POLYSACCHARIDES OF BROWN ALGAE

IV. FRAGMENTATION OF THE MOLECULES OF SARGASSAN AND PELVECYAN

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In preceding papers [1-2], the general characteristics of sargassan and pelvecyan, glucuronoglycans of the brown algae Sargassum pallidum and Pelvetia wrightii have been given. In order to obtain fragments simpler in composition and structure, we have attempted to perform the fragmentation of these polysaccharides.

Assuming the presence of a glucuronide chain in the polysaccharides [3, 4], we subjected these biopolymers to alkaline degradation. In order to exclude subsequent decomposition from the reducing end [5], the alkaline treatment was performed in the presence of sodium tetrahydroborate. In this case, decomposition of the polyuronide chain by the β-elimination mechanism [6] should be observed, with the formation of a large number of low-molecular-weight fragments. However, the action on sargassan and pelvecyan of 0.1 N caustic soda in the presence of 1% sodium tetrahydroborate for a prolonged period formed only high-molecular-weight fragments identical in saccharide composition with the initial polysaccharides. These fragments were stable to further alkaline treatment even at an elevated temperature. A similar phenomenon has been reported in the treatment of the initial polysaccharides with more concentrated alkali. This behavior shows the possible absence of a polyuronide chain in the structure of sargassan and pelvecyan, which agrees with Percival's results on the structure of such glucuronoglycans [7]. The fragments formed as a result of the alkaline treatment contained the polypeptide-forming part of the initial molecules.

When sargassan and pelvecyan were treated with dilute sulfuric acid under very mild conditions the hydrolysates contained xylose, fucose, and galactose, and also a number of oligosaccharides. Under these conditions, the polypeptide was split off and separated out in the form of a dark brown precipitate. The degraded polysaccharides precipitated with alcohol (yield about 30%) gave, on complete acid hydrolysis, galactose, mannose, xylose, fucose, and glucuronic acid (together with glucuronolactone). The mannose and glucuronic acid were present in the hydrolysate in considerably smaller amounts than the other monosaccharides.

Chromatography of the fragments obtained on Bio-Gels showed the considerable degradation of the molecules of the glucuronoglycans in the process of partial acid hydrolysis. The majority of the fractions corresponding to the peaks on the elution curves had the same monosaccharide composition; their hydrolysates differed only by their contents of the above-mentioned monosaccharides. However, on Bio-Gel P-30 there were only two peaks, and a hydrolysate of the second peak contained only xylose and fucose. The more severe hydrolysis of sargassan and pelvecyan with 0.4 N hydrochloric acid led to an increase in the amount of compounds corresponding to the second peak on Bio-Gel P-30 or Sephadex G-25. The formation of such a fragment may indicate the presence in sargassan and pelvecyan of sections of the carbohydrate chain consisting only of xylose and fucose residues, which corresponds to information on other glucuronoglycans of brown algae [3-4]. We have previously reported the isolation of low-molecular-weight oligosaccharides consisting of xylose and fucose.

When sargassan and pelvecyan were hydrolyzed with 0.5 N oxalic acid at 100° C, considerable amounts of fucose, xylose, and galactose were split out, the polypeptide precipitated, and oligosaccharide

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and polysaccharide fragments were formed. The latter were separated by precipitation with ethanol and subjected to additional fractionation and purification. As a result, we obtained a fragment from sargassan with a yield of 20%, $[\alpha]_D^{20}$ + 15° C (in water) and a fragment from pelvecyan with a yield of 15%, $[\alpha]_D^{20}$ – 25° C (in water) Chromatography on Bio-Gels showed that both fragments were fairly homogenous although they were characterized by appreciable polydispersity. Their homogeneity was also shown by the results of disc electrophoresis and chromatography on DEAE-cellulose. The molecular weights of both fragments were considerably lower than those of the initial polysaccharides. While the latter were not retained by Bio-Gel P-150, their fragments had elution volumes considerably greater than the free volume of this Bio-Gel. On Bio-Gel P-30, each compound gave a single peak with an elution volume equal to the free volume of the column.

The fragments obtained contained a large amount of residues of glucuronic acid (42-45%) and of mannose, and only traces of galactose, xylose, and fucose. As an example, Fig. 1 gives the results of the gas-liquid chromatography of the corresponding hydrolysates. Both polysaccharides contained sulfate groups (6.7-7.5%) and were distinguished by a very low nitrogen content (less than 1%), which shows the practical absence of the polypeptide component from them. In spite of the high content of glucuronic acid in the fragments investigated, attempts to obtain a glucuronan by partial hydrolysis were unsuccessful: the splitting out of the neutral monosaccharides was accompanied by the decomposition of the carbohydrate chain. This serves as an additional indication of the possible absence of a polyuronide chain as the basis of the molecules of sargassan and pelvecyan.

In the periodate oxidation of the fragments, about 0.5 mole of periodate per anhydro unit was consumed. The sodium tetrahydroborate reduction of the polyaldehydes obtained led to the formation of the corresponding polyalcohols, the content of sulfate groups in which was 3.0-4.6%, which shows that considerable desulfuration took place in the periodate oxidation process. In both cases, the complete hydrolysis of the polyalcohols gave glucuronic acid, glycolaldehyde and glycerol as the main decomposition products, together with a certain amount of mannose. The results obtained show the considerable oxidation of the mannose residues and the presence of glycosidic bonds at C-6 of these residues. In the glucuronic acid residues, the hydroxyls at C-3 are substituted by glycosidic bonds; the glucuronic acid probably does not contain sulfate groups, while part of the mannose residue is sulfated.

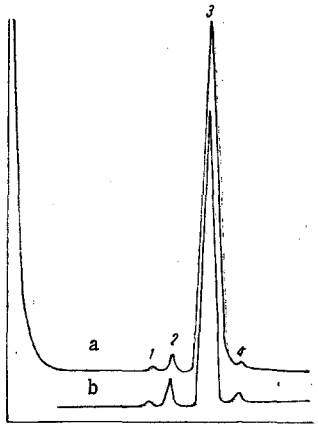

Fig. 1. Gas-liquid chromatography of hydrolysates of degraded sargassan (a) and pelvecyan (b) (the derivatives used were the acetates of the aldononitriles of the monosaccharides). Peaks: 1) fucose; 2) xylose; 3) mannose; 4) galactose.

EXPERIMENTAL

The general experimental conditions were analogous to those given previously [1]. Furthermore, the acetates of the corresponding aldononitriles were used for the gas-liquid chromatography of the monosaccharides [8]. The column, containing 15% of butanediol succinate on Chromosorb W (45-60 mesh), was washed with acid and silanized with chlorodimethylsilane; temperature programming was from 125 to 225°C.

Alkaline Degradation of Sargassan and Pelvecyan. A solution of 100 mg of one of the polysaccharides in 2 ml of 0.1N caustic soda was treated with 20 ml of sodium tetrahydroborate and the mixture was left at room temperature for 7-17 days. Then it was neutralized with 1 N acetic acid and studied by chromatography on various types of Bio-Gels and Sephadexes.

Partial Acid Hydrolysis of Sargassan and Pelvecyan. A. A mixture of 100 mg of polysaccharide and 2 ml of 1% sulfuric acid was heated at 100° C for 1 h. The reaction mixture was neutralized with barium carbonate, deionized with Amberlite IR-120 (H^+), and poured into ethanol (four volumes). The precipitate was separated off by centrifuging and subjected to chromatography on Bio-Gels. The supernatant solution was concentrated, and the resulting sirup was used for paper chromatography.

B. A mixture of 100 mg of polysaccharide and 2 ml of 0.4 N hydrochloric acid was heated at 90° C for 14 h. Then it was poured into ethanol, and the precipitate and the supernatant solution were studied as described above.

Preparation of the Sargassan and Pelvecyan Fragments. A. A mixture of 2.5 g of sargassan and 150 ml of 0.5 M oxalic acid was heated at 100° C for 30 min. Then it was dialyzed and the residue was freeze-dried and again hydrolyzed with oxalic acid under the same conditions for 1 h. The hydrolysate was dialyzed to neutrality. The combined dialysates were neutralized with calcium carbonate, deionized with Amberlite IR-20 (H^+), and evaporated. A mixture of mono- and oligosaccharides was obtained with a yield of 1.6 g. The undialysable residue was filtered, and the residue on the filter was washed with water and dried in vacuum. The polypeptide had the form of a brown powder; yield 0.15 g. The filtrate was freeze-dried, which led to a sargassan fragment with a yield of 0.45 g, $[\alpha]_D^{20}$ + 15°C (in water), containing 42.4% of glucuronic acid, 6.7% of sulfate groups, 0% of nitrogen, and 1.3% of ash.

The freeze-dried compound (10-20 mg) was hydrolyzed with 2 N sulfuric acid at 100° C for 6 h. The hydrolysate was shown by GLC (Fig. 1) and PC to contain, in addition to glucuronic acid and glucuronolactone, mannose (as the main component) and only traces of xylose and galactose.

B. Pelvecyan (1.0 g) was treated with oxalic acid as described above. The yields were: mixture of mono- and oligosaccharides 0.7 g; polypeptide 0.1 g; pelvecyan fragment 0.15 g; $[\alpha]_D^{20}$ – 25° C (in water); the latter contained 45.6% of glucuronic acid, 7.5% of sulfate groups, 0% of nitrogen, and 1.9% of ash. The hydrolysate of this fragment was shown by GLC (see Fig. 1) and PC to contain as the main components glucuronic acid, glucuronolactone, and mannose, and also traces of xylose, fucose, and galactose.

Periodate Oxidation of the Sargassan and Pelvecyan Fragments. Each polysaccharide (100 mg) was oxidized with 100 ml of 0.015 M sodium metaperiodate at 4° C and pH 5.4 in the dark. The consumption of periodate in both cases was 0.5 mole per anhydro unit. The polyaldehydes obtained were reduced with sodium tetrahydroborate to polyalcohols. Yield about 100 mg. The reduction products contained 3.0-4.6% of sulfate groups. The hydrolysates of both polyalcohols were shown by PC and GLC to contain glucuronic acid, glycolaldehyde, and glycerol, in addition to a small amount of mannose.

SUMMARY

It has been shown that the molecules of sargassan and pelvecyan each contain a linear high-molecular-weight fragment consisting of residues of glucuronic acid and mannose alternating with one another.

LITERATURE CITED

1. Yu. S. Ovodov, V. A. Khomenko, and T. F. Guseva, Khim. Prirodn. Soedin., 6, 285 (1970).
2. Yu. S. Ovodov and A. F. Pavlenko, Khim. Prirodn. Soedin., 6, 400 (1970).
3. B. Larsen, A. Haug, and T. J. Painter, Acta Chem. Scand., 20, 219 (1966).
4. B. Larsen, A. Haug, and T. J. Painter, Proc. 5th Intern. Seaweed Symp., Pergamon Press, Oxford (1966), p. 287.
5. R. L. Whistler and H. N. BeMiller, Advan. Carbohydrate Chem., 12, 289 (1957).
6. P. Albersheim, H. Neukom, and H. Deuel, Arch. Biochem. Biophys., 90, 46 (1960).
7. E. Percival, Chem. Ind. (London), 511 (1967).
8. V. M. Easterwood and B. J. L. Huff, Svensk Papperstidn., 72, 768 (1969).