POLYSACCHARIDES OF BROWN ALGAE

II. Sargassan From Sargassum Pallidum

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We have previously shown [1] that some brown algae from the Sea of Japan contain peptidoglucuronoglycans. In this paper we describe the isolation of such a compound, which we have called "sargassan," from the brown alga S. pallidum, which is widely distributed in the Sea of Japan, and we give this compound's characteristics.

After the elimination of pigments, low-molecular-weight impurities, and reserve polysaccharides, the extraction of the alga with hot water and 0.5% ammonium oxalate yielded a polysaccharide fraction consisting mainly of alginic acid (AA) and sargassan. The bulk of the AA was eliminated from the solution in the form of its potassium salt. Various methods of fractionation were used to separate the AA more completely. These included gel filtration on various types of Bio-gels (P-30, P-60, P-100, P-200). The elution curve on Bio-gel P-200 is given in Fig. 1. The second peak corresponds to the AA. However, the use of Bio-gels for preparative purposes does not appear possible, since irreversible absorption of the polysaccharides takes place, which leads to a low yield of sargassan.

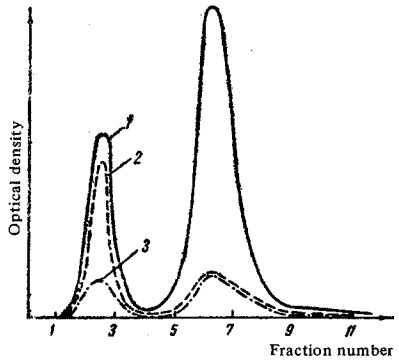

Fig. 1. Gel filtration of the total polysaccharide fraction on Bio-gel P-200. Optical density at (1) 490 mμ, at (2) 232 mμ, and at (3) 750 mμ.

Fractionation with ethanol or Cetavlon did not give satisfactory results. In the latter case, complete precipitation of the polysaccharides was observed, which shows their acidic nature.

Fractionation with copper acetate by a method described previously [2] was more successful. It led to the precipitation of the AA; the residual solution yielded sargassan containing only traces of AA and giving, on hydrolysis, galactose, mannose, fucose, xylose, glucuronic acid, glucurone, and only a very small amount of mannuronic and guluronic acids, which are present in AA. Even better results were achieved by fractionation with barium chloride or barium hydroxide with subsequent reprecipitation of the sargassan by ethanol from aqueous solution. The sargassan so obtained with $[\alpha]_D^{20}$ —40° (in water) was used for further study, although it contained a small amount of AA (table).

We can see from the table that sargassan contains a fairly large percentage of clucuronic acid and is, moreover, a sulfated polysaccharide.

The homogeneity of the sargassan was studied by various methods. Figure 2 gives the results of gel filtration of the sample obtained on Bio-gels. The elution curve for Bio-gel P-200 has two peaks. The first peak corresponds to sargassan; it is considerably larger than the second, corresponding to alginic acid. In ion-exchange chromatography on DEAE cellulose [4], sargassan gives practically a single peak showing the acid nature of the polysaccharide (Fig. 3). The electrophoresis of sargassan in a polyacrylamide gel by the method described previously [5] leads to almost a single zone, which also shows its homogeneity.

The monosaccharides present in the carbohydrate chain of sargassan (D-galactose, D-mannose, D-xylose, L-fucose, and D-glucuronic acid) were identified by paper chromatography in various systems, by gas-liquid chromatography (GLC) of the trimethylsilyl (TMS) ethers [6], by thin-layer chromatography (TLC) of the products of partial methylation of authentic samples and those under investigation [7], and also by TLC in layers of impregnated silica gel [8]; the glucuronic acid was also identified by paper electrophoresis [9].

Analytical Data for Purified Sargassan

Composition	Content, %			
	1*	1**	2*	2**
C	37,8	41,33	29,72	39,15
H	5,58	6,10	4,72	6,22
N	0,53	0,58	0,48	0,63
Sulfate	13,9	15,2	5,6	7,4
Uronic acids	21,9	24,0	24,2	32,0
Protein	7,0	7,7***; 3,6****	6,55	8,6***; 3,92****
Ash	8,5	0	24,1	0

*The sample was obtained by fractionation: 1) with copper acetate; 2) with barium chloride.
**Calculated to the ash-free material.
***Determined by Lowry's method [3].
****Calculated from the nitrogen content.

The relative amounts of galactose, mannose, xylose, and fucose in a sargassan hydrolysate were determined by GLC; they were approximately 1 : 3 : 3 : 2, respectively.

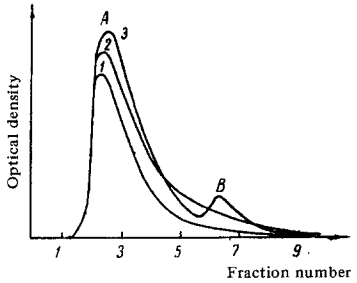

Fig. 2. Gel filtration of sargassan on Biogels (1) P-30, (2) P-100, and (3) P-200: A) peptidoglucuronoglycan; B) alginic acid.

The partial acid hydrolysis of sargassan gave a series of oligosaccharides. The isolation of some of them, consisting of xylose and fucose residues, shows the presence of a bond between these monosaccharide residues in the sargassan molecule.

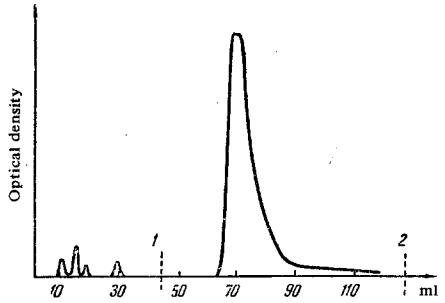

Fig. 3. Elution curve of the chromatography of sargassan on DEAE cellulose. Elution with (1) 0.5 M NaH_2PO_4 and (2) 0.05 → 0.03 M NaOH.

No amino sugar was detected in sargassan by paper chromatography, electrophoresis, or quantitative determination by the Elson–Morgan method [10].

We see from the table that sargassan contains 4–5% protein, which cannot be removed by Sevag's method [11]. The presence of the polypeptide component was confirmed by the results of hydrolysis with 6 N HCl with subsequent electrophoresis in pyridine–acetate buffer (pH 4.5) and paper chromatography in system 5 [12]. Sixteen amino acids were found and were identified by comparison with authentic samples. They were as follows (mM); histidine) 5.259, aspartic acid) 0.068, threonine) 0.073, serine) 0.091, glutamic acid) 0.057, glycine) 0.073, alanine) 0.03, leucine) 0.037, isoleucine) 0.036, valine) 0.008, cystine) 0.007, methionine) 0.003, tyrosine) 0.007, phenylalanine) 0.007, proline) 0.018, lysine) traces.

The coincidence of the maxima of the sargassan peak on the elution curves of ion-exchange chromatography and gel filtration and the results obtained by the phenol–sulfuric acid method [13] and also from the protein content [3] show the presence of a bond between the carbohydrate and peptide moieties of sargassan. In addition, when an aqueous solution of the polysaccharide was treated with sodium tungstate or trichloroacetic acid, and then with phenol, no precipitate was formed. In all cases, on acid hydrolysis and also when a solution of the polysaccharide was treated with Dowex 50 × 4 (H^+) cation-exchange resin at 80° C for 20 h, the polypeptide remained as an insoluble residue. The amount of protein in the supernatant solution fell considerably.

A special investigation is required to determine the nature of the bond between the peptide and polysaccharide components.

EXPERIMENTAL

Samples of polysaccharides were hydrolyzed with 2 N H_2SO_4 at 100° C for 15 h, with subsequent neutralization by barium carbonate and treatment with Amberlite IR-120 (H^+). The solutions were concentrated in vacuo at 40–45° C and used for chromatography.

Chromatography was carried out on paper in the following solvent systems: 1) ethyl acetate–pyridine–water–acetic acid (5 : 5 : 3 : 1), 2) butan-1-ol–ethanol–water (40 : 11 : 19), 3) butan-1-ol–pyridine–water (6 : 4 : 3), 4) ethyl acetate–acetic acid–water (3 : 2 : 1), 5) butan-1-ol–acetic acid–water (4 : 1 : 5); 6) n-propanol–ethyl acetate–water (7 : 2 : 1).

The spots were revealed with aniline hydrogen phthalate. TLC was carried out as described previously [8]. Gel filtration was performed in columns of Bio-gels prepared as described by Anderson and Stoddard [14] (Bio-gels from the firm Bio Rad Laboratories, California, USA).

GLC conditions: "Tsvet-2" chromatograph (OKBA [Experimental Design Bureau for Automation], Dzerzhinsk) with a flame ionization detector using a 1-m column containing 10% SE-30 on Chromosorb W silanized with dichlorodimethylsilane; temperature of the column was 190° C and of the inlet 250° C; rate of flow of the carrier gas (helium) 30 ml/min. The TMS derivatives of the monosaccharides were obtained in the usual way [6]. Electrophoresis in polyacrylamide gel was carried out as described by Pavlenko and Ovodov [5].

The amount of monosaccharides was determined by the phenol–sulfuric acid method [13], the uronic acids by decarboxylation [15], the proteins by Lowry's method [3], and C, H, N, and S by the classical analytical methods. The amino acids were identified by paper chromatography [15] and were determined quantitatively on a type 6020A amino acid analyzer (Czechoslovakia).

The S. pallidum was gathered in July–August 1968 in the sublittoral of the Sea of Japan (region of the Marine Experimental Station of the Institute of Biologically Active Substances of the Far Eastern Branch of the Siberian Branch of the Academy of Sciences of the USSR, Bay of Troits, Gulf of Pos'et).

Isolation and purification of sargassan. The freshly collected and comminuted algae (1 kg) were exhaustively extracted with methanol and covered with a 1% solution of formalin for 12 hr. The residue was treated several times with 0.2 N HCl to eliminate reserve polysaccharides, separated off, mixed with water, and neutralized with alkali. The polysaccharide was extracted successively with water and 0.5% ammonium oxalate solution at 75° C for 5 hr (two treatments). The combined solution was dialyzed against distilled water. A 5% solution of calcium chloride was added to the dialysate and it was left for several hours. The precipitate of calcium alginate was separated off, and the solution was dialyzed against distilled water for several days, treated three times by Sevag's method [11], concentrated, and freeze dried. This gave a polysaccharide fraction consisting of a mixture of AA and sargassan. Yield 16 g. Further fractionation was carried out with copper acetate, as described by Easterby and Jones [2], and

with barium chloride: to 1.6 g of crude polysaccharide in 50 ml of water was added 10 ml of a saturated solution of barium chloride. The precipitate formed was separated off, and the supernatant was dialyzed against distilled water until the reaction for chloride ion was negative. The solution obtained was freeze dried. Yield 0.5 g.

Isolation and identification of the monosaccharides. Sargassan (1.5 g) was hydrolyzed with 2 N H_2SO_4 (150 ml) as described previously. The syrup was deposited on a column of cellulose (2.8 × 50 cm) and was eluted with butanol two-thirds saturated with water, and then with butanol saturated with water. The fractions collected (10 ml) were studied by paper chromatography in system 1. The combined fractions were evaporated and were then purified by preparative paper chromatography in system 1. After elution with 50% aqueous ethanol, the following monosaccharides were obtained in the individual state: D-galactose, D-mannose, D-xylose, L-fucose, and D-glucuronic acid. They were identified by comparison with authentic samples, as described above.

Partial hydrolysis of sargassan. A mixture of 0.2 g of the polysaccharide and 22 ml of 1% H_2SO_4 was heated at 100° C for 1 hr. After cooling, the solution was treated with four volumes of ethanol. The precipitate was separated off by centrifuging, and the solution was evaporated to a small volume, neutralized with barium carbonate, and deionized with Amberlite IR-120 (H^+). The resulting syrup was used for the separation of the oligosaccharides on paper in system 5. The zones were eluted with 50% aqueous ethanol and evaporated to dryness. This yielded 2.5 mg of an oligosaccharide (I) giving fucose and xylose on hydrolysis, and 2 mg of an oligosaccharide (II) giving galactose and xylose on hydrolysis.

CONCLUSIONS

A sulfated peptidoglucuronoglycan, sargassan, has been isolated from the brown alga S. pallidum. The carbohydrate chain of this substance consists of D-galactose, D-mannose, D-xylose, L-fucose, and D-glycuronic acid residues. Some minor monosaccharide components may possibly be present in it. The peptides consist of 16 common amino acids.

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