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CARBOHYDRATES OF THE BROWN SEAWEED *DICTYOTA DICHOTOMA*

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Abstract—The fucose-containing polysaccharides of the brown alga *Dictyota dichotoma* were extracted with either trichloroacetic acid or HCl to give both water-soluble and water-insoluble materials. The latter had a high proportion (16 to 11%) of protein, and although all the sugars found in the water-soluble extracts were present, the major sugar in these water-insoluble polysaccharides was glucose. The water-soluble material extracted with HCl was a protein-free sulphated heteropolysaccharide. Complete removal of a glucan from the water-soluble extract was achieved by fractional precipitation with ethanol. The recovered glucan-free sulphated polysaccharide, which was rich in glucuronic acid, galactose, fucose and sulphate, showed high anticoagulating activity.

INTRODUCTION

Sulphated, fucose-containing polysaccharides have been studied in a number of brown seaweeds. In *Ascophyllum nodosum* [1, 2] the major polysaccharide constituent, ascophyllan, was shown to contain fucose (25%), xylose (26%), sodium uronate (19%), $NaSO_3$ (13%) and protein (12%). Later investigations of this alga report [3], a fucan composed of fucose (5 parts), xylose (1 part), glucuronic acid (1 part), ester sulphate (20%) and protein (3.8%)and [4] a second sulphated polysaccharide in which fucose, xylose, galactose, traces of mannose, glucuronic, mannuronic and guluronic acids and traces of firmly bound protein were found. From *Sargassum linifolium* Abdel-Fattah *et al.* [5] have isolated a sulphated heteropolysaccharide, sargassan, containing glucuronic acid, mannose, galactose, xylose, fucose and a protein moiety. More recently Hussein [6] has reported the isolation of two sulphated polysaccharides from *Colpomenia sinuosa*, both of which are bound to protein: one contains galactose (major), mannose, glucuronic acid, xylose, fucose (minor), glucose (traces) and the other glucose (major), glucuronic acid and galactose with traces of xylose and fucose. Mian and Percival [7] found that the fucans of 5 sequential extracts of each of *Himanthalia lorea*, *Bifurcaria bifurcata* and *Padina pavonia* comprised variable proportions of fucose, xylose, glucuronic acid, galactose (traces) and half-ester sulphate. Abdel-Fattah and Edrees [8] also reported a sulphated heteropolysaccharide composed of glucuronic acid, fucose (major), glucose, mannose and xylose and a protein moiety from *Padina pavonia*.

The present paper describes the isolation and purification of another sulphated heteropolysaccharide from the brown alga, *Dictyota dichotoma*.

RESULTS AND DISCUSSION

D. dichotoma contains *ca* 16% alginic acid, 2% crude laminarin, 20% acid-extractable polysaccharides, 2% mannitol, 7% lipids, 18% protein and 20% ash; 17% Ca and 4% Mg in ash. No low-molecular weight carbohydrates were found in the alcoholic extract after removal of mannitol. 2D PC (solvents A and B) of a hydrolysate of the algal material afforded *ca* 2.5% cysteic acid, 1% glutamic acid, 0.8% aspartic acid, 3% lysine, 0.5% glycine, 0.4% serine, 0.5% alanine, 0.3% threonine, 0.5% arginine, 0.2% tyrosine, 0.2% proline, 0.4% methionine, 0.4% leucine, 0.6% isoleucine, 0.5% phenylalanine and 0.2% valine. Complete acid hydrolysis of the algal material, after removal of mannitol followed by PC of the hydrolysate (solvent C) afforded mannuronic acid–guluronic acid–glucuronic acid–their lactones (14.7%), glucose (13%), fucose (6.3%), galactose (5.3%), mannose (3.7%) and xylose (2%). The presence of mannose as a constituent of the alga was confirmed by its identification, after elution from PC, as its crystalline phenylhydrazone [9] (mp and mmp 199–200°). Mannose and also the other mentioned sugars have been found as constituents of other brown seaweeds [5, 6, 8].

Acid-extractable water-insoluble and -soluble polysaccharides

The yields and composition of the different extracts are given in Table 1. The water-insoluble extracts (B)

Table 1. Polysaccharide compositions

	HCl extract			TCA extract	
	water-soluble		water-insoluble	water-soluble	water-insoluble
Constituents	(A)	(P)	(B)	(C)	(D)
% in alga	7.0	6.0	13.4	7.7	6.6
Ash	25	14	29	32	43.5
Protein	—	—	16	—	11
Carbohydrate	52	69.5	16	4.3	10
Glucuronic acid	31	25	17	21	15
Galactose	17	25	8	26	—
Glucose	8	7	34	5	42
Mannose	13	8	15	11	16
Xylose	9	12	11	9	9
Fucose	22	21	15	28	17

and (D) had low carbohydrate contents and were not examined further. The two water-soluble extracts (A) and (C) were very similar in yield and composition.

Partial purification of (A) (from 15 g alga) by elution through a column of Lewatit S 100 (H^+) resin gave material (P) (12 g) (Table 1). This purification increased the carbohydrate content and reduced the ash and the recovered polysaccharide contained 16% sulphate. Attempted fractionation of polysaccharide (P) by gradient elution from a DEAE-cellulose column with increasing concentration of NaCl (0–0.2 M) gave no significant fractionation. Similarly attempted fractional precipitation with cetylpyridinium chloride gave a low recovery (15.5%) of polysaccharide with little or no fractionation.

Fractionation of the sulphated polysaccharide (P) with ethanol afforded 6 fractions. Fractions 1 and 2 and 4–6 were very similar and were combined. The percentage recovery and composition of the resulting 3 fractions are given in Table 2. The polysaccharide defied further fractionation, indicating that the glucan-free product is one polysaccharide. The isolation of polysaccharide samples containing variable proportions of sugars is in agreement with the opinion of Mian and Percival [7] that brown seaweeds synthesize a wide spectrum of fucans.

A preparative fractionation of (P) with ethanol was carried out to obtain the purified glucan-free, sulphated heteropolysaccharide (R) which precipitated above 50% ethanol. The composition of this purified polysaccharide (R) which contained 16.6% sulphate is detailed in Table 2. It had $[\alpha]_D^{25°} -79.3°$. The purified polysaccharide had higher anticoagulating activity than heparin. Under conditions where human or sheep plasma coagulated after 1 hr in the presence of standard heparin solution, coagulation did not occur until after 4 days in the presence of the algal polysaccharide. A similar result was also reported for the sulphated heteropolysaccharide isolated from *Sargassum linifolium* [10].

EXPERIMENTAL

Dictyota dichotoma was collected in August 1972 from Roushdy at Alexandria. The alga was washed with H_2O to remove foreign substances, air dried and milled.

General. Chromatography on Whatman No. 1 paper was carried out with the following solvents. (A) *n*-BuOH–HOAc–H_2O [11] (4:1:5, upper layer), (B) PhOH–H_2O [12] (4.1), (C) *n*-BuOH–EtOH–H_2O [3] (40.11:19), (D) *n*-BuOH–C_5H_5N–H_2O [13] (6:4.3). Detection was affected with aniline hydrogen phthalate, ammoniacal $AgNO_3$ and ninhydrin reagents [14]. After ashing at 800°, Ca and Mg were determined complexometrically [15]. Lipids were isolated from the alga by Soxhlet extraction with *n*-hexane for 12 hr. Protein was determined for H_2O-soluble and H_2O-insoluble samples by the method of ref. [16] and by the micro-Kjeldahl method, respectively. Determination of amino acids in algal hydrolysate (in 6 N HCl for 24 hr at 105°) was done after 2D PC (solvents A and B) and elution from the chromatogram by the method of ref. [17]. Complete acid hydrolysis of algal material and polysaccharide samples was performed with H_2SO_4 [18]. Sugars in the hydrolysates were determined after descending PC (solvent C) by the method of ref. [19], using appropriate correction factors. Total carbohydrate content was determined by the PhOH–H_2SO_4 method [20] with conventional, graphical calibration. Inorganic sulphate liberated by hydrolysis [2] was determined by barium chloranilate [21].

Low-molecular weight carbohydrates. Mannitol was determined by extraction with boiling 85% EtOH for 24 hr [22]. After isolation, the mp and mmp were determined. It was also identified by PC (solvent A). After removal of crystalline mannitol the remaining alcoholic extract was concd and examined by PC (solvent A).

Crude laminarin. This was determined by the method of ref. [23]. On hydrolysis with 0.3 M HCl at 100° for 2 hr, the laminarin afforded mainly glucose (PC, solvent D).

Alginic acid. This was extracted according to the method of ref. [24].

Isolation of acid-extractable polysaccharides. Algal material (15 g) was extracted (× 3) while stirring with H_2O (150 ml) adjusted to pH 1.0 with either HCl or TCA at 80° for 3 hr. In each case, the filtered combined extracts were neutralized with satd aq. Na_2CO_3, followed by dialysis against distilled H_2O for 48 hr to form a ppt. in the dialysis bag. Centrifugation of the dialysed soln afforded the supernatant and the residue which represented the H_2O-soluble and H_2O-insoluble, acid-extractable polysaccharides, respectively. The residue was then dried under red. press. at 40°. The dialysed supernatant was concd to half its vol. and treated with 4 vol. of EtOH. The H_2O-soluble polysaccharides were then isolated by centrifugation and dried under red. press. at room temp. HCl afforded samples A and B while TCA afforded samples C and D for H_2O-soluble and H_2O-insoluble polysaccharides, respectively.

Prepn of partially purified HCl-extractable water-soluble polysaccharide material. The soluble polysaccharide in a second dialysis extraction (15 g) was percolated through a column of Lewatit S 100 (H^+) resin, and the effluent dialysed against distilled H_2O (48 hr) concd to half vol. and treated with 4 vol. EtOH. The partially purified polysaccharide (P) was isolated by centrifugation, washed with EtOH and then ether before drying under red. press. at 40°.

Fractionation of polysaccharide (P). A soln of polysaccharide (P) (0.4 g.) in H_2O (40 ml) was successively treated with different portions of EtOH until its concn reached 75%. Each ppt. was isolated by centrifugation and dried under red. press. at 40° (fractions 1–5). The final supernatant was dialysed against distilled H_2O and freeze-dried (fraction 6). Large scale prepn of material (R) (Table 2) precipitating from soln of polysaccharide (P) (3 g) at a concn of 50–75% EtOH was carried out, after removal of material precipitating at low concn of EtOH.

Assay for anticoagulating activity. The method of ref. [25] described for heparin sodium was used on a 1% aq. soln of polysaccharide. The time required for the clotting of human and sheep plasma was compared with that for a standard soln of heparin.

Table 2. Composition of major fractions of partially purified extract (P)

	Fractional precipitation with ethanol			
Fraction	1–2	3	4–5–6	Polysaccharide (R)
% recovery	12.8	20.3	45.2	40.0
Glucuronic acid	33.7	23	18–22	24.0
Galactose	19.6	12.2	25–31	25.2
Glucose	—	18.3	—	—
Mannose	9.3	10	8–9	9.7
Xylose	15	16.1	14–18	15.9
Fucose	22.4	20.4	24–33	25.2

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