POLYSACCHARIDES OF BROWN ALGAE

III. Pelvecyan from Pelvetia wrightii

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In a preceding communication [1] we described the isolation from the brown alga Sargassum pallidum of a sulfated peptidoglucuronoglycan called "sargassan" and gave its general characteristics. In this paper we report the preparation of a similar compound, which we have called "pelvecyan," from the brown alga P. wrightii and we characterize the latter in comparison with sargassan. We isolated pelvecyan in a manner similar to that described previously for the extraction of sargassan [1]. The bulk of the accompanying alginic acid (AA) was eliminated from the solution in the form of its calcium salt. More complete separation of the AA was achieved by fractionation with barium chloride and subsequent reprecipitation of pelvecyan with ethanol from aqueous solution. The pelvecyan obtained after dialysis and freeze-drying with $[\alpha]_D^{20}$ −57 ± 5° (in water) was used for further study, although it contained a small amount of AA and a fairly high percentage of ash. The pelvecyan was purified by chromatography on Dowex 50 × 4 cation-exchange resin (H^+ form), which led to freeing the sample from ash and to a decrease in the nitrogen content, probably because of the elimination of the NH_4^+ cation. On acid hydrolysis, samples of pelvecyan gave galactose, mannose, fucose, xylose, and a hexuronic acid, and practically no mannuronic or guluronic acids, which are present in AA. The analytical figures for both samples of pelvecyan are given in the table.

Composition	Content, %		
	1*	1a	2
C	31.8	37.5	—
H	4.55	5.38	—
N	0.81	0.95	0.40
Sulfate	13.4	15.9	17.1
Uronic acids	16.8	19.9	20.1
Protein	7.0**; 5.1***	5.9***	3.0**; 2.5***
Ash	15.4	0	0

*Samples obtained: 1) by fractionation with barium chloride, 1a) calculated to the ash-free material, 2) on treatment with Dowex 50 X 4 (H^+ form);
**by Lowry's method;
***from the nitrogen content.

The figures in the table show that pelvecyan, like sargassan [1], contains a fairly high percentage of hexuronic acid and, in addition, it is a sulfated polysaccharide. Like sargassan [1], it is practically homogenous, as shown by gel filtration through various types of Bio-Gels, by chromatography on DEAE-cellulose, by precipitation with Cetavlon, and by electrophoresis in polyacrylamide gel. Ultracentrifugation also shows the homogeneity of pelvecyan, although it shows its polydisperse nature as well.

We isolated the monosaccharides present in the carbohydrate chain of pelvecyan (D-galactose, D-mannose, D-xylose, and L-fucose) in the individual state and identified them by comparison with authentic samples using literature methods [1].

The hexuronic acid content is given in the table, and the relative amounts of galactose, mannose, xylose, and fucose in the hydrolysate of pelvecyan, approximately 3.5 : 1 : 4 : 6, were determined by the gas-liquid chromatography of the trimethylsilyl ethers of the corresponding polyols. It is obvious that pelvecyan differs from sargassan only in the ratio of xylose and fucose. Pelvecyan does not contain any amino sugars.

We see from the table that pelvecayn, like sargassan [1], contains 4–5% protein which is not removed by Sevag's method. The presence of a polypeptide component is confirmed by hydrolysis with 6 N HCl and by other methods [1]. We detected 16 amino acids: histidine, aspartic acid, threonine, serine, glutamic acid, glycine, alanine, leucine, isoleucine, valine, cystine, methionine, tyrosine, phenylalanine, proline, and lysine. The histidine is present in a predominating amount.

These results show the similarity of the polypeptide components of pelvecyan and sargassan. On chromatography on Dowex 50 × 4 cation-exchange resin (H^+ form), the content of polypeptide in pelvecyan decreases (see table). A similar observation applies to sargassan.

These results show that the polypeptide is not a structural fragment of the polysaccharide molecule. Sargassan and pelvecyan are probably complexes in which the polysaccharide and peptide components are bound to one another by ionic forces, the polysaccharide playing the role of a polyanion and the polypeptide the role of a polycation. The proof of this hypothesis requires additional investigations.

EXPERIMENTAL

The general experimental conditions were analogous to those given in the previous paper [1]. In addition, acetone–ethanol–isopropanol–0.05 M borate buffer, pH 10.0 (3 : 1 : 1 : 2) was used for the chromatography of the uronic acids. Ultracentrifuging was carried out in a G-120 analytical ultracentrifuge (MOM, Hungary, 50 000 rpm).

Source of the sample. P. Wrightii was collected in June–August 1969 in the sublittoral of the Sea of Japan (Bay of Troits, Gulf of Pos'et).

Isolation and purification of pelvecyan. Freshly collected and comminuted algae (1.5 kg) were treated as described previously [1]. After fractionation with barium chloride, pelvecyan was obtained with $[\alpha]_D^{20} -57.0 \pm 5°$ (in water). Yield 6 g (see table).

Chromatography of pelvecyan on Dowex 50 × 4 cation-exchange resin (H^+ form). Pelvecyan (50 mg) in 1 ml of water was deposited on a column (40 × 1.7 cm) of Dowex 50 × 4 ion-exchange resin (H^+ form, 100–200 mesh). The fractions containing the pelvecyan were eluted with water and freeze-dried. Yield 37 mg (see table).

Isolation and identification of the monosaccharides. Pelvecyan (1 g) was hydrolyzed with 2 N H_2SO_4 (100 ml) at 100° C for 15 hr. The hydrolysate was treated as in the previous work [1]. The monosaccharides obtained were identified by comparison with authentic samples using the methods given previously [1].

CONCLUSIONS

A sulfated peptidoglycuronoglycan, pelvecyan, has been isolated from the brown alga P. wrightii. Its carbohydrate moiety contains D-galactose, D-mannose, D-xylose, L-fucose, and hexuronic acid residues. The polypeptide component consists of 16 amino acids and may be bound to the polysaccharide moiety by ionic bonds.

Pelvecyan is similar in nature and properties to sargassan, which was isolated from the brown alga Sargassum pallidum.

REFERENCE

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