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SARGASSAN: A SULPHATED HETEROPOLYSACCHARIDE FROM *SARGASSUM LINIFOLIUM*

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Abstract—A new sulphated heteropolysaccharide containing glucuronic acid, mannose, galactose, xylose, fucose and a protein moiety has been extracted from *Sargassum linifolium* The polysaccharide extracted with HCl was richer in its carbohydrate and protein contents and contained lower amounts of ash than that extracted with oxalic acid

INTRODUCTION

IN 1955, Fischer and Dorfel[1] reported glucuronic acid as a constituent of many brown algal species Percival and McDowell[2] have also reported the presence of uronic acid-containing polysaccharides in all the major classes of seaweeds On the other hand, a highly purified preparation of fucoidan extracted from the brown seaweed *Himanthalia lorea* was found to contain, in addition to ester sulphate and fucose, about 5% galactose, 2% xylose and 3% uronic acid[3] Likewise, a purified preparation of *Macrocystis* fucoidan was shown by Schweiger[4] to contain proportions of galactose and xylose Accordingly, Schweiger concluded that the presence of a pure fucan as a major constituent of *Macrocystis* is unlikely.

Recently, Larsen *et al*[5] have reported the isolation of another fucose-containing polysaccharide, ascophyllan, from *Ascophyllum nodosum* This polysaccharide contained 25 2% fucose, 26% xylose, 19 2% sodium uronate, about 12% ester sulphate (as $NaSO_3$) and 12% protein Partial acid hydrolysis indicated a glucuronic acid backbone with side chains composed of fucose, xylose and half-ester sulphate. Additional sulphated glucuronoxylofucan containing L-fucose (49%), D-xylose (10%), D-glucuronic acid (11%), sulphate (20%) and protein (3 8%) has also been recently isolated from the cell-walls of *Ascophyllum nodosum*[6] This was achieved by extraction with ammonium oxalate-oxalic acid at 80° after removal from the weed of laminaran, fucoidan and the major part of alginic acid

According to these recent studies, many authors[2,5,6] are now of the opinion that the type and variety of algal fucose-containing polysaccharides is much wider than originally believed Therefore, Percival and McDowell[2] consider it undesirable to extend the name

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[2] PERCIVAL, E and MCDOWELL, R H (1967) *Chemistry and Enzymology of Marine Algal Polysaccharides*, Academic Press, London
[3] BERNARDI, G and SPRINGER, G F (1962) *J Biol Chem* **75**, 237
[4] SCHWEIGER, R G (1962) *J Org Chem* **27**, 4267, 4270
[5] LARSEN, B, HAUG, A and PAINTER, T J (1966) *Acta Chem Scand* **20**, 219
[6] PERCIVAL, E (1968) *Carbohyd Res* **7**, 272

fucoidan to include the fucose-containing polysaccharides The present paper describes the isolation of a new sulphated heteropolysaccharide containing glucuronic acid, fucose and other sugars from *Sargassum linifolium*

RESULTS AND DISCUSSION

The percentage composition of *Sargassum linifolium* was found to be 22 15% alginic acid, 3 01% laminaran, 6 32% mannitol, 17 22% crude protein and 19 58% ash (inc Ca^{2+} and Mg^{2+}) Acid hydrolysis of the algal material afforded mannuronic acid, glucuronic acid, guluronic acid and their respective lactones as well as galactose, glucose, mannose, xylose and fucose No low MW carbohydrates were found in the algal alcoholic extract after removal of mannitol Mannuronic and guluronic acids were thus derived from alginic acid while glucose was from laminaran On the other hand, glucuronic acid, mannose, galactose, xylose and fucose constituted other polysaccharide materials All these sugars, except mannose and xylose, were found as constituents of *Sargassum linifolium* using algal samples which had been freshly collected periodically[7] in 1967–8 The apparent absence of mannose and xylose among the hydrolysis products of the aforementioned algal samples might be due to the unsuitability of the solvent used Mannose has been also reported as a constituent of the brown algal species *Fucus vesiculosus*,[8] *Macrocystis pyrifera*[4] and *Ascophyllum nodosum*[9 10]

pH 1 0 was found to be the best for obtaining a maximum yield of the acid-extractable polysaccharides, i e extraction either with HCl solution at 80° for 3 hr or with oxalic acid solution at 90° for 4 hr In both cases, three successive extractions of the residue gave only a further 10% of material

Complete acid hydrolysis of the water soluble polysaccharide afforded glucuronic acid, galactose, glucose, mannose, xylose and fucose Chromatographic separation of these sugars was achieved in butanol–ethanol–water, with the papers being developed for 48 hr Besides allowing for a sharp separation of galactose, glucose and mannose, this time of development was also suitable for fucose detection Preparative chromatography in the same system on Whatman 3 MM paper gave sufficient mannose for its characterization. The eluate containing mannose was evaporated under reduced pressure and then heated in a sealed tube with 0 3 N HCl at 97° for 4 hr [11] PC of the hydrolysate revealed only mannose, showing that complete acid hydrolysis had occurred and that there was no confusion with the aldobiouronic acid, glucuronosyl-fucose which has a similar R_f The isolated mannose was, further characterized as its phenylhydrazone (m p and m m p 199–200°) Mannose has previously been detected in fucoidan[4] and in a sulphated polysaccharide[10] isolated from the brown algal species *Macrocystis pyrifera* and *Acsophyllum nodosum*, respectively

The proportions of sugars in the algal polysaccharide differed according to the conditions of extraction and the extracting agent, but it was consistently rich in glucuronic acid, galactose and fucose HCl was more efficient than oxalic acid in extracting products containing high proportions of glucuronic acid and galactose On the other hand, extraction with oxalic acid afforded products rich in glucose, mannose, xylose and fucose In both cases, glucose was a minor component A rise in the temperature of the extraction led to

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[8] DEWAR, E T (1954) *Chem Ind (London)* 785
[9] MANSKE, R H F (1930) *J Biol Chem* **86**, 571
[10] LARSEN, B , HAUG, A and PAINTER, T (1970) *Acta Chem Scand* **24**, 3339
[11] PERILA, O and BISHOP, C T (1961) *Can J Chem* **39**, 815

significant increase in glucuronic acid and decrease in fucose; extraction of combined mannose and combined galactose was also favoured at high temperatures

Hydrolysis of the acid-extractable water insoluble polysaccharide yielded only glucose This polysaccharide may thus be laminaran, which is known to be a component of *Sargassum linifolium* and other brown algae [7]

Although the acid-extractable water soluble polysaccharide were deionized by dialysis, it contained high amounts of ash Furthermore, by purification and treatment with ion exchange resins the product still contained appreciable amounts of ash The presence of such high amounts of ash must be attributed to the presence of sulphate Indeed sulphate was found in the purified polysaccharide extracted with HCl at 80° for 3 hr· 11 45% by titrating the intact polysaccharide with cetylpyridinium chloride[12] and 15 40% by determination with barium chloranilate[13] after hydrolysis with HCl, respectively. The agreement between the values of sulphate determined by the two methods indicates that all or most of the sulphate is present as half-ester

Purification of the polysaccharide increased its carbohydrate content but did not lead to the removal of any of the sugar components. On the other hand, deproteinization with trichloroacetic acid did not achieve complete removal of protein and 3 3% (Folin reagent)[14] were still present in the purified material Moreover, the product had a brown colour which could not be removed by treatment with charcoal. However, it gave negative reaction with ninhydrin [15] These results collectively suggest the presence of a protein linked to the polysaccharide The presence of protein moieties in sulphated polysaccharides isolated from brown algae has recently been reported [5,6]

EXPERIMENTAL

Specimen Sargassum linifolium was collected by Edfina Co for canned foods from the Alexandrian coast during 1969 The algae were washed with running water to remove foreign substances, spread and left in the sun for several days and finally milled The values were calculated on a dry wt basis

Determination of ash, calcium and magnesium After ashing at 800° Ca and Mg were determined complexometrically according to the method of Flaschka [16]

Protein Organic N was determined by Kjeldahl's method and multiplied by 6 25 In case of the purified polysaccharide, protein determination was done by the method of Lowry *et al* [14]

Mannitol This was determined by extraction with boiling 85% EtOH for 24 hr [7] After isolation, the m p of crystalline mannitol and m m p were determined It was also identified chromatographically using *n*-BuOH–HOAc–H_2O (12 3 5)[17] and Dedonder reagent [18]

Low MW carbohydrates After removal of crystalline mannitol the remaining alcoholic extract of the algal material was concentrated under reduced pressure at 35° and then examined by PC *n*-BuOH–pyridine–H_2O (6 4 3)[19] was used while detection of spots was achieved by spraying with aniline diphenylamine phosphoric acid and aniline oxalate [20]

Laminaran This was determined by isolation from algae according to the method of Black *et al* [21] The isolated laminaran was partially hydrolysed with 0 3 N HCl at 100 for 2 hr The hydrolysate was then chromatographed on Whatman No 1 paper using *n*-BuOH–pyridine–H_2O (6 4 3) The glucose produced was detected by spraying with ammoniacal $AgNO_3$ [18]

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Alginic acid Alginic acid was extracted with Na_2CO_3 according to the method of Abdel-Fattah *et al* [22] After isolation by precipitation with HCl, alginic acid was washed with EtOH, ether and finally dried

Isolation of acid-extractable polysaccharides Acid soluble polysaccharides were isolated by extraction with HCl or oxalic acid solution The extraction conditions for obtaining maximum yield of the products were determined by altering the pH of the extracting medium, temperature and time of extraction In each process, 15 g of the algal material were extracted while stirring with 150 ml of the agent After filtration, the extract was neutralized with satd Na_2CO_3 and dialyzed against dist H_2O for 48 hr During that period a H_2O insoluble polysaccharide precipitated in the dialysis bag Centrifugation of the dialyzed solution afforded the residue and the supernatant which represented the H_2O insoluble and H_2O soluble acid-extractable algal polysaccharides, respectively The H_2O soluble acid-extractable polysaccharide was isolated by treating the dialyzed supernatant with 4 vol of absolute EtOH, followed by centrifugation The isolated H_2O soluble and H_2O insoluble polysaccharide materials were then dried under reduced pressure at room temp and weighed

Determination of the proportion of sugars in the isolated polysaccharides Complete acid hydrolysis of the isolated polysaccharides was achieved according to the method of Haug and Larsen [23] The hydrolysis products were then chromatographed on Whatman No 1 paper (50 cm length) using *n*-BuOH–EtOH–H_2O (40 11 19)[6] by descent After developing the paper for 48 hr, the separated sugars were determined quantitatively [24]

Total carbohydrate The carbohydrate content of each of the products was determined by the phenol–H_2SO_4 method[25] and the quantities were read off graphs constructed from measurements on solutions containing the appropriate sugars in the appropriate proportions

Aldehydic end groups This was done according to the method of Chanda *et al* [26]

Preparation of purified acid-extractable H_2O soluble polysaccharide material The algal material (15 g) was extracted with water adjusted to pH 1 0 with HCl (150 ml) at 80° for 3 hr After neutralizing the clarified extract with satd solution of Na_2CO_3, followed by dialysis against dist H_2O (48 hr), the dialyzed solution was centrifuged and the residue discarded The supernatant was then percolated through a column of Lewatit S 100 (H^+) resin and the effluent dialyzed against dist H_2O (48 hr) Thereafter, the dialyzed solution was concentrated under vacuum at 40° to half its vol and treated with 4 vol of EtOH The precipitate isolated by centrifugation, was dissolved in H_2O and trichloroacetic acid solution was added to give a final concentration of 10% The precipitated proteins were centrifuged out and removal of excess trichloroacetic acid from the supernatant was achieved by extraction (3 ×) with equal vol of Et_2O The aqueous layer was then separated and dialyzed for 2 days against dist H_2O Thereafter, the dialyzed solution was concentrated, under reduced pressure at 40°, to half its vol and treated with 4 vol of EtOH The purified polysaccharide was isolated by centrifugation, washed with EtOH, Et_2O and finally dried under vacuum at 40°

Determination of sulphate Sulphate was determined on the purified polysaccharide by two methods The intact polysaccharide material was titrated with cetylpyridinium chloride, in the presence of sufficient acid to suppress the ionization of the carboxyl groups[12] and the total inorganic sulphate liberated by hydrolysis with HCl was determined by barium chloranilate [13]

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