Phytochemistry, Vol 23, No 11, pp 2531–2533, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00

CARBOHYDRATES OF *PADINA TETRASTROMATICA**

N V S A V PRASADA RAO, K V SASTRY and E VENKATA RAO†

Department of Pharmaceutical Sciences, Andhra University, Visakhapatnam, India

(*Revised received* 6 *April* 1984)

Key Word Index—*Padina tetrastromatica*, Phaeophyceae, brown seaweed, sulphated heteropolysaccharide

Abstract—Extraction with hydrochloric acid (pH 2 5) of the brown alga *Padina tetrastromatica* afforded water-soluble and water-insoluble polysaccharides The water-soluble polysaccharide was fractionated using cetyltrimethyl ammonium bromide and chromatography on DEAE-cellulose and Sephadex G-100 A neutral laminaran like glucan and two new sulphated heteropolysaccharides comprising D-glucuronic acid, L-fucose, L-rhamnose, D-xylose, D-arabinose, D-galactose, D-glucose and half-ester sulphate were obtained The alginic acid isolated from this brown seaweed was found to be predominantly of poly 1 → 4β-D-mannuronic acid type The water-soluble sulphated polymer showed high anticoagulant activity

INTRODUCTION

Studies on marine brown algae have been carried out extensively because of the commercial importance of their carbohydrate constituents like alginic acid, mannitol and laminaran Although alginic acid was known to be the major polysaccharide of these algae, isolation of others such as fucose containing polysaccharides have also been described [2, 3] Mian and Percival reported [4] that fucans extracted from *Padina pavonia, Himanthalia lorea* and *Bifurcaria bifurcata* comprised variable proportions of fucose, xylose, galactose, glucuronic acid and half-ester sulphate Abdel-Fattah *et al* reported [5] the isolation of a sulphated heteropolysaccharide attached to a protein moiety and two new glycoproteins from *P pavonia* Recently Magdel-Din Hussein reported [6] for the first time glucose as a constituent sugar in a sulphated heteropolysaccharide of brown algae in addition to glucuronic acid, fucose, xylose, mannose and galactose This paper deals with the isolation and purification of sulphated heteropolysaccharides containing uronic acid, galactose, mannose, glucose, arabinose, xylose, fucose, rhamnose and half-ester sulphate in variable proportions from a local alga, *Padina tetrastromatica* Alginic acid was also isolated and characterized

RESULTS AND DISCUSSION

The composition of *P tetrastromatica* was found to be as follows 30% ash, 8 5 ppm sodium, 10 0 ppm potassium, 3 5 ppm zinc, 2 0 ppm nickel, 1 3 ppm manganese, 232 ppm calcium, 2 5 ppm magnesium, 20 0 ppm iron, 1 0 ppm copper, 0 25% phosphorus, 3% mannitol, 2 1% crude laminaran, 14 3% alginic acid, 5% total lipids and 16 1% crude protein 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 afforded uronic acids (mannuronic acid + glucuronic acid, 18 1%), glucose (6 1%), fucose (4 1%), galactose (2 3%), mannose (2 0%), xylose (1 1%) and traces of rhamnose and arabinose

Alginic acid

This was extracted with 1% aq sodium carbonate solution and purified by potassium chloride fractionation The purified sodium alginate has viscosity of 260 cps (1% aq solution) Periodate oxidation studies of alginic acid demonstrated the presence of (1 → 4) linked mannuronic acid units However guluronic acid and gulose were present in traces in the hydrolytic products of oxo-alginic acid and reduced oxo-alginic acid respectively This, together with the results of acid hydrolysis of the alginic acid shows that mannuronic acid constituted the major portion of the polymer

Acid extractable polysaccharides

Acid extraction (HCl, pH 2 5) of *P tetrastromatica* led to the isolation of a water-insoluble (A) and water-soluble (B) polysaccharide materials Polysaccharide A (~ 0 1%) was rich in ash (39 1%) and comprised 25% carbohydrates and 10 7% protein Acid hydrolysis of A gave (PC, solvent A) glucuronic acid, galactose, mannose, xylose, arabinose, fucose and rhamnose in the mol proportions of about 11 7 15 9 21 0 3 6 1 0 18 5 1 3 respectively In view of the poor yield, insolubility and high ash content further work on A was not carried out

The water-soluble polysaccharide B (~ 2%) after partial purification comprised 55% carbohydrates, 8 2% protein and 4 9% sulphate Acid hydrolysis of B (PC, solvent A) gave glucuronic acid (31 1%), galactose (9 3%), glucose (28 5%), xylose (2 1%), fucose (13 1%) and rhamnose (2 0%) Preliminary fractionation of B with cetyltrimethylammonium bromide gave a major fraction B_1 (protein, 2 1%) and a minor fraction B_2 (protein, 8 1%) The ratio of B_1 to B_2 was 9 1

Fractionation of B_1 on DEAE-cellulose (Cl^-) and

*"Studies on Indian Seaweed Polysaccharides", Part 2 For Part 1 see ref [1]

†To whom correspondence should be addressed

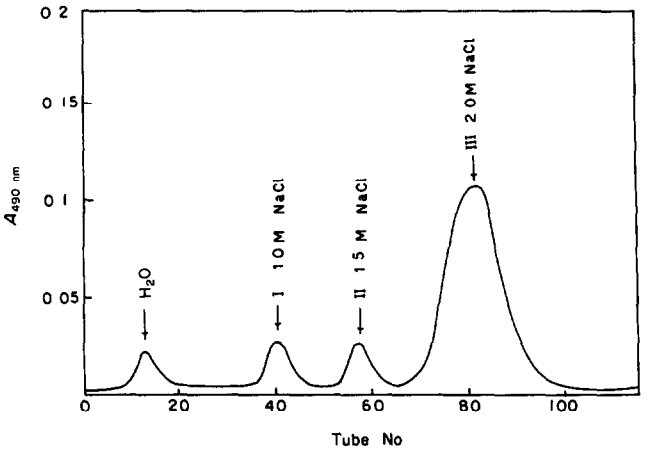

Fig 1 Chromatography of water-soluble polysaccharide on DEAE-cellulose

elution with water followed by graded elution with 0–2 M sodium chloride gave four fractions (Fig 1) The aq eluant (~ 1%) contained a glucan presumably laminaran Analysis of each of the remaining three fractions revealed no significant differences in the proportions of the individual monosaccharides Hence one of the fractions B_1-III which was obtained in fairly large amount from the 2 M sodium chloride eluant was taken up for further study It gave a single peak over a column of Sephadex G-100 eluted with 1 M sodium chloride and also moved as a single band in paper electrophoresis indicating the homogeneity of the polysaccharide

Analysis of the pure polysaccharide, B_1-III, $[\alpha]_D$ −79° (H_2O, *c* 1 0) showed 65 4% carbohydrates, 2% protein, 20% ash and 5 9% half-ester sulphate The low sulphate content of this polymer is comparable with the sulphate content (~ 6%) of the heteropolysaccharide reported from *Dictyota plagiogramma* by Percival *et al* [7], but differs from that of *P povonia* (SO_4^{2-}, 18 6%) Acid hydrolysis of the polysaccharide B_1-III afforded glucuronic acid, fucose, rhamnose, xylose, mannose, galactose and glucose in the mol ratios 13 4 6 6 1 34 1 0 7 6 5 1 14 4 respectively

IR spectra of the polysaccharide B_1-III gave stretch bands at 2960 cm^{-1} (C–H band in methyl groups of fucose and rhamnose) at 1650 cm^{-1} (carboxyl groups) and very weak absorption at 1240 cm^{-1} (half-ester sulphate) In paper electrophoresis fraction B_2 moved as a single band showing its homogeneity Acid hydrolysis of the polymer gave (PC, solvent A) the same monosaccharides in same mol proportions that are present in B_1-III fraction To the best of our knowledge this is the first report of a brown algal polysaccharide containing rhamnose and arabinose among other sugars

The water-soluble sulphated polysaccharide showed high anticoagulant activity Under conditions where plasma coagulated after 2 hr in the presence of standard heparin solution, coagulation did not occur until 58 hr in the presence of the water-soluble polysaccharide

EXPERIMENTAL

Padina tetrastromatica, a local brown alga, was collected in February, 1982 at Rishikonda near Visakhapatnam coast The alga was washed with H_2O, air dried and milled

General The homogeneity of the polysaccharide was tested by paper electrophoresis (6 5 V/cm^2) using C_5H_5N–HOAc buffer (0 05 M) at pH 6 0 PC on Whatman No 1 was performed in the solvent systems (A) *n*-BuOH–C_5H_5N–H_2O, 6 4 3, (B) upper layer of *n*-BuOH–EtOH–H_2O, 4 1 5, (C) EtOAc–C_5H_5N–H_2O, 10 4 3, (D) MeCOEt–HOAc–H_2O (9 1 1) saturated with boric acid and (E) PhOH saturated with H_2O Detection was effected with 1 alkaline $AgNO_3$ and 2 *p*-anisidine HCl Determination of the sugars in the acid hydrolysate was done by descending PC and elution from the chromatograms, uronic acids were determined by reaction with carbazole–H_2SO_4 and neutral sugars with PhOH–H_2SO_4 Determination of neutral sugars in the acid hydrolysate was also done by GC (FID) after converting them into their alditol acetates Resolutions were performed in glass columns containing 3% ECNSS-M on Gas-Chrom Q (100–200 mesh) at 190° After ashing, metals were determined using Shimadzu Atomic Absorption Spectrophotometer AA-640-12 model The algal material was wet-ashed with H_2SO_4–$HClO_4$–HNO_3 (1 2 3) for total phosphorus estimation by the method of Fiske and Subbarao Protein was determined for H_2O-soluble and H_2O-insoluble samples by Lowry and microkjeldhal methods respectively Lipid content was determined by Soxhlet extraction with *n*-hexane for 12 hr Sulphate was estimated by the method using barium chloranilate [8]

Carbohydrates of low MW Mannitol was determined by extraction with boiling 85% EtOH for 24 hr [9] After isolation mp and mmp was determined and also identified by PC (solvents A and B) After removal of crystalline mannitol the alcoholic extract was concd and then examined by PC (solvents A, B and C)

Crude laminaran This was determined by extraction according to the method of ref [9] On hydrolysis with 1 M HCl at 100° for 3 hr laminaran afforded mainly glucose

Alginic acid Determination of alginic acid was achieved according to method of ref [10] The actual isolation and periodate oxidation of alginic acid were done following the method of ref [11]

Isolation of acid extractable polysaccharides Algal material (500 g) was twice extracted at 85° for 2 hr with H_2O adjusted to pH 2 5 After filtration the combined extracts were neutralized with satd Na_2CO_3 and the ppt (A) formed was collected by centrifugation, which represented H_2O-insoluble polysaccharide (0 47 g) The supernatant was poured into 4 vols of EtOH to obtain polymer B (H_2O-soluble polysaccharide, 10 2 g) which was collected at the centrifuge It was redissolved in H_2O (25 ml)

and treated with TCA (25 ml of 25% soln) The precipitated proteins were removed from the supernatant by centrifugation and excess TCA was removed by extraction with Et_2O (3 × 50 ml) Then the soln was dialysed against H_2O for 2 days and lyophilized to yield 9 3 g

Fractionation of the acid extractable H_2O*-soluble polysaccharide* A soln of B (2 1 g, recovered after TCA treatment) in H_2O (200 ml) containing Na_2SO_4 (0 6 g) was treated with 1% aq cetyltrimethylammonium bromide until no further precipitation occurred The ppt (Fraction B_1) was isolated by centrifugation in the presence of celite while the supernatant (Fraction B_2) was dialysed against H_2O and lyophilized Polysaccharide material was recovered from the ppt (B_1) by several extractions with 2 M NaCl soln Excess cetrimide was then removed by precipitation with 1 M KCNS, followed by dialysis of the supernatant and precipitation of the polysaccharide (B_1 1 6 g)

A soln of B_1 (1 5 g) in H_2O (60 ml) was added to a column (3 × 35 cm) of DEAE-cellulose (Cl^- form) After allowing the polysaccharide soln to drain in, the column was washed with H_2O until the effluent was free from carbohydrate The column was then eluted with 0–2 M NaCl soln till the eluate gave a negative test for carbohydrates The carbohydrate content in each fraction was estimated by the PhOH–H_2SO_4 reaction After dialysis against H_2O the polysaccharide solns were concentrated, precipitated with 4 vols EtOH, isolated by centrifugation and dried under vacuum (yield 1 3 g) The major fraction, obtained after DEAE-cellulose fractionation, was applied as a concd soln on a column of Sephadex G-100 Then it was eluted with 1 M NaCl soln and processed further as above

Assay for anticoagulation activity The method [12] described for heparin sodium was used on 0 5% aq solns of H_2O-soluble sulphated polymer The time required for the clotting of blood plasma was 58 hr, compared with that of 2 hr for standard soln of heparin

Acknowledgements—The authors thank Dr M Umamaheswara Rao, Reader, Department of Botany, Andhra University for identification of the seaweed, Prof C V N Rao, Head of the Department of Macromolecules, IACS, Calcutta for the GLC results, and the CSIR, New Delhi for providing Senior Research Fellowship (to N V S A V P R)

REFERENCES

1 Prasada Rao, N V S A V, Sastry, K V and Venkata Rao, E (1982) *Ind J Pharm Sci* **44**, 144
2 Haug, A and Larsen, B (1963) *Acta Chem Scand* **17**, 1653
3 Percival, E and McDowell, R H (1981) in *Encyclopedia of Plant Physiology* (Tanner, W and Loewus, F A, eds) Vol 13B, pp 277–316 Springer, Berlin
4 Mian, A T and Percival, E (1973) *Carbohydr Res* **26**, 133
5 Abdel-Fattah, A F and Edrees, M (1977) *Phytochemistry* **16**, 939
6 Magdel-Din Hussein, M, Abdel-aziz, A and Mohamed Salem, H (1980) *Phytochemistry* **19**, 2131
7 Percival, E (1980) *Phytochemistry*, **20**, 3865
8 Lloyd, A G (1959) *Biochem J* **72**, 133
9 Black, W A P, Cornhill, W J, Dewar, E T and Woodward, F N (1951) *J Appl Chem* **1**, 505
10 Cameron, E, (1948) *J Soc Chem Ind* **67**, 161
11 Drummond, D W, Hirst, E L and Percival, E (1962) *J Chem Soc* 1208
12 *The United States Pharmacopeia* (1980) Twentieth Revision, p 365 Mack Publishing Company, Easton