Phytochemistry, Vol. 20, No. 7, pp. 1579–1582, 1981.
Printed in Great Britain.

0031-9422/81/071579-04 $02.00/0

CHEMISTRY OF THE POLYSACCHARIDES OF THE BROWN SEAWEED *DICTYOPTERIS PLAGIOGRAMMA**

ELIZABETH PERCIVAL, MD. ANISUR RAHMAN† and HELMUT WEIGEL

The Bourne Laboratory, Royal Holloway College (University of London), Egham, Surrey, TW20 OEX, U.K.

(*Received* 7 *November* 1980)

Key Word Index—*Dictyopteris plagiogramma*; Phaeophyceae; seaweeds; polysaccharides; laminaran; fucans; alginic acid.

Abstract—Laminaran, fucose-containing polysaccharides ('fucans') and alginic acid were isolated from *Dictyopteris plagiogramma*. The laminaran comprised G- and M-chains (ratio 3:1). The 'fucans' were present in four extracts of a four-step sequential extraction procedure and all contained slightly differing proportions of fucose, xylose, galactose, mannose, glucuronic acid residues and half-ester sulphate. Non-reducing chain ends as well as the positions of glycosidic linkages to fucose, xylose and glucuronic acid are the same as previously reported for other 'fucans'. Galactose and mannose occur mainly as trisubstituted residues with substitution at 0-1, 0-3, 0-4 and at 0-1, 0-3, 0-6, respectively.

INTRODUCTION

Dictyopteris plagiogramma (Montague) Vickers, an alga belonging to the Phaeophyceae, order Dictyotales, family Dictyotaceae, is commonly found in the sub-tropical waters around South America. The alga grows to a height of about 25 cm on rocks, old corals and shells. The plants are erect and profusely branched. The only previous chemical studies on this genus, known to the authors, are concerned with aspects of alginic acid [2] and laminaran [3]. The material used in the present study was harvested off the coast of Brazil.

RESULTS AND DISCUSSION

The dried and powdered alga was sequentially extracted as detailed earlier [4]. The ethanolic extracts were devoid of carbohydrate. As the alga was extensively washed before drying, it is probable that low MW carbohydrates, such as D-mannitol, were removed during the washing. Extraction with cold H_2O, hot H_2O, dilute aqueous HCl, and aqueous Na_2CO_3 gave, respectively, the polysaccharide materials **A**, **B**, **C** and **F** (alginic acid).

The polysaccharides **A** and **B**, comprising about 7.6% of the dry alga, were of similar compositions, the only obvious difference being in their uronic acid contents (**A**, 20%; **B**, 12%). The hydrolysates of each contained the same monosaccharides and that of **B** contained D-glucose, fucose, D-glucuronic acid, mannose, xylose and galactose in the approximate mol.-percentages of 37.0, 37.0, 9.3, 7.4, 6.2 and 3.1, respectively. HPLC of the polysaccharide materials **A** and **B** gave essentially identical results, revealing that each contained the same two polydisperse components, significant quantities of which had, when compared with standard dextrans, apparent M_r values ~3000 and ~30000.

A and **B** also gave similar patterns (carbohydrate, uronic acid and half-ester sulphate content, as well as proportions of component monosaccharides; see Table 1) when fractionated on columns of DE-52 cellulose. However, water eluted a glucan of the laminaran type (total from **A** and **B** ~3% of dry alga; see below for characterization), whereas the polysaccharides eluted with increasing concentration of KCl were heteropolymers devoid of glucose.

The polysaccharide material (**C**) (5.8% of dry alga) gave on hydrolysis also D-glucose, fucose, D-glucuronic acid, mannose, xylose and galactose, but in contrast to **A** and **B** in the approximate mol.-percentages of 3.1, 41.7, 20.8, 8.3, 10.4 and 15.6, respectively. These and the corresponding values for the fractions obtained from **A** and **B** show that

Table 1. Fractionation of polysaccharide material **B** on DE-52 cellulose

Eluant	Recovery (%)	Carbohydrate (%)	Uronic acid (%)	Sulphate content (%)
H_2O	36.0	85*,†	nil	nil
0.1 M KCl	2.1	55‡,§	17	n.d.
0.2 M KCl	15.4	66‡,§	16	4
0.3 M KCl‖	20.9	73‡,§	12	7
0.5 M KCl	7.3	45‡,§	10	9
0.8 M KCl	3.5	46‡,§	8	n.d.
M KCl	1.9	20‡,§	n.d.	n.d.

*Hydrolysate contained only glucose.

†Carbohydrate content based on glucose as a standard.

‡Hydrolysate contained all constituent monosaccharides except glucose.

§Carbohydrate content based on an artificial mixture of fucose–galactose–mannose–xylose–glucuronic acid in the ratio 5:1:1:1:2.

‖Examined by HPLC.

n.d. = Not determined.

*Part V in the series "Carbohydrates of Brown Seaweeds". For Part IV see ref. [1].

†Present address: Department of Applied Science, University of Rajshahi, Bangladesh.

most, if not all, of the extracted glucose-containing polysaccharide material is in fact a laminaran.

Characterization of laminaran obtained from **B**

HPLC revealed that significant quantities of the glucan had, when compared with standard dextrans, apparent $M_r \sim 3000$ (cf. M_r of unfractionated **A** and **B**). Its classification as a laminaran is based on the following. The glucan had $[\alpha]_D - 11.3°$ (*c* 0.23, H_2O). PC of a hydrolysate revealed glucose and a small amount of a non-reducing material, and the derived poly-*O*-TMSi-alditols were shown by GLC to be the corresponding derivatives of glucitol and mannitol. After treatment of the hydrolysate as well as a synthetic mixture of D-glucose and D-mannitol (in the molar ratio 30:1) with D-glucose oxidase, PC revealed that the two oxidized materials contained D-gluconic acid and mannitol in approximately the same proportion (visual estimation). This indicated that a fairly high proportion of the glucan consisted of M-chains. However, all attempts to separate the M-chains from G-chains on a DEAE-Sephadex–molybdate column [5] were unsuccessful.

The small extent of oxidation by periodate of the glucan shows that its glucopyranosyl units are indeed joined through (1 → 3)-linkages. The glucan had DP 14, as determined by the Timell method [6], a value which is of the same order of magnitude as that which can be estimated from HPLC. It is also close to the DP (15; determined from periodate oxidation results) of the laminaran of *Bifurcaria bifurcata* [4] which is devoid of M-chains. On the assumption that the G-chains and M-chains of the glucan of *Dictyopteris plagiogramma* have similar DP, it can then be calculated that the ratio of G-chains to M-chains in the glucan is *ca* 3.

Characterization of fucose-containing polysaccharides

The fucose-containing polysaccharides ('fucans') obtained by sequential and by direct alkali extraction comprised, respectively, *ca* 10 and 6% of the dry weight of the alga. Each contained varying proportions of constituent fucose, galactose, mannose, xylose, glucuronic acid and half-ester sulphate. Their sulphate content (~5%) was considerably lower than that (>10%) of the majority of the examined fucans of brown seaweeds. Fractionation experiments with the 'fucans' of the present investigation yielded only fractions with slightly different proportions of all the constituents.

HPLC of a major fraction obtained from material **B** (see Table 1) gave a single peak corresponding to a range of apparent M_r between ~25000 and ~40000, when compared with standard dextrans (maximum peak height corresponding to $M_r \sim 30000$). Thus, the two peaks in the HPLC of the original materials **A** and **B** corresponded to the laminaran (lower apparent M_r) and 'fucan' (higher apparent M_r).

Comparison of the gel filtration pattern of the polysaccharide material **C**, the polyalcohol derived from it by oxidation with periodate followed by reduction with $NaBH_4$, and of the polyalcohol after mild treatment with acid revealed no change in apparent molecular size until the degradation by acid had proceeded for ~50 hr. The complete hydrolysate of the polyalcohol contained all the constituent monosaccharides of **C**, but only traces of glucuronic acid. These results indicate that periodate oxidation had taken place predominantly at the peripheral part of the polysaccharide material and that glucuronic acid occurs as non-reducing end groups and/or as (1 → 4)-linked units in the polymer.

Methylation by the Hakomori method of **C** appeared to cause a small reduction in apparent molecular size without concomitant formation of oligosaccharides. The hydrolysate of the methylated **C** contained, in addition to a component of low PC migration rate (*O*-methyloligouronic acid), the neutral *O*-methyl-monosaccharides shown in Table 2(a). The components of the *O*-methyloligouronic acid are shown in Table 2(b). The results confirm the occurrence of (1 → 4)-linked D-glucuronic acid. They also show that fucose, xylose and D-glucuronic acid are mutually linked in the polysaccharide.

The linkages to fucose, xylose and D-glucuronic acid are the same as those reported [7] for 'fucans' which are devoid of galactose and mannose. Galactose and mannose occur mainly as trisubstituted residues; in galactose substitution is at 0–1, 0–3, 0–4 (major) and 0–1, 0–3, 0–6, whereas in mannose substitution is at 0–1, 0–3, 0–6. It was not ascertained whether these constitute branching-units and/or mono-sulphated chain-units. In addition, (1 → 6)-linked mannose is a minor chain-unit. Sulphated galactose units and (1 → 3)-linked galactose have been reported to occur in the 'fucans' of *Sargassum linifolium* [8] and *Desmarestia aculeata* [9] respectively. In addition, (1 → 2)-linked xylose, (1 → 3)-linked fucose, (1 → 4), (1 → 4,6)-, (1 → 2,3,4)-linked galactose, non-reducing end group galactose as well as (1 → 2)-, (1 → 6)-, (1 → 2,4)- and (1 → 2,3)-linked mannose seem to occur in the 'fucans' of *Pelvetia* and *Sargassum* [10].

Table 2. Products of methylation analysis of polysaccharide material **C**

(a) By direct hydrolysis of methylated polysaccharide*, †

Fucose (vl)
2-*O*-Methylfucose (l)
3-*O*-Methylfucose (m)
3,4-Di-*O*-methylfucose (s)
2,3,4-Tri-*O*-methylfucose (s)
Xylose (s)
2,3-Di-*O*-methylxylose (m)
2,3,4-Tri-*O*-methylxylose (s)
2,4-Di-*O*-methylgalactose (s)
2,6-Di-*O*-methylgalactose (l)
2,3,4,6-Tetra-*O*-methylgalactose (s)
2,4,-Di-*O*-methylmannose (m)
2,3,4-Tri-*O*-methylmannose (s)

(b) From *O*-methyloligouronic acid, isolated from direct hydrolysate of methylated polysaccharide material*, †

Fucose (m)
2-*O*-Methylfucose (s)
Xylose (s)
D-Glucuronic acid (m)
2-*O*-Methyl-D-glucuronic acid (s)
3-*O*-Methyl-D-glucuronic acid (s)
2,3-Di-*O*-methyl-D-glucuronic acid (l)

*Letters in parentheses indicate GLC peak size of alditol product: vl = very large, l = large, m = medium, s = small.

†Structure established by GLC/MS of corresponding acetylmethylalditol.

Alginic acid

Sequential extraction of the alga gave an alkali- and ethanol-soluble material (**D**) containing only 4% carbohydrate but 4,5-unsaturated acids [11], indicating that alginic acid had been degraded. The alginic acid (**F**) (14% of dry wt of alga; as alginic acid), shown to contain mannuronic and guluronic acids (by electrophoresis of a hydrolysate), had a very low viscosity (η_{rel} 1.6, *c* 1%) as anticipated from the degradation. Direct alkali extraction of the alga gave a slightly higher yield of alginic acid (18.7%). The materials extracted with 1% aq. Na_2CO_3–0.1% aq. NaOH and with 3% aq. Na_2CO_3 had η_{rel} 48 and 21 (*c* 1%), respectively. These values are considerably lower than those found ($\eta_{rel} > 300$, *c* 1%) [12] for alginic acids obtained from other brown seaweeds. We therefore assume that the alginate in the alga was degraded during the drying process and storage.

The results reported here confirm that species of brown seaweeds from different genera and of different morphological forms synthesize the same types of polysaccharides, namely, laminaran, sulphated fucose-containing polysaccharides ('fucans') and alginic acid. The major differences occur in the 'fucans' from the different genera. All contain fucose, xylose and glucuronic acid. Some contain, in addition, galactose and mannose. They are all polydisperse and differ considerably in the proportion of their component monosaccharides and in the degree of half-ester sulphation. Comparison with results reported from other laboratories is, however, sometimes difficult as the results depend on the method of extraction used and, more importantly, on the degree of purification and fractionation of the extracted materials.

EXPERIMENTAL

Algal material. Dictyopteris plagiogramma (Montague) Vickers was harvested in May 1976 from *ca* 2 m of water at Marakazawa, nr. Natal, Brazil, washed and sun dried.

General methods. Details of analytical methods are given in refs. [4] and [9].

Isolation of carbohydrates by sequential extraction of alga. The dried, powdered alga (39 g) was sequentially extracted as described in ref. [4], except that the extraction with aq. ammonium oxalate and the treatment with chlorite were omitted. The white powders obtained after dialysis and freeze-drying of the cold aqueous (**A**, 0.55 g), hot aqueous (**B**, 2.42 g) and acidic extracts (**C**, 2.25 g) had, respectively, carbohydrate, 63, 64 and 66; uronic acid, 20, 12 and 20; half-ester sulphate, 6, 6 and 4%. The alkaline extract gave a greenish material (**D**, 0.5 g; carbohydrate, 4%; obtained by concentration of material soluble in 20% aq. EtOH), a polysaccharide material (**E**, 0.35 g; carbohydrate, 69; uronic acid, 17; half-ester sulphate, 4%) soluble in 2% aq. $CaCl_2$ and calcium alginate (**F**, 6.9 g).

Isolation of alginic acid by direct alkaline extraction of alga. The dried, powdered alga (25 g) was extracted as previously reported [1] except that the preliminary treatment with $CaCO_3$ was omitted. Following extraction with 1% aq. Na_2CO_3–0.1% aq. NaOH and with 3% aq. Na_2CO_3, calcium alginate (4.1 g and 1.5 g, respectively) was obtained in addition to polysaccharide materials (0.91 and 0.59 g, respectively).

Monosaccharide components of polysaccharide materials **A**, **B** *and* **C**. A portion of each material was separately hydrolysed. The hydrolysates were analysed by PC and paper electrophoresis in borate electrolyte (pH 10). The neutral monosaccharides in a portion of each hydrolysate were converted into poly-*O*-TMSi-alditols [by reduction with $NaBH_4$, followed by trimethylsilylation with Me_3SiCl–$(Me_3Si)_2$], which were identified by GLC. Similarly, the neutral monosaccharides in the hydrolysates of materials **B** and **C** were converted into poly-*O*-acetylhexitols (by reduction with $NaBH_4$ followed by acetylation with Ac_2O–pyridine). These were also identified and their approximate mol.-percentages calculated from GLC peak areas. Further portions of the hydrolysates of materials **B** and **C** were fractionated, using Whatman No. 3MM paper and solvent [1], into neutral and acidic saccharides. Portions of the neutral saccharides were fractionated on Whatman No. 1 paper using solvent [2] and each monosaccharide determined by the PhOH–H_2SO_4 method and by GLC. Portions of acidic materials were esterified (with CH_2N_2), reduced (with $NaBH_4$) and hydrolysed. PC revealed a product with a migration rate identical with that of glucose. Its reaction with D-glucose oxidase showed it to be D-glucose.

HPLC of polysaccharide materials **A** *and* **B**. Separate solutions of materials **A** and **B** (7.8 and 7.2 mg/cm^3, respectively) in 0.1% HOAc were filtered through Millipore (pore size 0.45 μm). HPLC of portions of the filtrates (40 μl) was performed on a Waters ALC 202 chromatograph, fitted with a column (600 × 2 mm i.d.) packed with Lichrosphore Si 100 with a flow pressure of 1500 psi and a flow rate of 0.1–0.2 cm^3/min.

Fractionation of polysaccharide materials **A** *and* **B** *on DE-52 cellulose.* The materials **A** (206 mg) and **B** (507 mg), separately dissolved in water (40 cm^3), were applied to columns (30 cm × 4.5 cm i.d.) of DE-52 cellulose (pre-swollen microgranular). The columns were eluted with H_2O, and 0.1 M, 0.2 M, 0.3 M, 0.5 M and M KCl, as described previously [4]. The carbohydrate, uronic acid and half-ester sulphate contents of each of the fractions were determined [4] (see Table 1), and the monosaccharides produced by hydrolysis were characterized by PC and GLC of derived poly-*O*-TMSi-alditols (cf. above).

Periodate oxidation of laminaran obtained from polysaccharide material **B**. The glucan (29.5 mg; obtained from the aq. eluate of the above fractionation) was treated with 0.015 M $NaIO_4$ (20 cm^3) in the dark at 2° for 5 days and then at ambient temp. for 2 days. At time intervals aliquots were withdrawn for the estimation of periodate reduced. At the times quoted the values had become constant. For comparison, the laminaran (32.5 mg) obtained from *Bifurcaria bifurcata* [4] was likewise treated. At 2°, the laminarans of *Dictyopteris plagiogramma* and of *Bifurcaria bifurcata* reduced, respectively, 0.255 and 0.193 mol. of $NaIO_4$ per anhydroglucose unit. The corresponding figures for ambient temp. are 0.374 and 0.354.

Periodate oxidation of **C**. The material **C** (170 mg in 170 cm^3 H_2O) was oxidized with 0.03 M $NaIO_4$ (170 cm^3) in the dark at ambient temp. Examination of aliquots showed that the oxidation was complete after 7 days. Excess periodate was reduced by the addition of ethylene glycol (1 cm^3). The polyaldehyde was reduced with $NaBH_4$ as usual. Treatment of the product with $NaIO_4$ resulted in no further reduction of $NaIO_4$. A portion (15 mg) of the polyalcohol (136 mg), obtained after dialysis and freeze-drying, was hydrolysed with 90% HCO_2H and the hydrolsate analysed by PC. The remainder of the polyalcohol was treated with M CF_3CO_2H at ambient temp. After 25, 50 and 75 hr, portions of the reaction mixture were subjected to gel filtration using Sepharose 4B.

Methylation of **C**. The material **C** (23.5 mg) was twice methylated by the Hakomori method. Portions (*ca* 2 mg) of the once and twice methylated materials were chromatographed on Sepharose 4B (column size 16.5 cm × 1.3 cm i.d.). Further portions of the methylated materials were separately hydrolysed and the hydrolysates examined by PC and, after conversion into *O*-acetyl-*O*-methylalditols, by GC/MS [see Table 2(a)]. The remainder of the hydrolysate of the twice methylated material was

fractionated on Whatman No. 3MM paper using solvent [2] and a component of low chromatographic mobility was obtained. This was esterified (with CH_2N_2), reduced (with $NaBH_4$) and hydrolysed (with M H_2SO_4). The hydrolysate was neutralized with *N*-methyldioctylamine (5% in $CHCl_3$). A portion of the hydrolysate was examined by PC and, after conversion into *O*-acetyl-*O*-methylalditols, by GC/MS [see Table 2(b)].

Acknowledgements—The authors thank Dr. M. Durairatnam, Departomento de Oceanografia e Limnologia, Universidade Federal do Rio Grande do Norte, Natal, Brazil, for characterization of the alga, and Dr. Paul Finch for helpful discussions.

REFERENCES

1. Carlberg, G. E., Percival, E. and Rahman, M. A. (1978) *Phytochemistry* **17,** 1289.
2. Fischer, F. G. and Dörfel, H. (1955) *Hoppe-Seyler's Z. Physiol. Chem.* **301,** 224; **302,** 186.
3. Shah, H. N. and Rao, A. (1969) *Curr. Sci.* **38,** 413.
4. Jabbar Mian, A. and Percival, E. (1973) *Carbohydr. Res.* **26,** 133.
5. Stark, J. (1976) *Carbohydr. Res.* **47,** 176.
6. Timell, T. E. (1960) *Svensk. Papperstidn.* **63,** 668.
7. Jabbar Mian, A. and Percival, E. (1973) *Carbohydr. Res.* **26,** 147.
8. Abdel Fattah, A. F., Hussein, M. M. E. and Salem, M. H. (1974) *Carbohydr. Res.* **33,** 19.
9. Percival, E. and Young, M. (1974) *Carbohydr. Res.* **32,** 195.
10. Pavlenko, A. F., Kurika, A. V., Khomenko, V. A. and Ovodov, Yu. S. (1974) *Khim. Prir. Soedin.* 142.
11. Weisbach, A. and Hurwitz, J. (1959) *J. Biol. Chem.* **234,** 705.
12. Percival, E. and McDowell, R. H. (1967) *Chemistry and Enzymology of Marine Algal Polysaccharides*, p. 117. Academic Press, London.