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CARBOHYDRATES OF THE BROWN SEAWEEDS
PART III. *Desmarestia aculeata*

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ABSTRACT

Mannitol, sucrose, and laminitol have been isolated from ethanolic extracts of the brown seaweed *Desmarestia aculeata* and characterised, and rhamnose, sedoheptulose, glucose, fructose, and 2-*O*-methyl- and 3-*O*-methyl-fucose have been identified by their chromatographic mobilities and g.l.c. retention times. Laminarin, alginic acid, and "fucans" were isolated also and characterised. The laminarin contained 1.7% of mannitol end-groups, and the fucans a relatively high proportion of galactose which was present as end-group and (1→3)-linked units.

INTRODUCTION

Earlier studies[1] of *Himanthalia lorea*, *Birfurcaria bifurcata*, and *Padina pavonia* have shown, as in other brown algae, that mannitol is the major carbohydrate of low molecular weight metabolised by these weeds. Glucose and *myo*-inositol were also detected in *Bifurcaria*. The polysaccharides in all three genera were similar to those found in other members of the Phaeophyceae, namely, laminarin, a family of glucuronoxylofucans ("fucans")*, alginic acid, and cellulose. The laminarins all appeared to be devoid of mannitol-terminated chains, and the proportions of laminarin and fucans differed in the three genera.

Little chemical investigation of the carbohydrates of species of *Desmarestia* has been reported. Mannitol, laminitol, 1-*O*-β-D-glucosyl-D-mannitol and 1,6-di-*O*-β-D-glucosyl-D-mannitol were isolated by Bouveng and Lindberg[2] from *D. aculeata*. Haug[3] found that 12% of sodium alginate could be extracted with 3% aqueous sodium carbonate from *D. aculeata* (collected in August), but that if the weed was pre-treated with formaldehyde the yield of alginate was only 4.7%. This author also reported a mannuronic to guluronic acid ratio of 0.85. We now describe a systematic investigation of the carbohydrates of two samples of *D. aculeata*, a member of the *Desmarestiaceae*[4]. *D. aculeata* is a slender, branched alga (length, 30–180 cm) which grows near the low-water mark. One sample (~250 g of damp weed) was harvested in late August 1971 at Rustington, Sussex, and the other (~50 g of damp weed) in March 1972 at Farr Bay, Sutherland.

*Throughout this paper the term "fucan" connotes a glucuronoxylofucan.

RESULTS AND DISCUSSION

Carbohydrates of low molecular weight. From the ethanolic extracts of the Southern sample of *D. aculeata*, a syrupy mixture of carbohydrates of low molecular weight, corresponding to 13% of the dry weight of the weed, was obtained. Nine substances were detected in the extract and, as in other Phaeophyceae, the major constituent was mannitol (~9%). Mannitol, laminitol, and relatively large amounts of sucrose were obtained crystalline and characterised. 3-*O*-Methyl- and 2-*O*-methylfucose, rhamnose, glucose, fructose, and sedoheptulose, all present in very small amounts, were isolated by paper chromatography and were identified by their mobilities, colour reactions (Table I), and the behaviour of their Me_3Si derivatives on g.l.c.

TABLE I

COMPOSITION OF THE ETHANOL EXTRACT OF *D. aculeata*

Compound[a]	*$AgNO_3$*	*Aniline oxalate*	*Urea–HCl*	*Orcinol/trichloroacetic acid*
I, 2-*O*-Methylfucose	+	+		
II, 3-*O*-Methylfucose	+	+		
III, Unknown	+	+		
Rhamnose	+	+		
Sedoheptulose	+	+		+
Fructose	+	+	+	
Mannitol	+			
Glucose	+	+		
Sucrose	+	+	+	

[a]Where identified, the mobility (p.c.) was identical with appropriate standards run as controls in solvents *1*, *2*, and *3*.

The ethanolic extract from the Scottish sample closely resembled that of the Southern sample on chromatographic analysis, and was not examined further. Sucrose has been found[5] previously in *Cladostephus sp.* The identity of D-glucose was confirmed with D-glucose oxidase[6], but it is possible that both it and fructose were derived by hydrolysis of some of the sucrose. Sedoheptulose and methylated fucoses have not been reported before in any members of the Phaeophyceae; 2-*O*-methylfucose has been found in a number of polysaccharides of higher plants[7]. Unfortunately, the amount of the methylated fucoses was too small to allow unequivocal characterisation.

Laminarin. Unlike the laminarin isolated from the three weeds investigated in Part I of this series, the present glucan was found in the acidic as well as the aqueous extracts. It was separated from the fucans in both extracts, and the combined yields are given in Table II. The Southern weed, harvested in August, had the highest content of laminarin, as would be expected from the known, gradual build-up of this polymer from April to September[8]. However, even the weed harvested in March had a much

higher content of laminarin than the weeds from the other three genera previously investigated (see Table II). Complete hydrolysis of the *Desmarestia* laminarins gave mannitol as well as glucose. Paper chromatography of a partial hydrolysate revealed the presence of gentiobiose, laminari-biose, -triose, and -tetraose, together with isomaltose. The last-named disaccharide has been shown[9] to be a reversion product produced under the hydrolytic conditions used. The presence of the laminarisaccharides shows that the *D. aculeata* laminarin, like other algal laminarins, contains (1→3)-linked glucose residues. Gentiobiose in the hydrolysate indicates that, like so many algal laminarins, it also contains a proportion of (1→6)-linked units.

TABLE II

YIELDS[a] OF SEAWEED POLYSACCHARIDES

Species	*Laminarin*	*"Fucans"*	*Alginic acid*	*"Fucans"*		
				Alkali	*Ammonium oxalate*	*Chlorite*
Himanthalia lorea	0.02	18.0	16.0	1.8	1.0	1.0
Bifurcaria bifurcata	0.20	17.0	16.0	1.8	1.0	1.0
Padina pavonia	0.07	5.0	13.0	2.0	1.0	2.5
Desmarestia aculeata (August collected)	5.6	2.2	12.0	12.5	—	—
(March collected)	1.8	4.8	16.0	2.2	4.4	1.1

[a]Percentage yields, based on the dry weight of the different seaweeds.

Hitherto, mannitol, as a constituent of laminarin, has only been reported in glucans extracted from species of *Laminaria*; all other algal laminarins, in spite of extensive investigation, are apparently devoid of mannitol. In *Laminaria* laminarin, the mannitol is linked through C-1(6) to C-1 of the glucose residues at the reducing end of some of the glucan chains, giving non-reducing molecules[10]. The present laminarin contains 1.7% of mannitol and gave a value of 3.08% for reducing power compared with 2.1% for *Laminaria hyperborea* laminarin which has a mannitol content of 2.7%. A typical laminarin devoid of mannitol has a value of ~5% for the reducing power. From these figures, it may be deduced that the mannitol is linked as in the laminarin from species of *Laminaria* and gives rise to non-reducing molecules.

After separation from the glucose in the laminarin hydrolysate, the mannitol was found to be contaminated with a considerable proportion of fructose. Although mannose has been detected in some algal laminarins[11], this is the first time that fructose has been found as a constituent. Chromatographic analysis of the unhydrolysed laminarin showed the absence of any absorbed mannitol, sucrose, or fructose. It was thought that fructose might be attached to the polymer molecule so as to give chains terminated with a molecule of sucrose. However, all attempts to detect sucrose in a partial hydrolysate of the laminarin proved unsuccessful. Furthermore, the reducing power was too high to accommodate fructose as well as mannitol at the

reducing end of the chains. The part that fructose plays in the overall structure of the laminarin must await further study.

Glucuronoxylofucans. The percentage yields of the "fucans" in the different extracts are given in Table II. Apart from the "fucan" extracted by alkali from the weed harvested in August, the yields resemble those from *Padina pavonia* and species of *Laminaria*, and are much lower than those from *Himanthalia* and *Bifurcaria* (Table II). Hydrolysates of the "fucans" from *Desmarestia* contained a higher proportion of galactose than those of the "fucans" isolated in Part I of this series[1]. The polysaccharide isolated from the alkali extract was richest in galactose, and the molar proportions of galactose, fucose, xylose, and glucuronic acid in a hydrolysate were 2:1:0.13:1.7. The galactose was separated from a hydrolysate and characterised as the D form with D-galactose oxidase.

Fractionation of the acid extract on DEAE-cellulose gave a recovery, after removal of 53% of laminarin, of 35% in the 0.3M potassium chloride eluate, 11.5% in the 0.5M eluate, and only 1% in the M eluate. The 0.3M fraction had a uronic acid content of 28% and a small content of sulphate. This is a higher content of uronic acid than for the corresponding fraction separated from the other genera[1]. The 0.5M fraction contained 22% of uronic acid and ~10% of sulphate, and in the M fraction the sulphate content was 13%. Although the sulphate contents are lower, the general trend in this fractionation is the same as that obtained from other genera of the Phaeophyceae[1].

The mixture of methyl glycosides derived from the methylated, reduced, and re-methylated sugars in the 0.3M potassium chloride extract and from the similarly treated, galactose-rich, alkali-extracted "fucan" mentioned above, contained the same methylated fucoses, xyloses, and glucoses on g.l.c. analysis as those detailed in Part II of these studies[1,2], namely, 2-, 3-, 3,4-di-, and 2,3,4-tri-*O*-methylfucose, 2,3-di- and 2,3,4-tri-*O*-methylxylose, and 2,3,6- and 2,3,4,6-tetra-*O*-methylglucose. In addition, tentative evidence for the presence of 2,3,4,6-tetra-*O*-methyl- and 2,4,6-tri-*O*-methylgalactose was also obtained, but the overlap of their characteristic peaks with those of other methylated sugars, particularly methyl tetra- and tri-*O*-methylglucosides, made unequivocal characterisation impossible. A portion of the galactose-rich material was methylated and hydrolysed without reduction of the uronic acid. Strong peaks with the retention times of the methyl 2,3,4,6-tetra-*O*-methylgalactosides (column *1*, *T* 1.8; column *2* 2.0) and methyl 2,4,6-tri-*O*-methylgalactosides (column *1*, *T* 3.8, 4.2; column *2* 2.6, 2.3) were given by the compounds in this hydrolysate after conversion into the methyl glycosides. The presence of these two sugars was confirmed by their conversion into the methylated alditol acetates followed by g.l.c. and mass spectrometry. The majority of the galactose in the galactose-rich fraction is therefore present as end-group and (1→3)-linked galactose residues. These results were confirmed by periodate-oxidation studies.

The hydrolysate from polyalcohol I (70% recovery), obtained after periodate oxidation and reduction of the galactose-rich "fucan", contained (paper chromatography) no xylose and the proportion of galactose to fucose had decreased. After a

further periodate oxidation of polyalcohol I and reduction of the derived polyaldehyde the proportion of galactose in the hydrolysate of the polyalcohol II (80% recovery), although still present, appeared to be further decreased. Practically all the uncleaved glucuronic acid had disappeared in polyalcohol II. The incomplete oxidation of the glucuronic acid and end-group galactose in polyalcohol I may be attributed to acetal formation between these two sugars and cleaved units in the polyaldehyde[13].

Although galactose has been reported as a constituent of the "fucans" extracted from *Macrocystis pyrifera*[14] and in trace quantities in the "fucans" investigated in Part II[12], this is the first time that evidence has been advanced for the way in which it is linked in the macromolecule.

Apart from these fractions having a high content of galactose, the *Desmarestia* "fucans" appear to have a structure very similar to that of the "fucans" examined in Part II[12], in that they are polydisperse and comprise a family of macromolecules of similar, general structure but containing different proportions of uronic acid and half-ester sulphate.

Alginic acid. The quantity of alginic acid separated (Table II) was approximately the same as reported by Haug[3] for this species. However, initial treatment of the Scottish sample with formaldehyde did not appear to affect the yield appreciably. Mannuronic and guluronic acids and their lactones were detected as the sole constituents in hydrolysates of each sample.

EXPERIMENTAL

Details of analytical methods are given in Part I[1]. In addition, urea–HCl and orcinol–trichloroacetic acid[15] sprays were used to detect hexuloses and heptuloses, respectively. The methylated alditol acetates from the alkali-extracted material were also injected into a 3% OV225 glass column (9 ft) at 170° fitted into a Perkin–Elmer F11 gas chromatograph combined with an RMS4 mass spectrometer. The mass spectra were recorded at an inlet temperature of 200°, ionising potential of 80 eV, ionising current of 80 μamp, and an ion-source temperature of 240°.

Isolation of the carbohydrates. — Samples of weed were plunged into ethanol immediately after collection. Portions of the Southern sample and of the Scottish material were then sequentially extracted as detailed[1] in Part I, except that the formaldehyde treatment was omitted for the Southern weed, as were the ammonium oxalate extraction and the chlorite treatment. The percentage yields of the polysaccharides, compared with those separated from the other genera in Part I, are given in Table II.

80% Ethanol extract. — After removal of the ethanol, a solution of the dried residue in water was deionised with Biodeminrolit (CO_3^{2-}) resin and extracted several times with ether. Evaporation of the aqueous solution gave a yellow syrup from which mannitol crystallised. After removal of the crystals, the mother liquors were analysed by p.c. Nine substances were detected with different sprays and dip reagents (see Table I). A portion of the syrup was fractionated on Whatman 3MM paper by development with solvent *4*, and the substances with the mobilities of sucrose, sedoheptulose, and laminitol were eluted. After concentration of the eluants, sucrose and laminitol

crystallised. The laminitol had m.p. and m.m.p. 260°, and properties [M_G (pH 10) 0.78; R_F 0.77 (solvent *1*), 0.62 (solvent *2*), 0.43 (solvent *4*), yellow spot on a blue background on detection with Bromocresol Purple–borate[16]] identical with those of the authentic compound. The sedoheptulose was characterised by p.c. (ethyl acetate–pyridine–water, 8:2:1)[17] and by g.l.c. (column *4*) as the Me_3Si derivative. A second aliquot of the mother liquors was fractionated on 3MM paper with solvent *3*, and the materials (I–III) (see Table I) were separated and identified by p.c. (solvent *2*) and g.l.c.

Characterisation of the laminarin. — This polysaccharide was separated from the aqueous calcium chloride and from the acid extracts by fractionation on DEAE-cellulose as in the previous study[1]. Two separate aliquots were hydrolysed (1) with 0.5M sulphuric acid for 3 h at 100°, and (2) with 0.25M acid for 1 h at 70°. The hydrolysates were worked up in the usual way and analysed by p.c. (solvents *1*, *2*, and *5*), using a variety of detection sprays, and by g.l.c. The second hydrolysate was examined for oligosaccharides. Laminari-biose, -triose, and -tetraose, gentiobiose, mannitol, isomaltose, and laminaribiosylmannitol were used as reference standards. The product with the mobility of mannitol in the first hydrolysate was separated on 3MM paper and analysed by p.c. and g.l.c. The proportion of mannitol in the laminarin was measured[18]. The reducing power of the *Desmarestia* laminarin and that of laminarin from *Laminaria hyperborea* were determined[19] in parallel experiments.

Characterisation of the glucuronoxylofucans ("fucans"). — Each of the extracts was subjected to hydrolysis and the hydrolysates were analysed by p.c. and g.l.c. The acid extract of the Southern sample was fractionated on a column[1] of DEAE-cellulose and the various fractions were analysed for sulphate and uronic acid content.

The 0.3M KCl fraction and the alkali-extracted "fucan" (see Table II) were subjected to Hakomori methylations[20]. The 0.3M KCl fraction and half of the alkali-extracted fucan were reduced with lithium aluminium hydride and re-methylated[12]. The derived, methylated glycosides and methylated alditol acetates from the three methylated materials were analysed by g.l.c. (columns *1*, *2*, and *6*). The methylated, alkali-extracted fucan, in which the uronic acid had not been reduced to glucose and re-methylated, was analysed on the OV 225 column and subjected to mass spectrometry.

The alkali-extracted "fucan" (500 mg) was oxidised by periodate in the presence of 2.5% of 1-propanol for 18 h under the conditions used previously[12]. After destruction of the excess periodate with ethylene glycol, the polyaldehyde was reduced with borohydride[12] and the derived polyalcohol (360 mg) was recovered in the usual way. An aliquot (86 mg) was hydrolysed with 0.5M sulphuric acid in an atmosphere of carbon dioxide and the hydrolysate was examined by p.c. The polyalcohol (274 mg) was re-oxidised with periodate and the derived polyalcohol II (212 mg) recovered as before. An aliquot was hydrolysed and analysed by p.c.

The sodium carbonate extracts. — These extracts were treated as in Part I[1], and the yields of calcium alginate and of the fucan are given in Table II. Aliquots of the alginate were hydrolyzed and analyzed by p.c.

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