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CARBOHYDRATES OF THE BROWN SEAWEED *PADINA PAVONIA*

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

Abstract—The carbohydrate composition of the brown alga *Padina pavonia* was investigated. Periodate oxidation of the isolated alginic acid showed that only 0.496 mole of the oxidant was reduced per anhydro-uronic acid residue. Extraction with HCl (pH 1.5) afforded water-soluble and water-insoluble polysaccharide materials. The former, when fractionated with cetylpyridinium chloride, gave a sulphated heteropolysaccharide material containing glucuronic acid, fucose (major), glucose, mannose, and xylose, and also a mannan linked to a protein moiety. The water-insoluble material was an acid glycoprotein, the carbohydrate portion of which consisted of glucuronic acid (major) and galactose (minor).

INTRODUCTION

Brown algae contain a variety of polysaccharides. Although alginic acid has long been known to be the major polysaccharide component of these algae, yet the isolation of others such as fucose-containing polysaccharides have been described [1–4]. Studies on the latter, however, are not comprehensive.

This paper deals with the structural features of alginic acid isolated from a local alga, *Padina pavonia*. We also report the isolation of a sulphated heteropolysaccharide containing glucuronic acid, fucose, and a protein moiety, and two new glycoproteins, namely, a neutral glycoprotein containing mannose, and an acid glycoprotein containing glucuronic acid.

RESULTS AND DISCUSSION

P. pavonia contains *ca* 15% alginic acid, 2% crude laminarin, 8% mannitol, 8% acid-extractable polysaccharides, 12% crude protein and 29% ash. No low MW carbohydrates were found in the alcoholic extract after removal of mannitol. Complete acid hydrolysis of the algal material, after removal of mannitol, followed by PC. of the hydrolysate (solvent C) afforded mannuronic acid (9.7%), guluronic acid + glucuronic acid (5.7%), glucose (3%), galactose (3.2%), mannose (1.2%), fucose (1.2%), and xylose (0.2%). These results are in partial agreement with those found for *P. pavonia* by Mian and Percival [4]. However, the latter authors did not find mannose residues in the fucans of 5 sequential extracts of the alga collected in Jamaica. In the present work, the presence of mannose as a constituent of our alga was confirmed by its identification, after elution from PC, as its crystalline phenylhydrazone [5] (mp and mmp 199–200°). Furthermore, mannose has also been found as a constituent of the brown seaweed *Sargassum linifolium* [3]. Such differences in the composition of the same alga collected from different regions may be due to seasonal changes or to differences in climatic conditions.

Alginic acid

Oxidation of alginic acid by periodate resulted in reduction of 0.496 mole of oxidant per anhydrouronic acid unit in 30 hr; no further reduction occurred by extending the reaction time to 100 hr. The low reduction of periodate indicates the formation of acetal-linkages during oxidation [6, 7].

Acid hydrolysis of the polytricarboxylic acid gave (PC, solvents C, D, E) L(+) threaric, erythraric, glyoxylic, mannuronic and guluronic acids as well as mannurone and gulurone. The isolation of erythraric and threaric acids demonstrated the presence of (1 → 4)-linked mannuronic and/or guluronic acid residues in alginic acid. However, the detection of mannuronic acid, guluronic acid and their lactones among the hydrolysis products of the polytricarboxylic acid provided additional evidence for the formation of acetal-linkages during oxidation of alginic acid by periodate. Further support of these conclusions was obtained by reduction of the oxo-alginic acid, followed by acid hydrolysis, which yielded (PC, solvents F, G and H) erythritol, threitol, mannose and gulose.

Acid-extractable water-soluble polysaccharides

The HCl-extractable, H_2O-soluble polysaccharide material A (7.2%) was rich in ash (22%) and contained SO_4^{2-} (mean 12%) and protein (4.3%, after TCA treatment). Acid hydrolysis of A gave (PC, solvent C) *ca* 16% fucose, 14% mannose, 11% glucuronic acid, 1.5% glucose, and a trace of xylose. Thus A is a sulphated heteropolysaccharide material containing a protein moiety. 2D-PC (solvents A and B) of a hydrolysate of A revealed a qualitative amino acid composition similar to that of the crude, dried weed, namely, cysteic acid,

aspartic acid, glutamic acid, serine, glycine, alanine, threonine, tyrosine, leucine, isoleucine, phenylalanine, valine, methionine, histidine, and tryptophane.

Fractionation of A (1.2 g) with cetylpyridinium chloride gave a major sulphated fraction **A1** (SO_4^{2-}, 0.6 g) and a minor neutral fraction **A2** (0.15 g). The ratio of **A1** to **A2** was 4:1. **A1** was richer in protein (7.6%) than **A2** (4.3%).

Hydrolysis of **A1** gave (PC, solvent C) glucuronic acid (21.8%), fucose (28%), and minor amounts of glucose, mannose, and xylose; glucuronic acid and fucose comprised 43% and 56% of the total carbohydrate of our fucan. Compared with the fucans isolated from *P. pavonia* by Mian and Percival [4], **A1** was devoid of galactose and contained only trace amounts of xylose. Such differences may again be related to seasonal changes and also to the extraction conditions. Indeed, the last-named authors reported the isolation of fucans with different extractants, from *P. pavonia*, comprising variable proportions of fucose, xylose, glucuronic acid, galactose, and half-ester sulphate.

A2 contained protein (4.3%) and on acid hydrolysis it gave (PC, solvent C) mannose (52%) and minor amounts of fucose and xylose; mannose comprised 88% of the total carbohydrate. **A2** can therefore be considered as a mannan linked to a protein moiety, i.e. a neutral glycoprotein.

Acid-extractable water-insoluble polysaccharide

The HCl-extractable, water-insoluble material **B** (1%) differed from **A** in containing higher ash (32%) and protein (32.3%) contents, and a minor carbohydrate component (3.4%). Acid hydrolysis of **B** gave (PC, solvent C) glucuronic acid (3%) and galactose (trace); galactose was not a component of **A**. Thus, **B** is an acid glycoprotein containing glucuronic acid (major) and galactose (minor).

As far as we are aware, nothing has yet been reported on the isolation from *P. pavonia* of either an acid glycoprotein containing glucuronic acid (**B**) or a neutral glycoprotein containing mannose (**A2**).

EXPERIMENTAL

Padina pavonia, a brown alga, was collected in April 1972 from Roushdy at Alexandria. The alga was washed with H_2O to remove foreign substances, and then air dried and milled.

General. Chromatography on Whatman No. 1 paper was performed with the following solvent systems: A *n*-BuOH–HOAc–H_2O [8] (4:1:5, upper layer), B PhOH–H_2O [9] (4:1), C *n*-BuOH–C_5H_5N–H_2O [10] (6:4:3), D EtOAc–HOAc–HCO_2H–H_2O [11] (18:4:1:5), E EtOAc–HOAc–H_2O [11] (3:1:3), F EtCOMe–HOAc–H_2O [12] (9:1:1) satd with boric acid, G *n*-BuOH–EtOH–H_2O (40:11:19), H EtOAc–HOAc–HCO_2H–H_2O (18:3:1:4). Detection was effected with aniline hydrogen phthalate, ammoniacal $AgNO_3$, aniline–xylose and ninhydrin reagents [13]. Ash contents were determined by heating polysaccharide samples to constant wt at 800°. Protein was determined for H_2O-soluble and H_2O-insoluble samples by the method of ref. [14] and by the micro-Kjeldahl method, respectively. Complete acid hydrolysis of algal material and polysaccharide samples was performed with H_2SO_4 [15]. Determination of sugars in the acid hydrolysates was done after ascending PC (solvent C) and elution from the chromatograms; uronic acids were determined by reaction with carbazole [16], xylose with orcinol [17] and galactose, mannose, glucose and fucose with L-cysteine-H_2SO_4 [18, 19]. Sulphate was determined by two methods; the polysaccharide material was titrated with cetylpyridinium chloride [20] while the total inorganic sulphate liberated by hydrolysis [2] was determined by barium chloranilate [21]. All solns were concnd *in vacuo* at 40° in a rotary evaporator.

Low-MW 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 concnd and then examined by PC (solvent C).

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 C).

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

Periodate oxidation of alginic acid. (a) Alginic acid (0.54 g) dispersed in cold acetate buffer (200 ml, pH 3.8) was treated with 30 mM $NaIO_4$ (200 ml) and the reaction mixture was left, with occasional shaking, for 100 hr in the dark at 2°. Aliquots (5 ml) were withdrawn at intervals and the periodate consumed was determined [25]. (b) Alginic acid (3 g/100 ml H_2O) was added to Na metaperiodate (15.8 g/100 ml H_2O), and the mixture was stored at room temp. for 24 hr with continuous shaking. The oxo-alginic acid, isolated by precipitation with *t*-BuOH was further oxidised with Br_2 as reported earlier [11]. The final hydrolysate was chromatographed using solvents C, D, and E. (c) Following the method of ref. [12] the periodate-oxidised alginic acid was reduced and the product hydrolysed. The final hydrolysed solution was concentrated and chromatographed using the solvents F, G, and H.

Isolation of acid-extractable polysaccharides. Algal material was twice extracted while stirring with H_2O adjusted to pH 1.5 with HCl at 80° for 1.5 hr. After filtration, the combined extracts were neutralised with satd aq Na_2CO_3, followed by dialysis against H_2O for 72 hr; a ppt. formed in the dialysis bag. Centrifugation of the dialysed soln afforded the supernatant (**A**) and the residue (**B**) which represented the H_2O-soluble and H_2O-insoluble, acid-extractable polysaccharides, respectively. The residue was then dried under red. pres. at 40°. The supernatant (**A**) was treated with TCA to give a final conc of 12.5%. The precipitated proteins were removed from the supernatant by centrifugation and excess TCA was removed by extraction (×3) with an equal vol. of Et_2O. The aq. layer was then dialysed for 3 days against H_2O and the dialysed soln lyophilized.

Fractionation of the acid-extractable water-insoluble polysaccharide. A solution of A (1.2 g) in H_2O (100 ml) containing Na_2SO_4 (0.4 g) was treated with aq. 2.5% cetylpyridinium chloride until no further precipitation occurred. The ppt. (fraction **A1**) was isolated by centrifugation in the presence of celite while the supernatant (fraction **A2**) was dialysed against H_2O and freeze-dried. Polysaccharide material was recovered from the ppt. (**A1**) by several extractions of the celite with 4 M KCl. Excess cetylpyridinium chloride was then removed by precipitation with M K thiocyanate, followed by dialysis of the supernatant against H_2O. The dialysed soln was then freeze-dried.

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