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CARBOHYDRATES OF THE SEAWEEDS, *DESMARESTIA LIGULATA* AND *D. FIRMA**

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Abstract—Crystalline mannitol and some oligosaccharides were separated from ethanolic extracts of *Desmarestia ligulata* and *D. firma*. Laminaran, 'fucans' and alginic acid were also isolated from both species. The laminaran from *D. ligulata* comprised both M- and G-chains but no M-chains were found in the laminaran from *D. firma*. In both species the amount of 'fucan' was small, particularly in *D. firma*. Both 'fucans' contained glucuronic acid, galactose, xylose and fucose and that from *D. ligulata* also contained mannose. After sequential extraction of *D. ligulata* with water, acid and alkali evidence was obtained for the presence of cellulose, a uronan, and protein in the residual material.

INTRODUCTION

Both *Desmarestia ligulata* and *D. firma*, members of the Desmarestiaceae, grow below low water tide mark and can only be collected by divers. *D. ligulata* has a branched thallus whereas in *D. firma* the thallus is largely unbranched. The two species may also be distinguished by the delicate papery texture of *D. firma* at the end of the growing season, in contrast with the much coarser cartilaginous texture of *D. ligulata* [2]. Both species contain free sulphuric acid, thought to be present in the vacuolar sap [3], which causes the weeds to break down rapidly on exposure to air. Neither species has been previously investigated chemically, partly because of the difficulty of collection, and also because of the need to neutralise or destroy the sulphuric acid immediately on removal of the weed from seawater. It seemed desirable therefore to repeat experiments on *Desmarestia ligulata* and *D. firma* which have been carried out on *D. aculeata* [1] a species which is devoid of sulphuric acid. Comparison of the results should demonstrate any differences in the carbohydrates among the different species of *Desmarestia* and reveal any effect that free sulphuric acid might have on these metabolites.

RESULTS AND DISCUSSION

Even in the presence of solid barium carbonate the ethanolic extracts of *Desmarestia ligulata* (A) were very acid. It was found subsequently that this difficulty could best be overcome by using 80% ethanol containing 1% triethanolamine which had a pH 10, and which remained at this pH after the addition of fresh weed of A. Removal of the weed into water gave a neutral solution indicating that the amine had removed all the free sulphuric acid. This method was also used for the extraction of freeze-dried *Desmarestia firma* (B) weed. Gravimetric determination of the sulphate in the ethanol gave 5.8% of free sulphuric acid in the dry weight of this weed.

Crystalline mannitol, 3.4% of the dry wt of A and 5.4% of B, was separated from the ethanolic extracts of both species. The residual syrups (*ca* 5 mg carbohydrate from A) was shown by PC and GLC to contain xylose, mannose and galactose together with some oligosaccharides. The last were combined and after hydrolysis gave galactose (major) xylose, mannose and myoinositol. The residual syrup from B. contained D-glucose, monouronic acid and its lactone (with the same PC and ionophoretic [4] mobilities as mannuronic acid and mannuronolactone) and oligosaccharides consisting of mannitol, xylose (major), glucose and galactose (trace). Although both 1-*O*-D-mannitol-β-D-glucopyranoside and 1,6-*O*-D-mannitol di-(β-D-glucopyranoside) have been found in a number of brown seaweeds [5] the above oligosaccharides appear to be unique to the present algae. They are possibly either precursors of 'fucans' or fragments hydrolysed by sulphuric acid from these saccharides during extraction. Sprays specific for glucose, ketoses and heptuloses gave negative results (except for glucose in B) indicating the absence of these carbohydrates in the ethanolic extracts. This is in contrast to the extract from *D. aculeata* [1] where sucrose, fructose and sedoheptulose were all present.

The aqueous extract from A (2.7% of the dry wt) had a carbohydrate content of 76% (glucose graph) and contained glucose, galactose, mannose, fucose and xylose. Glucose and galactose were confirmed as the D-sugars by the appropriate oxidase spray. L-Fucose, D-xylose and D-galactose are normally found in 'fucans' from brown seaweeds, but as mannose is rare it was separated from a hydrolysate and characterised as D-mannose by its mobility on ionophoresis in borate buffer and as the crystalline phenylhydrazone with mp and mmp with authentic material of 188°. The presence of glucuronic and mannuronic acids in the hydrolysate of

* Part IV in a series 'Carbohydrates of the Brown Seaweeds'. For Part III see Ref. [1].

Table 1 Fractionation of the aqueous extract from *Desmarestia ligulata*

Fraction	Recovery %	Carbohydrate %	Uronic acid content %	Sulphate content %
Aqueous	2.5	85*		
0.1 M KCl	12.5	83†	14.8	3
0.2 M KCl	41	70†	22.5	3
0.3 M KCl	18	87†	17.0	7.5
0.5 M KCl	11	71‡	3.6	20
	85	90		

* Compared to a glucose graph.

† Compared to a graph based on the carbohydrate composition of the 0.2 M KCl fraction.

‡ Compared to a graph based on the carbohydrate composition of the 0.5 M KCl fraction.

this extract was confirmed by PC and ionophoretic mobility [4]. After fractionation of the aqueous extract on DE- cellulose five fractions were separated (Table 1). The aqueous fraction, after hydrolysis, contained glucose with a trace of a non-reducing substance with the mobility of mannitol indicating the presence of laminaran with M- and G-chains (i.e. chains terminating in mannitol and glucose respectively). Treatment of a hydrolysate with glucose oxidase converted the glucose into gluconic acid allowing the detection of mannitol by PC to be more definitive. Similar treatment of a synthetic mixture of glucose and mannitol in the ratio of 25:1 gave an identical chromatogram to that from the aqueous fraction, confirming the presence of M-chains in the laminaran from A. The total yield of laminaran was 0.08% of the dry wt of the weed. Similarly the aqueous fraction from the separated aqueous extract of B (Table 2) contained mainly glucose with traces of mannuronic acid and appeared to be devoid of mannitol. To confirm the absence of mannitol a sample was analysed by elution from a DEAE-cellulose-molybdate column [6]; no fractionation occurred. The presence of M-chains is not invariable although the laminaran obtained from *D. aculeata* also contained M-chains while that from *Bifurcaria bifurcata*, *Himanthalia lorea* and *Padina pavonia* [7] did not. All four KCl fractions from the aqueous extract of A contained fucose, galactose, mannose, xylose and glucuronic acid. The ratios of these sugars in the 0.2 M KCl fraction was 15:1.5:0.4:1:11, and in the 0.5 M KCl fraction 9.7:2.7:1.4:1:trace respectively. These proportions are in agreement with the uronic acid and sulphate contents (Table 1) since in all previous studies of 'fucans' the higher proportions of sulphate are always accompanied by a higher proportion of fucose and a lower proportion of glucuronic acid.

Table 2. Fractionation of the aqueous extract from *Desmarestia firma*

Fraction	Recovery (mg)	Carbohydrate content (%)*	Uronic acid content (%)	Sulphate content (%)
Aqueous	11	83	—	—
0.3 M KCl	466	53	17†	1
0.5 M KCl	22	78	7‡	8
1.0 M KCl	—			
	499	90		

* Glucose graph; † Mannuronolactone graph; ‡ Glucuronic acid graph.

The aqueous extract from B isolated as a white solid in 3.2% yield of the dry wt of the weed contained glucose (major), galactose, fucose, xylose, glucuronic and mannuronic acids but was devoid of mannose. The results of fractionation on DE-cellulose are given in Table 2. The 0.3 M KCl fraction contained glucose (major), fucose, galactose, xylose, glucuronic and mannuronic acids. Quantitative GLC analysis of this hydrolysate revealed that at least half of it was glucose. This makes the total laminaran content of the aqueous extract about 1.8% of the dry wt. The 0.5 M KCl fraction contained fucose (major), galactose, xylose and glucuronic acid.

The acid extract from A isolated in 2.7% yield of the dry wt, had a carbohydrate content of 72% (read off a graph made from a soln with the composition of the 0.2 M KCl fraction of the aqueous extract). A hydrolysate contained fucose, galactose, xylose, mannose and glucuronic acid together with a small amount of mannuronic acid. Fractionation of an aliquot of the hydrolysate on a cellulose column resulted in the same elution pattern as obtained from the aqueous extract, except that the water eluate contained no carbohydrate.

The acid extract from B (1.4% of the dry wt) consisted of mainly mannuronic acid together with traces of glucose, fucose, galactose and xylose.

The alkaline extracts of the residual material from A contained 13.9% and from B 16% of alginic acid. Ionophoresis [4] of hydrolysates confirmed the presence of guluronic acid and mannuronic acid. The viscosities of 1% aqueous solns of the derived sodium alginates at 25° from A and B were 1.7 and 1.5 cps respectively. These very low values indicate that the alginic acid has been much degraded. An estimation of the mannuronic to guluronic acid in the sodium alginate of A gave an M/G ratio of 0.06. The high guluronic acid content supports this degradation since mannuronic acid residues are more readily hydrolysed by acid. Degradation is also confirmed by earlier findings in similar extracts. The so-called 'fucan' separated from the alkaline extract of B was mainly degraded alginic acid.

In an attempt to avoid degradation the alginic acid was extracted from the algae directly after neutralisation of the free sulphuric acid. Two samples of alginate were separated from each weed; those from A had viscosities of 300 (0·1% NaOH extract) and 150 cps (Na_2CO_3 extract) and from B 56 and 68 cps respectively. The M/G ratios [13] of the former extracts were 0.47 and 0.40 indicating a guluronic acid content of 67 and 71%. While it is recognised that the method overestimates the guluronic acid content it is still considered that a certain amount of degradation of the alginic acid has occurred. Nevertheless these results indicate a guluronic acid content of more than 50% for the alginates from both weeds. The loss of mannuronic acid is supported by the presence of this acid and low molecular weight alginic acid in the 'fucans' separated from the direct alkaline extracts.

The residue remaining after the sequential extraction of A contained 27% protein (based on a N_2 content of 4.4%) and gave a red/brown colour with Herzberg's

Table 3. Approximate percentages of the dry weight of the polysaccharides isolated from three species of *Desmarestia*

Species	Laminaran	'Fucan'	Alginic acid
D. aculeata			
August col.	5.6	14	12
March col.	1.8	9	16
D. ligulata	0.08	6	19 (16)
D. firma	1.8	1.9*	23 (17)

Figures in parentheses are the yields from the sequential extractions.

* Derived from aqueous and acid extracts only.

stain indicating the presence of cellulose, confirmed by the presence of D-glucose in a hydrolysate. Uronic acid was also detected in the hydrolysate. Quantitative determination of the 25° hydrolysate gave 11% and of the 100° hydrolysate 1.3% uronic acid.

The approximate percentage yields of the different carbohydrates based on the dry wt of three species of *Desmarestia* are given in Table 3. The most striking feature of these results is the low yield of 'fucans' particularly from B. This low yield cannot be explained by the hydrolytic effect of the sulphuric acid after death of the alga since if this were so fragments of these 'fucans' would have been found in the ethanolic extracts. It can only be concluded that A, B and *D. aculeata* synthesise less 'fucan' than most other brown seaweeds [7]. However, *Durvillia* species [9] also appear to be virtually devoid of 'fucans'.

The presence of mannose in the 'fucan' from A is in striking contrast to the 'fucan' of other species of *Desmarestia*, although it has been reported in the 'fucans' of *Sargassum linefolum* [10] *S. pallidum* [11] and *Pelvetia wrightii* [12].

It must be stressed that the alginate content in the growing weed of the species under investigation is probably considerably higher than the estimated value (Table 3), since the results clearly show that some degradation does occur during storage and extraction.

Finally it can be concluded that the presence of sulphuric acid in the two species of *Desmarestia* has no effect on their carbohydrate metabolism.

EXPERIMENTAL

Algal material. Desmarestia ligulata (A) was collected on 17 July 1973 from deep water at Port Erin, Isle of Man, and immediately plunged into boiling EtOH containing solid $BaCO_3$.

Desmarestia firma (B) was collected from False Bay, Cape Town, South Africa from 10 m depth on 27th March 1975, and was freeze-dried immediately after removal from seawater.

General methods. Details of analytical methods are given in Parts 1 [7] and III [1].

Isolation of carbohydrates. Samples of both weeds were sequentially extracted as detailed in Part I [7] except that the $C_2O_2(NH_4)_2$ extraction and chlorite treatments were omitted and alginic acid was extracted directly from the dried weed with alkali.

Ethanol extract. All the EtOH extracts of A were combined. The residual weed was air-dried and weighed (dry wt 55.7 g). During the EtOH and H_2O extractions of A, solid $Ba(CO_3)_2$ was added in an attempt to keep the pH between 5 and 7. Freeze-dried B (50 g) was extracted with 80% EtOH (500 ml) containing 4% triethylamine at 25% pH 7.0, acidified with M HNO_3 and the sulphate ppted with $BaCl_2$.

The combined EtOH extracts from each weed were treated as in a previous publication [1]. Crystalline mannitol (1.9 g from A and 2.7 g from B) was separated and the residual syrups analysed by PC solvents (1) and (3), and (A) (E) (F) [7] and galactose oxidase, heptose and ketose sprays [1], and by GLC of the sugar and alditol TMSi derivatives on column 4. Spots with $R_{mannitol}$ less than 1.0 were eluted from PC, combined and the mixture hydrolysed. The hydrolysates were analysed by PC and GLC as above.

Aqueous extract. The combined extracts, after dialysis, were freeze-dried to a white powder (1.62 g from A, and 1.60 g from B). An aliquot of each was hydrolysed and the hydrolysate examined by PC and GLC as for ethanolic extracts and by ionophoresis at pH 6.7 and 10.

An aliquot of the extract from A (100 mg) was hydrolysed and the sugars separated on 3 MM chromatography paper in solvent (3). The 'mannose' fraction was eluted and mixed with phenylhydrazine. The derived phenylhydrazone was recrystallised from EtOH.

Fractionation of the aqueous extracts. Aliquots of the white powders (430 mg from A and 480 mg from B) were fractionated on DE-cellulose columns [7]. The carbohydrate, sulphate and uronic acid contents were determined for each fraction, and after hydrolysis each fraction was analysed by PC and GLC as before. Hydrolysates of the 0.2 M KCl and 0.5 M KCl fractions from A were separated on 3 MM paper in solvents (3) and (1), eluted and the relative proportions of the sugars measured.

The acidic extracts. After appropriate treatment [7] the combined extracts from each weed were freeze-dried to white powders (1.5 g from A and 0.7 g from B), analysed and the A extract fractionated as for aqueous extracts above.

Alkaline extracts. (1) The residual solids remaining after the acid extractions were exhaustively extracted with 3% Na_2CO_3 soln. The derived Na alginates were treated with a 2% soln of $CaCl_2$ [7]. From this Ca alginate (9.3 g from 55.7 g A and 8.6 g from 50 g B and 'fucans' 345 mg A and 960 mg B) were separated. (2) The dry powdered alga (5.0 g A and 5.4 B) was suspended in a saturated soln of $CaCO_3$ (150 ml) with stirring and pH maintained at 5–7 by the addition of solid $CaCO_3$. After dialysis (3 days) the supernatant was freeze-dried to a white solid (20 mg from A and 22 mg from B). A soln of 1.8% formalin (2 ml) was added to each residual alga, the mixture stirred and stood for 30 min. The supernatants were decanted off solid Na_2CO_3 (1.5 g) and 0.1% NaOH soln (1.5 ml) added to the residual weeds, and the mixtures kept at 60° for 2 hr with stirring. After dilution the residues were filtered and the solns neutralised (pH 6.5) with dilute HCl and diluted to 800 ml. A 2% $CaCl_2$ soln (100 ml) was added to each mixture with stirring and the ppt gelatinous Ca alginates were removed by centrifugation, washed with dilute $CaCl_2$ and suspended in H_2O and freeze-dried to a brownish powder of Ca alginate (692 mg from A and 1.22 g from B). The supernatants from the centrifugations were dialysed until the dialysate was free from Ca^{++}, and the solns freeze-dried to white solids ('fucans', 173 mg from A and 30 mg from B). The residues were extracted with 3% Na_2CO_3 solns (150 ml each) at 70° for 3 successive 3 hr periods. The combined solns were treated as before and gave Ca alginates (223 mg from A and 90 mg from B) and 'fucans' (45 mg from A and negligible amount from B). (3) A second portion of B (10.9 g) was exhaustively extracted with 80% EtOH containing triethylamine to maintain pH 7–8, and treated *ca* 18 hr with formaldehyde. The recovered material was then subjected to alkaline extraction as above. From the 0.1% NaOH extraction 2.15 g of Ca alginate and 119 mg of 'fucan' were separated. From the subsequent Na_2CO_3 extract 823 mg of Ca alginate and 302 mg of 'fucan' were separated.

Conversion of calcium alginate to sodium alginate. The respective Ca alginates were converted to Na alginates by

leaching with acid. The derived alginic acids were suspended in H_2O with vigorous stirring and titrated with 0·1 M NaOH to pH = 7 so that all the alginic acid dissolved. The solns were dialysed for 2 days and freeze-dried.

Estimation of the guluronic to mannuronic acid ratio. The Na alginates were subjected to sequential partial hydrolysis [13]. The guluronic acid contents of the supernatants and the residues (the latter converted into soluble Na alginate) were determined by the carbazole method measuring the chromophore produced in H_2SO_4 with and without added borate at 55° [14]. Standard carbazole graphs were prepared for polyguluronic acid and mannuronolactone.

Examination of the residual solid. The solid remaining after the sequential extraction of the A [7] was tested for cellulose by Herzberg's stain. Aliquots of the residue (2 × 50 mg) were hydrolysed with 72% H_2SO_4 (5 ml each) at 25° and at 100° for 1 hr. The uronic acid contents of the hydrolysates were determined and the hydrolysates were investigated by PC and ionophoresis.

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