses have very similar retention times, and this peak area represents all three. This also applies to the two mono-*O*-methylrhamnoses, and to tetra-*O*-methylglucose and 2,4-di-*O*-methylxylose. [c]2,3-Di-*O*-methylrhamnose absent. [d]4-*O*-Methylrhamnose absent. [e]These sugars absent. [f]2,4-Di-*O*-methylxylose absent.

The two acidic polysaccharides differ in the linkage, as well as in the proportion, of their xylose residues. They both contain about the same amount of end-group xylose, but the rest of the xylose in *Urospora* is 1,4- and 1,2,4-linked, whereas the xylose residues in *Codiolum* are 1,4- and 1,3-linked with no xylose residues at branch points.

In both polysaccharides, the glucose, and hence the glucuronic acid, is 1,4-linked, with an appreciable amount being present as end groups. Subjection of uronic acid-containing polysaccharides to a second Hakomori methylation results in considerable degradation; base-catalysed elimination and transformation of the uronic acid into a 4,5-unsaturated acid occurs[4]. When such a sugar as 1,3-linked rhamnose is attached to C-4 of the uronic acid, β-elimination of the uronic acid leaves the reducing sugar with a good leaving-group at position 3, and this will undergo further β-elimination and ultimately be converted into a furan derivative. A chain of (1→3)-linked rhamnose residues attached to C-4 of glucuronic acid would be completely degraded in this way. Application of this procedure to the *Urospora* rhamnan gave a chloroform- and a water-soluble fraction, each containing the same

amount of carbohydrate. Analysis of the hydrolysed chloroform-soluble material revealed 2,3,4-tri- and 2,3-di-*O*-methylxylose, and degraded fragments having molecular weight less than that of a methylated monosaccharide. Hydrolysis of the water-soluble material gave 2,3-di-*O*-methylxylose, 2-*O*-methylrhamnose, and unmethylated rhamnose, together with a negligible amount of small, degraded fragments. Quantitative measurements indicated that ~50% of the residues had been degraded. A possible formula and scheme that is in keeping with these and all the previous results, and noting that *Urospora* rhamnan resisted complete methylation, is illustrated in Fig. 1. Such a molecule is broken into small fragments and oligosaccharides when subjected to the above treatment. Although it must be emphasised that this is only one of a number of possible formulae that could fit the known facts, the alkaline-degradation results permit the conclusion that all three sugars are mutually linked, that the glucuronic acid is present in the inner part of the molecule with glycosidically linked rhamnose (and possibly xylose) attached to C-4 of the uronic acid residues, and that some of the xylose is present as side chains.

Acid-extracted polysaccharides. — The small amount of polysaccharide (3.3% of the dry weight) extracted by acid from *Urospora wormskioldii* gave a purple colour with I_2/KI, and 2,3,4,6-tetra-, 2,3,6-tri- (major), and 2,3-di-*O*-methylglucose were characterised in a hydrolysate of the methylated polysaccharide, indicating an essentially amylopectin-type polysaccharide.

The polysaccharides extracted from *Codiolum* with acid constituted 16% of the dry weight, had $[\alpha]_D$ −5° and a sulphate content of 5.5%, and contained D-mannose and D-glucose in the proportions of ~5:1. Treatment with Fehling's solution gave a copper complex from which a polysaccharide containing mainly mannose residues was recovered. From the solution, a glucan containing a small proportion of mannose was precipitated. After a second treatment with Fehling's solution, small proportions of mannose and glucose persisted in the glucan and mannan, respectively.

The *Codiolum* glucan was recovered in 11% yield from the fractionation. It had a carbohydrate content of 87%, was devoid of sulphate, and gave a purple colour with I_2/KI solution. It had $[\alpha]_D$ +131°; specific rotations reported for amylopectins from different green algae vary from 154–205°. The relatively low $[\alpha]_D$ value for the glucan described here is probably due to the presence of a small proportion of the mannan. After methylation of the glucan and hydrolysis, 2,3,4,6-tetra-, 2,3,6-tri-, and 2,3-di-*O*-methylglucoses were characterised in the proportions of ~1:21:1. Traces of methylated mannoses were also detected. The methylated glucoses confirm the glucan to be 1,4-linked, with 1,4,6-branch points and an average chain-length of 23, typical of an amylopectin.

The mannan from *Codiolum* was recovered in 58% yield from the fractionation. It had $[\alpha]_D$ −25°, and carbohydrate and sulphate contents of 82 and 6.5%, respectively. Methylation analysis gave 2,3,4,6-tetra-, 2,3,6-tri-, 3,6-di- and 2,3-di-*O*-methylmannoses, together with small proportions of the methylated glucoses derived from the methylated amylopectin. From measurements of the peak areas of the methylated alditol acetates in g.l.c., the relative proportions of the above *O*-methyl-

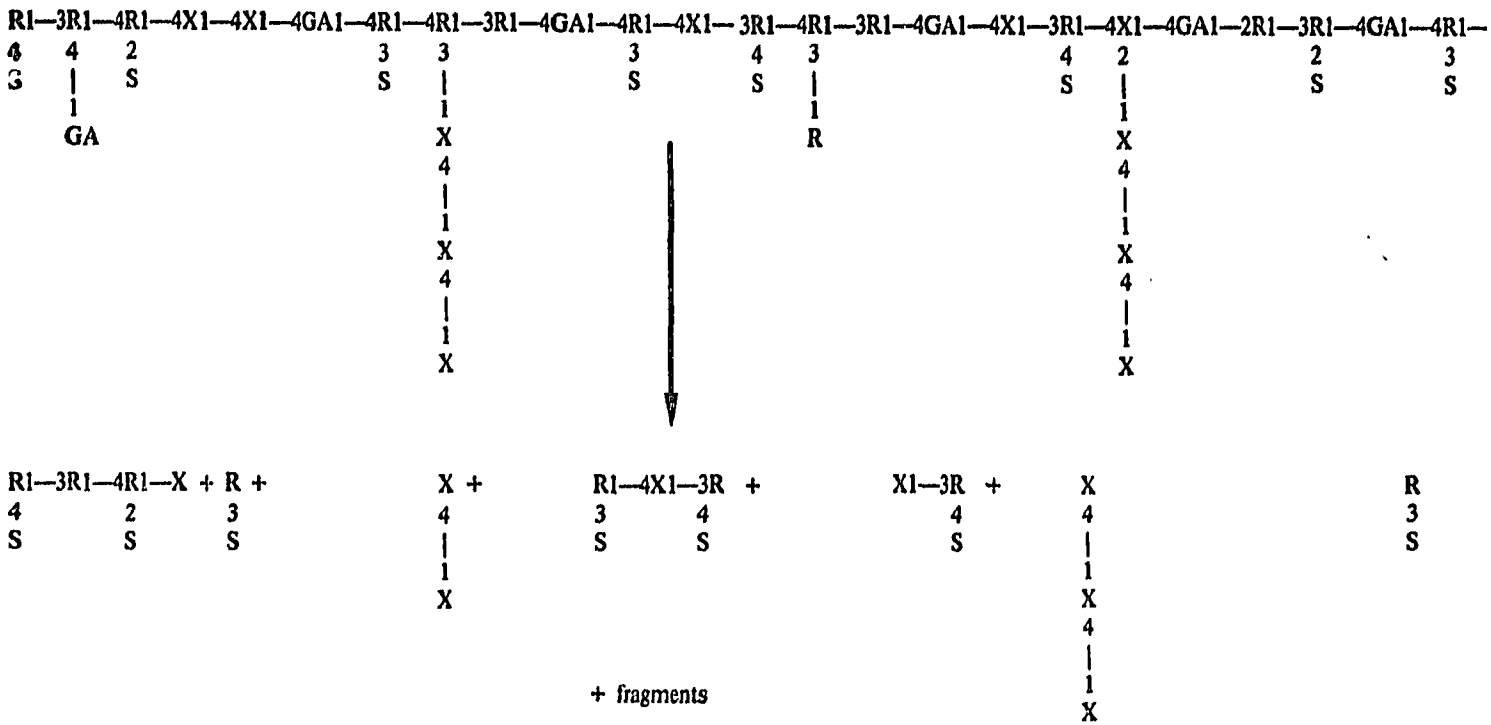


Fig. 1. A possible structure for the glucuronoxylorhamnan from *Urospora*. Key: GA = glucuronic acid residue, R = rhamnose residue, X = xylose residue, and S = half-ester sulphate group.

mannoses were found to be 1:13:1.5:1. The mannan is therefore (1→4)-linked, sulphated and/or branched at C-2 and C-6, and has an average chain-length of ~16. If all the di-*O*-methylmannose residues were derived from sulphated units, a sulphate content of 8.3% is required. As the sulphate content is lower than this value, it appears that both sulphated residues and branch points are present. After desulphation, the mannan was recovered in 86% yield, and the sulphate content had been decreased to 0.6%. The approximate relative proportions of *O*-methylmannoses in the hydrolysate of the methylated desulphated mannan had changed from 1:13:1.5:1 to 1:13:0.2:1. The fact that the peak for 3,6-di-*O*-methylmannose had almost disappeared in the products from the desulphated material, whereas that for 2,3-di-*O*-methylmannose remained the same, provides evidence that the mannan is sulphated at C-2 and has branch points at C-6. This structure requires a sulphate content of 5.2%, which is less than that originally present. However, measurement of the sulphate content after one and two Hakomori methylations showed that each methylation lowered the sulphate content by 1–1.5%.

Periodate-oxidation and methylation studies of the 0.1M- and 0.2M-KCl fractions of the aqueous extract of *Codiolum* (Table III)[5] revealed that they also contained a sulphated, branched, 1,4-linked mannan, which was apparently more easily extracted from the weed than the above mannan.

The residue. — The freeze-dried, brownish solids remaining after sequential extraction of the two algae had a high content of nitrogen. These residues and the solids remaining after hydrolysis both gave a reddish brown colour with Herzberg's stain[6], indicative of cellulose. The hydrolysate of the residue from *U. wormskioldii* contained mainly glucose, together with small proportions of rhamnose, xylose, and glucuronic acid, whereas the hydrolysate of the residue from *Codiolum* contained mannose, glucose, and traces of rhamnose.

Comparison of the polysaccharides isolated from the two life forms and from other green seaweeds. — Some of the carbohydrates isolated from the two life forms of this seaweed, and characterised, are very similar to those reported for *Urospora penicilliformis*[1]. The monosaccharides are the same in each of the three species. The proportion of maltosaccharides in the presently described algae is very much smaller than in *U. penicilliformis*, and oligosaccharides containing 1,3-linked glucose and rhamnose appear to be unique to *Codiolum*. Amylose was isolated from *U. penicilliformis*, but not in the present experiments, although the aqueous fractions from the cellulose columns gave blue colours with I_2/KI. These fractions were, however, mixtures of polysaccharides in which 1,3- and 1,4-linked, and triply linked, glucose occurred.

The sulphated glucuronoxylorhamnans metabolised by these two life forms, by *U. penicilliformis*[1], and by *Ulva lactuca*[7], *Enteromorpha compressa*[8], and *Acrosiphonia centralis*[9] have many features is common. They appear to consist of a family of polydisperse heteropolysaccharides built up on the same general plan, but with various proportions of the individual sugars, extent of branching, and sulphation.

The major difference between the polysaccharides of *U. wormskioldii* and

Codiolum and those of *U. penicilliformis* is the virtual absence of a mannan in the first, and the presence of a unique, sulphated, branched (1→4)-linked β-D-mannan in the second. In contrast, *U. penicilliformis* synthesises a (1→3)-linked α-D-mannan; a small proportion of 1,3-linked mannose units was detected in the hydrolysate of the methylated neutral polysaccharides from *Codiolum*, indicating the possible presence of such a polysaccharide. This difference in polysaccharides in the two life forms is surprising. The *Codiolum*, however, is an erect form, and it is possible that the mannan is a skeletal polysaccharide that gives rigidity to the alga, particularly as mannose is found with glucose in the residue after aqueous and acid extraction.

EXPERIMENTAL

General. — The general methods[10], and methods for methylation of polysaccharides and their analyses have been described previously[11]. G.l.c.–m.s. was performed with a F11 gas chromatograph coupled to a Hitachi RSM-4 mass spectrometer[11], and with a Pye 107 gas chromatograph (helium carrier-gas) coupled to a V.G. Micromass 12F mass spectrometer operating at 70 eV with a trap current of 50–100 μA and a pressure of $\sim 10^{-6}$ Torr. For the chemical-ionisation spectra, the ion source was operated at 150°, 50 eV, and an emission current of 1000 μA under a pressure of 0.4–0.5 Torr with isobutane as the reactant gas. The molecular weights of the methylated sugars were determined by this means[5]. Uronic acid was determined by a modified carbazole method[12]. Sulphate (expressed as percentage of carbohydrate) was determined by the method of Jones and Letham[13], after digestion of the polysaccharide[14]. Reduction of the uronic acid residues in a polysaccharide was effected by the method of Taylor and Conrad[15], as the ethyl(3-dimethylaminopropyl)carbodiimide complex.

Extraction of the algae. — The algae were plunged into ethanol immediately on harvesting, and were received in this ethanolic solution a few weeks after collection. They were removed from the alcohol (which was added to the ethanolic extracts) and air-dried. The weights of the air-dried materials (1.5 g of *U. wormskioldii* and 4.75 g of *Codiolum*) are regarded as the dry weights of the algae. Each sample was separately ground to a fine powder under liquid nitrogen, and extracted as illustrated in the flow chart. The fifth ethanolic extract was virtually colourless, and contained a negligible amount of carbohydrate. After partitioning of the ethanolic extracts between toluene and water, the almost colourless, aqueous ethanol layers were concentrated (precipitated inorganic salts were removed) to give cream-coloured solids (Table I).

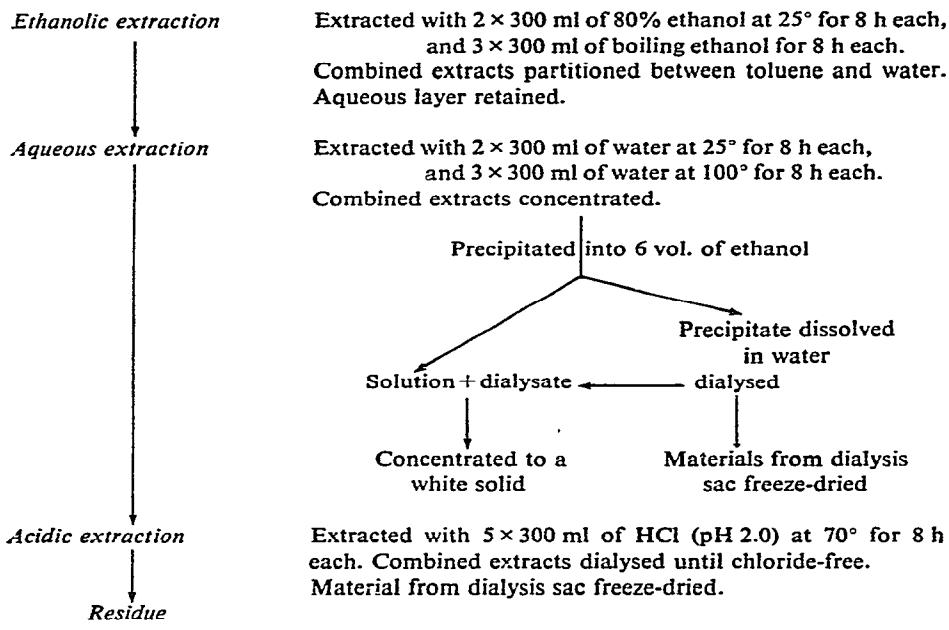
Ethanolic extracts. The carbohydrates in these extracts were characterised as in a previous publication[1].

Aqueous extracts. The polysaccharides and carbohydrates of low molecular weight extracted by water were separated by precipitation of the polysaccharides with ethanol (Table I). They were each isolated as white solids.

Water-soluble carbohydrates of low molecular weight. — P.c. revealed that the carbohydrates of low molecular weight from the aqueous extract of *Urospora* were

Flow Chart

Dry weight of weed: 0.87 g of *U. wormskioldii* and 4.75 g of *Codiolum*



similar to those found in the ethanolic extract, and the two materials were combined. The *Codiolum* extract was devoid of monosaccharides. Its carbohydrate, sulphate, and nitrogen contents were determined. An aliquot was hydrolysed, and the constituent sugars were characterised by p.c. (solvents *A–C*; sprays *1*, *4*, and *6*)[10], and by g.l.c. of the trimethylsilylated sugar and alditol derivatives. An aliquot was methylated, and the derived methylated sugars were converted into the alditol acetates, which were characterised by g.l.c.–m.s.

Water-soluble polysaccharides. — Hydrolysis of these white polysaccharides yielded rhamnose, xylose, glucuronic acid, glucose, and mannose in the approximate proportions of 8:6.5:3.5:1:trace for *Urospora* and 10:5:1:2:10 for *Codiolum*.

Each extract [72 mg (containing 56.7 mg of carbohydrate) from *Urospora*, and 408 mg (containing 228 mg of carbohydrate) from *Codiolum*] was separately fractionated on columns of DEAE-cellulose (Cl^- form, 200 g of grade DE-52, preswollen) by graded elution with water and increasing concentrations of potassium chloride. Each eluate was dialysed, concentrated, and freeze-dried. The carbohydrate, sulphate, and uronic acid contents of each fraction were measured (Table II), and the constituent sugars in the respective fractions were separated and characterised by p.c. and g.l.c.–m.s. (Table III). The rhamnose had $[\alpha]_D$ +7.8° (*c* 0.32, water) and the mannose had $[\alpha]_D$ +15.6° (*c* 0.41, water). Glucose was confirmed as the D sugar by its reaction with D-glucose oxidase. The uronic acid was shown to be glucuronic acid from its mobility[16] on ionophoresis in borate buffer

containing Ca^{2+}. The D configuration was confirmed when the glucose obtained on reduction was found to react with D-glucose oxidase.

The 0.3M- and 0.5M-KCl fractions from each of the algae were essentially the same (Table III), and were combined.

Methylation of the neutral polysaccharides in the aqueous eluates. Aliquots of each of these (Table III) were methylated, the derived methylated sugars were characterised as their alditol acetates by g.l.c.–m.s., and their approximate, relative proportions were determined from the peak areas in g.l.c.

Examination of the acid polysaccharides. — (*a*) *Attempted removal of sulphate from the glucuronoxylorhamnan with alkali.* An aliquot of the acid polysaccharide in water (5 ml) was treated with alkali[17]. After dialysis and freeze-drying, the recovered material was hydrolysed, and the constituent sugars were identified by p.c. in several solvents and by g.l.c. as the trimethylsilylated sugar and alditol derivatives.

(*b*) *Reduction of the uronic acid in glucuronoxylorhamnan.* Aliquots of the polysaccharides (38.2 mg from *Urospora*, and 28 mg from *Codiolum*) were reduced as the carbodiimide complexes. After dialysis and freeze-drying, the derived polysaccharides (34 and 25 mg, respectively) were analysed for uronic acid and constituent sugars.

(*c*) *Desulphation of the reduced acid polysaccharide.* An aliquot (15 mg) of the reduced acid polysaccharide from *Urospora* was dispersed in dry 0.08M methanolic hydrogen chloride (10 ml), and the suspension was vigorously shaken for 18 h in a sealed container at 25°. The residual solid was filtered off, treated twice more with fresh, methanolic hydrogen chloride, and then washed with dry methanol. After dissolution in water and dialysis, the material from the dialysis sac was freeze-dried to a crisp, white solid (12 mg).

(*d*) *Methylation of the glucuronoxylorhamnan.* Aliquots of the combined 0.3M- and 0.5M-KCl eluates (Table II) from each of the algae, of the reduced polysaccharides, and of the reduced, desulphated polysaccharide were methylated separately, and the products analysed in the usual way.

(*e*) *Alkaline degradation of the methylated acid polysaccharide.* An aliquot (40 mg) of the methylated acid polysaccharide from *Urospora*, which had been methylated by the Hakomori method, was dissolved in methyl sulphoxide (3 ml), and 2.0M methylsulphinyl carbanion[4] (2 ml) was added. The mixture was set aside overnight, and then neutralised with 50% acetic acid (6 ml) and extracted with chloroform. The carbohydrate contents of the chloroform and aqueous solutions were measured. The two solutions were separately taken to dryness, and the residual solids hydrolysed and analysed in the usual way.

The acid extracts. — These extracts were analysed in the same way as for the alcohol-insoluble polysaccharides from the aqueous extracts.

Fractionation of the acid extract from Codiolum. The remainder of the extract (755 mg) was dissolved in 4M NaOH (75 ml), and freshly prepared Fehling's solution was added with stirring until the formation of the blue, gelatinous copper complex was complete. The complex was removed by filtration, and the slightly blue filtrate

was dialysed for 3 days (with frequent changes of deionised water), concentrated, and freeze-dried to a white solid (glucan). The precipitate was washed with water and suspended in cold water (100 ml), and the copper complex was decomposed by the addition of 5M HCl. The polysaccharide was recovered by precipitation with ethanol (4 vol.). After centrifugation, the solid was dissolved in water, and the solution was dialysed for 2 days, and freeze-dried to a white solid (mannan). An aliquot of each fraction was hydrolysed, and the products were examined by p.c. The fractionation procedure was repeated on the glucan and on the mannan.

The glucan was methylated once by the Hakomori method, and the product analysed.

The mannan. Two separate aliquots (20 mg of each) of the mannan were methylated once and twice, respectively, by the Hakomori method. The sulphate content of each methylated mannan was determined. The remainder of the methylated materials were hydrolysed, and the methylated sugars were characterised in the usual way.

The mannan (100 mg) was partially desulphated by shaking with 0.08M methanolic hydrogen chloride (50 ml) for 12 h. The solid was filtered off, and washed with dry MeOH. The process was repeated ($\times 3$), and the final product (86 mg) was analysed for sulphate. An aliquot was methylated by the Hakomori method.

The residue. — Aliquots of the residues remaining after the sequential extractions of the algae were hydrolysed, and the products were analysed by p.c.

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