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SULPHATED HETEROPOLYSACCHARIDES FROM *PADINA PAVONIA*

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Key Word Index—*Padina pavonia*; Phaeophyceae; brown seaweed; heteropolysaccharide sulphate; glucuronic acid; galactose; fucose; glycoproteins.

Abstract—Extraction with hydrochloric acid (pH 2.5) of the brown alga *Padina pavonia* afforded water-soluble and water-insoluble polysaccharides comprising D-glucuronic acid, L-fucose, D-xylose, D-mannose, D-glucose and D-galactose residues. The water-soluble polysaccharide was fractionated by using ethanol, and cetylpyridinium chloride and by chromatography on DEAE-cellulose. A neutral laminaran-like glucan, a sulphated heteropolysaccharide composed of the aforementioned sugars and a protein moiety were obtained. The isolated heteropolysaccharide showed high anticoagulation activity.

INTRODUCTION

The sulphated heteropolysaccharides of brown algae have been the subject of many studies [1–6]. Mian and Percival [7] reported that 'fucans' which were present in five sequential extracts of *Padina pavonia*, *Himanthalia lorae*, and *Bifurcaria bifurcata* comprised variable proportions of fucose, xylose, glucuronic acid, galactose (traces), and half-ester sulphate. The present paper deals with the chemical composition of the local seaweed *Padina pavonia*, and the isolation and purification of sulphated heteropolysaccharide containing glucuronic acid, fucose, xylose, mannose, glucose and galactose residues.

RESULTS AND DISCUSSION

The composition of *Padina pavonia* was found to be as follows: 18% ash, 2.5% calcium, 2.6% magnesium, 0.7% sodium, 0.4% phosphorus, 240 ppm potassium, 166 ppm manganese, 120 ppm zinc, 50 ppm copper, 2.3% mannitol, 1.5% crude laminaran, 15% alginic acid, 17% total lipids and 25% crude proteins. Traces of free glucuronic acid, glucose and xylose were detected (solvent A) in the alcoholic extract of alga after removal of mannitol. Paper chromatography (solvents B and C) of the hydrolysate of the algal material afforded 29, 16, 16, 15, 15, 14, 14, 12, 11, 10, 9, 8, 3, 2, 2, 2 and 1 mg/g dry alga of glycine, threonine, phenylalanine, serine, aspartic acid, leucine, alanine, glutamic acid, isoleucine, tyrosine, methionine, valine, arginine, cysteine, lysine, histidine, tryptophan and proline, respectively. Complete acid hydrolysis of the algal material, after removal of the low MW carbohydrates, followed by paper chromatography (solvent A) of the hydrolysate afforded uronic acids (mannuronic acid + guluronic acid + glucuronic acid) (16.3%), glucose (10.4%), fucose (3.8%), galactose (3.3%), mannose (2.4%) and xylose (1.6%).

Acid extraction (HCl, pH 2.5) of *P. pavonia* led to the isolation of water-insoluble (A) and water-soluble (B) polysaccharide materials. The polysaccharide A (~8%) was rich in ash (36.7%) and comprised 21% total carbohydrates and 15.7% protein. The ash of A was found to contain 3.8% calcium and 0.7% magnesium. Acid hydrolysis of A gave (PC, solvent A) glucuronic acid, glucose, mannose, galactose, xylose, and fucose in the proportions of about 36.1, 19.0, 14.2, 12.8, 9.8, and 8.4% respectively. A similar hydrochloric acid-extractable water-insoluble polysaccharide has been, however, isolated from the local brown algal species *Colpomenia sinuosa* [6].

The water-soluble polysaccharide B (~70%) after partial purification gave polysaccharide P (6% weight of alga) which comprised 74.5% of total carbohydrates, 11.8% protein, 17.6% sulphate. Determination of calcium and magnesium in the ash of P gave 0.2 and 2.3% of these cations, respectively. Acid hydrolysis of P gave (PC, solvent A) glucuronic acid (33.6%), fucose (17.9%), xylose (15.3%), mannose (13.9%), glucose (11.2%) and galactose (8.2%). The presence of glucose was confirmed with D-glucose oxidase.

Three fractionation methods of P were attempted. Precipitation with increasing concentrations of ethanol from 17 to 75% gave six fractions, all of which contained the monosaccharides present in the original material, with no significant differences in their proportions. Attempted fractionation with cetylpyridinium chloride resulted in excessive loss of polysaccharide material, 27 mg carbohydrate being recovered from 1.5 g P (i.e. from 1.1 g carbohydrate). Even so, each of the seven fractions separated contained all the monosaccharides present in P, again with no significant fractionation.

Preliminary fractionation of P on DE-52 cellulose and elution with water followed by gradient elution from 0 to 2 M NaCl and then graded elution with 0.25 and 0.5 M NaOH gave 12 fractions. The aqueous eluant contained ~5% of glucan thought to be laminaran. Analysis of each of the remaining 11 fractions revealed minor differences in the proportions of the individual monosaccharides, but no fraction was devoid of any of the monosaccharides. It can

be concluded from these fractionation experiments that, as with 'fucans' from other species of brown seaweeds [7], extract P contains a family of similar macroheteromolecules. However, this is the first time that glucose has been found to be a constituent of this type of heteropolymer.

As a result of these experiments, large scale removal of the glucan from P on DE-52 cellulose was carried out. After elution with water, the acid polysaccharide (~4% of the alga) was eluted with 2 M NaCl. Analysis of the pure polysaccharide revealed that it had $[\alpha]_D - 76°$ and contained 76.6% total carbohydrates, 4.8% protein and 18.6% SO_4. Acid hydrolysis of this algal polymer afforded glucuronic acid, fucose, xylose, mannose, glucose, and galactose in the molar ratio 2.8:1.5:1.5:1.2:1.2:1.0, respectively. IR spectra of purified P gave stretch bands at 2960 cm^{-1} (C—H bond in methyl group of fucose), at 1240 and 1650 cm^{-1} (half ester sulphate and carboxyl groups); and at 810–840, 860–885 (indicative of both half-ester sulphate groups and α- and β-linkages) thus confirming many of the other results.

The new isolated algal polysaccharide had higher anticoagulation activity than heparin. Under conditions where human and sheep plasma coagulated after 1 hr in the presence of standard heparin solution, coagulation did not occur until after 3 days in the presence of the algal product. Similar results were, however, found for the sulphated polysaccharides isolated from *Chordaria firma* [8] and *Sargassun linifoliun* [9].

EXPERIMENTAL

General. Details are given in a previous paper [9]. The following additional techniques were used. After ashing, metals were determined using atomic absorption spectrophotometry. For determination of total phosphorus, the algal material was wet ashed with H_2SO_4–$HClO_4$–HNO_3 (1:2:3). The phosphorus content of the resulting ash was determined by the method of ref. [10]. Lipids were isolated from the alga by Soxhlet extraction with petrol (bp 40–60°) for 12 hr. Characterization of glucose was achieved using the method of ref. [11]. Determination of amino acids in the algal hydrolysate (hydrolysis with 6 N HCl for 24 hr at 105°) was done after PC (solvent Band C) and elution from the chromatogram by the method of ref. [12]. Determination of IR spectrum of the purified heteropolysaccharide was carried out according to the method of ref. [13].

Padina pavonia was collected in August 1974 from Roushdy at Alexandria. The alga was washed to remove foreign substances and then dried and milled.

Carbohydrates of low MW. Mannitol was determined by extraction with boiling 85% EtOH for 24 hr [14]. After isolation, the mp and mmp were determined. It was also identified by PC (solvent B). After removal of crystalline mannitol, the remaining alcoholic extract was concd and examined by PC (solvents A and B).

Crude laminaran. This was determined by extraction according to the method of ref. [15]. On hydrolysis with 0.3 N HCl at 100° for 2 hr, the resulting crude laminaran afforded mainly glucose (PC, solvents A and B).

Alginic acid. Determination of alginic acid was achieved according to the method of ref. [16].

Preparation of the partially purified acid-extractable water-soluble polysaccharide material P. The algal material (330 g) was treated as for *Sargassum linifolium* [9] except that the pH was 2.5 and the extraction was at 90°, instead of pH 1 and 80°. The residue (A) which was discarded in *Sargassum* studies was analysed in the present studies. The partially-purified water-soluble polysaccharide P (20 g) was isolated as before.

Preparation of the purified polysaccharide. A soln of P (8 g) in H_2O (300 ml) was added to a column (5 × 28 cm) of DEAE-cellulose (D52) (Cl^-). After allowing the polysaccharide soln to drain in, the column was washed with H_2O until effluent was free from carbohydrates [9]. The column was then eluted with 2 M NaCl till the eluate gave negative test for carbohydrates [9]. After dialysis against dist. H_2O, the polysaccharide soln was concd to half its vol., pptd with 4 vol. EtOH, isolated by centrifugation and dried under vacuum (yield ~5 g).

Assay for anticoagulation activity. The method [9] described for beparin sodium was used on a 1% aq. soln of the pure polysaccharide. The times required for clotting of human and sheep plasma were compared with those of a standard heparin soln.

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