Pakistan J. Sci. Ind. Res., Vol., 19, Nos. 5–6, October–December 1976

CHEMICAL COMPOSITION OF THE BROWN SEAWEED CYSTOSEIRA BARBATA AND ISOLATION OF A SULPHATED HETEROPOLYSACCHARIDE

M. M. Hussein, N. M. El-Sayed and A. F. Abdel-Fattah

National Research Centre, Dokki, Cairo, A.R. Egypt

(Received May 15, 1976; revised August 23, 1976)

Abstract. The composition of the brown seaweed *Cystoseira barbata* was investigated. A sulphated heteropolysaccharide containing glucuronic acid, galactose, mannose, fucose, xylose, glucose and a protein moiety has been extracted from the alga with HCl or trichloroacetic acid. In both cases, glucose was a minor component. The proportion of sugars in the algal polysaccharide differed according to the extracting agent and the conditions of extraction.

Brown seaweeds contain a variety of polysaccharides. Attention has recently been focussed on sulphated polysaccharides and various brown algal species have been investigated for this purpose. Larsen *et al.*[1] reported the isolation of a fucose-containing polysaccharide, ascophyllan, from *Ascophyllum nodosum*. This polysaccharide contained fucose 25.2%, xylose 20%, sodium uronate, 19.2% about ester sulphate 12% and protein 12%. Another sulphated polysaccharide containing fucose 49%, xylose 10%, glucuronic acid 11% sulphate 20% and protein 3.8% has also been isolated from the cell-walls of *Ascophyllum nodosum*.[2]

Recently, Abdel-Fattah *et al.*[3] reported on the isolation from the brown alga *Sargassum linifolium* of a new sulphated heteropolysaccharide, sargassan, composed of D-glucuronic acid, D-mannose, D-xylose, and L-fucose residues, as well as a protein moiety. Evidence was obtained[4] which indicated that the carbohydrate part of sargassan involves a backbone composed of D-glucuronic aicd and D-mannose residues, with side chains containing residues of D-galactose, D-xylose and L-fucose, and having sulphate groups attached to some galactose and fucose residues.

The present paper deals with the composition of the acid-extractable polysaccharides of the brown alga *Cystoseira barbata* collected from Rasel-Hekma locality. This locality, which is about 230 kilometres from Alexandria, is unique in being mostly dominated with *Cystoseira barbata*. As far as we are aware, no studies have yet been reported on the composition of this brown algal species from Rasel-Hekma locality.

Materials and Methods

Specimen. Cystoseira barbata was collected in July 1973 from Rasel-Hekma locality, Mediterranean sea coast, Egypt. The alga was washed with running water to remove foreign substances, spread and dried in the air and finally ground. The values were calculated on a dry weight basis.

Determination of Ash, Calcium and Magnesium. After ashing at 800° Ca and Mg were determined complexometrically according to the method of Flaschka.[5]

Amino Acids. The algal material was hydrolysed in 6N HCl in a sealed tube for 16 hr at 105°. After removal of HCl by evaporation at 100° with occasional addition of water, two-dimensional paper chromatography of the hydrolysate was performed using the solvent mixture n–butanol–acetic acid–water (4:1:5, v/v)[6] and phenol–water (4:1, w°v).[7] Detection of spots was achieved with ninhydrin.[8]

Proteins. Organic N was determined by kjeldahl's method and multiplied by 6.25. In case of water soluble samlpes, protein determination was done by the method of Lowry *et al.*[9]

Lipids. Total lipids were isolated by Soxhlet extraction with n-hexane for 12 hr.

Mannitol. This was determined by extraction with boiling 85% ethanol for 24 hr.[10] After isolation, the m.p. of crystalline mannitol and mixed m.p. were determined. It was also identified chromatographically using n-butanol–acetic acid–water (12:3:5, v/v)[11] and Dedonder reagent.[12]

Low-molecular Weight 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 paper chromatography. n-Butanol–pyridine–water (6:4:3, v/v.)[13] was used while detection of spots was effected by spraying with aniline phthalate.[8]

Laminaran. This was determined by isolation from the algal material according to the method of Black *et al.*[14] The isolated laminaran ws partially hyrdolysed with 0.3N HCl at 100° for 2hr. The hydrolysate was then chromatographed on Whatman paper No. 1 using n-butanol–pyridine–water (6:4:3).[13] The glucose produced was detected by spraying with ammoniacal silver nitrate.[12]

Alginic Acid. It was extracted with Na_2CO_3 according to the method of Abdel-Fattah *et al.*[15] After isolation by precipitation with HCl, alginic acid was washed with ethyl alcohol, ether and finally dried.

Fucoidan-like Polysaccharide. The dried ground algal material (50 g) was stirred with HCl solution (500 ml) at pH2 and 100° for 3 hr.[16] After filtration, the algal residue was reextracted in the same way and the two extracts were combined and neutralized with NaOH followed by concentration under vacuum to small volume. Ethyl alcohol

TABLE 1. COMPOSITION OF *Cystoseira barata*.

Ash	Ca^{2+} in ash	Mg^{2+} in ash	Protein	Total lipids	Mannitol	Laminaran	Alginic acid	Fucoidan-like polysaccharide
18·40	34·81	6·04	8·00	0·37	1·38	1·81	32·45	11·7

(2 vol) was then added and the polysaccharide isolated by centrifugation was washed with alcohol, ether and finally dried under vacuum at 35°.

Isolation of Acid-extractable Polysaccharides. Acid soluble polysaccharides were isolated by extraction with HCl or trichloroacetic acid solution. Four separate 15 g quantities of the algal material were extracted (twice) while stirring with 150 ml of the agent, separately, at 20° for 4 hr and 80° for 3 hr. After filtration, the extract was neutralized with saturated Na_2CO_3 and dialyzed against distilled water for 48 hr. During that period a water–insoluble polysaccharide precipitated in the dialysis bag. Centrifugation of the dialyzed solution afforded the residue and the supernatant which represented the water-insolauble (A) and water-soluble acid-extractable (B) algal polysaccharides, respectively. The water-soluble acidextractable polysaccharide was isolated by treating the dialyzed supernatant with 4 volumes of absolute ethyl alcohol, followed by centrifugation. The isolated water-soluble and water-insoluble polysaccharide materials were then dried under reduced pressure at room temperature and weighed.

Determination of the Proportion of Sugars in the Isolated Polysaccharides. Complete acid hydrolysis of the isolated plysaccharides was achieved according to the method of Haug and Larsen.[17] The hydrolysis products were then chromatographed on Whatman paper No. 1 using n-butanol–ethanol–water (40:11:19, v/v).[2] by descent. After developing the paper for 48 hr. the separated sugars were determined quantitatively.[18]

Total Carbohydrate. The carbohydrate content of each of the isolated products was determined by the phenol–H_2SO_4 method.[19] In the case of the water-insoluble polysaccharides, it was determined as glucose while in case of the water-soluble products the quantities were read off graphs constructed from measurements on solutions containing the appropriate sugars in the appropriate proportions.

Purification of Acid-extractable Water-soluble Polysaccharide Material. The water-soluble product isolated by extraction with HCl solution at 80° for 3 hr was used. It was dissolved in water, then percolated through a column of Lewatit S 100 (H^+) resin and the effluent dialyzed against distilled water (48 hr). Thereafter, the dialyzed solution was concentrated under vacuum at 40° to half its volume and treated with 4 volumes of ethyl alochol. The precipitate isolated by centrifugation, was dissolved in water 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 volume of ether. The aqueous layer was then separated and dialyzed for 2 days against distilled water. Thereafter, the dialyzed solution was concentrated, under reduced pressure at 40°, to half its volume and treated with 4 volumes of ethyl alcohol. The purified polysaccharide was isolated by centrifugation, washed with ethyl alcohol, ether and finally dried under vacuum at 40.°

Determination of Aldehydic End Groups. This was done according to the method of Chanda *et al.*[20]

Determination of Sulphate. Sulphate was determined on the purified polysaccharide by barium chloranilate method.[21]

Results and Discussion

The data recorded in Table 1 indicated that alginic acid comprised the major component of the brown alga *Cystoseira barbata.* Generally, the alga was characterized by containing an exceptionally high amount of alginic acid and low amounts of proteins, mannitol and lipids as compared with the same algal species and other brown algae collected previously from the Alexandrian coast.[3,10] Such variation in the quantitative composition of the same algal species may be due to differences in ecological conditions between places of algal collection.

The qualitative amino acid composition of alga revealed the presence of 17 amino acids, i.e. glutamic acid, aspartic acid, serine, arginine, lysine, histidine, glycine, threonine, alanine, tyrosine, leucine, isoleucine, phenylalanine, methionine, valine, proline, and tryptophane. Such amino acid composition is similar to that reported for *Sargassum linifolium.*[22] On the other hand, no low-molecular weight carbohydrates were found in the alcoholic extract of alga after removal of mannitol. This is in agreement with that reported earlier for the same algal species and other brown algae.[3,10,13]

Extraction of the algal material with HCl at pH 2 and 100°C for 3 hr provided a polysaccharide designated as fucoidan-like polysaccharide. By complete acid hydrolysis of this polysaccharide and paper chromatography it afforded glucuronic aiod, glucose, galactose, mannose, xylose and fucose. Isolation of fucose containing polysaccharides from other brown algal species has also been reported.[1–3,23]

The acid-extractable polysaccharides were investigated by extracting the algal material at pH 1.0 and 20° or 80° with HCl or trichloroacetic acid solution. In each case water–soluble and water–insoluble polysaccharide materials were isolated. As indicated in Table 2 the water insoluble polysaccharide material comprised a minor componenet of the alga. Generally HCl afforded water insoluble products richer in their

TABLE 2. SOME CHARACTERISTICS OF THE ACID-EXTRACTABLE POLYSACCHARIDE MATERIALS

Prodcut	Extracting agent	Temp of extraction (°C)	Time of extraction (hr)	Yield (%)	Ash (%)	Ca^{2+} in ash (%)	Mg^{2+} in ash (%)	Protein (%)	Total carbohydrate (%)	A.E.G. (meq/g)
Water insoluble	HCl	80	3	1·95	18·7	31·7	6·1	10·7	21·7	..
		20	24	1·49	29·6	31·4	6·3	25·4	22·3	..
	T.C.A.	80	3	1·92	18·0	39·9	2·8	3·0	19·7	..
		20	24	2·94	27·6	37·5	7·6	13·3	20·0	..
Water-soluble	HCl	80	3	10·08	21·0	41·4	4·9	8·6	49·0	1·1
		20	24	12·41	32·7	31·4	4·0	8·2	3·0	1·0
	T.C.A.	80	3	5·14	25·9	16·9	3·1	11·2	28·1	1·1
		20	24	5·23	35·1	24·4	3·6	6·4	27·1	1·0

T.C.A. trichloroacetic acid, A.E.G. aldehydic end groups, meq. milliequivalents.

TABLE 3. RELATIVE PROPORTION OF MONOSACCHARIDES IN THE ACID-EXTRACTABLE WATER-SOLUBLE ALGAL POLYSACCHARIDE MATERIALS.

Extracting agent	Temp of extraction (°C)	Time of extraction (hr)	Relaitve proportions of monosaccharides (%)					
			Glucuronic acid	Galactose	Mannose	Fucose	Xylose	Glucose
HCl	80	3	27·77	20·22	20·55	12·28	14·96	4·22
	20	24	23·42	25·16	15·49	19·35	11·31	5·27
T.C.A.	80	3	30·46	17·58	13·35	20·10	14·67	3·84
	20	24	25·34	23·66	8·98	27·04	13·16	1·82

T.C.A., trichloroacetic acid.

protein contents than those isolated by extraction with trichloroacetic aicd. In either case, extraction at 20° afforded water-insoluble products rich in ash and protein. Hydrolysis of any of the isolated water-insoluble products yielded only glucose. Water-insoluble glucan also was found in other brown algae.[3]

HCl was more efficient than trichloroacetic aicd in extracting the water-soluble polysaccharide material (Table 2). In any case, all the products were characterized by containing high amounts of ash although they were deionized by dialysis.

Acid hydrolysis of each of the water soluble products yielded glucuronic acid, galactose, mannose, fucose, xylose and glucose. The proportion of the sugars in these products differed according to the extracting agent and the conditions of extraction (Table 3). These products may thus be considered as different polysaccharides or a family of polysaccharides built up on the same general plan but with differences in fine structures. All the water soluble products were consistently rich in glucuronic acid and galactose and contained minor amounts of glucose. In addition, at 80°, HCl and trichloroacetic acid favoured the extraction of combined mannose and combined fucose, respectivley. In both cases, extraction of combined fucose was favoured at 20°. All these sugars were also found as constituents of polysaccharides isolated from other brown algae but in other proportions.[1–3,23]

Purification of the water-soluble polysaccharide, isolated by extraction at 80° for 3 hr, increased its carbohydrate amount content (68%) and the product contained the same sugar components. Although purification involved treatment with ion exchange resins and deproteinization with trichloroacetic acid, yet the product still contained appreciable amounts of ash (15.37%) and protein(6.2%). Such remaining ash may be attributed to the presence of sulphate (13.64%) as well as high glucuronic acid content. On the other hand, the purified product gave negative reaction with ninhydrin[8] and had a brown colour which could not be removed by treatment with charcoal. These results collectivley suggest the presence of a protein linked to the polysaccharide. The acid-extractable water-soluble products is therefore a sulphated heteropolysaccharide contining a protein moiety. Isolation of sulphated heteropolysaccharides containing protein moieties from other brown seaweeds have recently been reported.[1–3,23]

References

1. B. Larsen, A. Haug and T.J. Painter, Acta Chem. Scand., **20,** 219 (1966).
2. E. Percival, Carbohydrate Res., **7,** 272 (1968).
3. A.F. Abdel-Fattah, M.M. Hussein and H.M. Salem, Phytochemistry, **12,** 1995 (1973).
4. A.F. Abdel-Fattah, M.M. Hussein and H.M. Salem, Carbohydrate Res., **33,** 19 (1974).
5. H. Flaschka, Mikrochim. Acta **39,** 38 (1952).

6. S.M. Partridge, Biochem. J., **42,** 238 (1948).
7. K.H. Slotto and J. Primosigh, Nature (London), **168,** 696 (1951).
8. R.J. Block, E.L. Durrum and U. Zweig,*A Manual of Paper Chromatography and Paper Electrophoresis* (Academic, New York, 1955), p. 127.
9. O. H. Lowry, N.J. Rosebrough, A.L. Farr and R.J. Randall, J. Biol. Chem, **193**, 265 (1951).
10. A.F. Abdel-Fattah and M.M. Hussein, Phytochemistry, **9,** 721 (1970).
11. I. Smith, *Chromatographic and Electrophoretic Techniques* (Heinemann, London, 1960), vol. I, p. 246.
12. R. Dedonder, Bull. Soc. Chim. Fr., 874 (1952).
13. E.J. Bourne, P.G. Johnson and E. Percival, J. Chem. Soc., 1561 (1970).
14. W.A. P. Black, W.J. Cornhill, E. T. Dewar and F.N. Woodward, J. Appl. Chem., **1,** 505 (1951).
15. A.F. Abdel-Fattah, M.M. Hussein and H.M. Salem U.A.R. J. Chem., **14,** 185 (1971).
16. W.A.P. Black, E.T. Dewar and F.N. Woodward, J. Sci. Food Agr., **3,** 122 (1952)
17. A. Haug and B. Larsen, Acta Chem. Scand., **16,** 1908 (1962).
18. C.M. Wilson, Anal. Chem., **31,** 1199 (1959).
19. M. Dubois, K. A. Gillies, J. K. Hamilton, P.A. Rebers and F. Smith, Anal. Chem., **28**, 350 (1956).
20. S. K. Chanda E.L. Hirst, J.K. N. Jones and E.G. V. Percival, J. Chem., Soc., 1289 (1950).
21. A.G. Lloyd, Biochem. J., **72,** 133 (1959).
22. A.F. Abdel-Fattah, M.M. Hussein and H.M. Salem, Carbohydrate Res., **33,** 9 (1974).
23. M.M. Hussein, Phytochemistry, **14,** 1865 (1975).