POLYSACCHARIDES OF BROWN ALGAE

VI. A STUDY OF SARGASSAN AND ITS FRAGMENTS BY GAS-LIQUID CHROMATOGRAPHY

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The sulfated polysaccharide sargassan has been isolated previously from the brown seaweed Sargassum pallidum [1]. In the investigation of its structure use has been made of Smith degradation starting from the partially desulfated sargassan with the subsequent comparison of the low- and high-molecular-weight products formed. Some analytical figures for these compounds are given in Table 1.

The quantitative carbohydrate composition of sargassan and its fragments has been determined by gas-liquid chromatography (GLC) applied to acetates of the aldononitriles. In the present paper we show the applicability of this method to the analysis of hydrolyzates of sulfated glucuronoglycans. On obtaining acetates of aldononitriles from the monosaccharides formed on complete acid hydrolysis of the polysaccharides under study, we found that glucuronic acid cannot be determined by the GLC method. In view of this, the amount of this component was determined by decarboxylation according to Anderson [3]. It was established that the presence of a uronic acid in the mixture has no effect on the quantitative determination of the other monosaccharides by GLC.

The complete acid hydrolysis of the polysaccharides investigated took place under fairly severe conditions. Consequently, it was necessary to introduce a suitable correction for the degradation of the monosaccharides during hydrolysis. For this purpose, artificially prepared mixtures of monosaccharides were analyzed by the given method with and without treatment under the conditions of acid hydrolysis. It can be seen from Table 2 that considerable degradation of the monosaccharides, especially xylose, takes place on hydrolysis.

The quantitative calculation was performed by the method of an internal standard, for which L-rhamnose was used. The calibration coefficients (see Table 2) were determined with the degradation of the monosaccharides during hydrolysis taken into account. As an example, Fig. 1 shows a chromatogram of a hydrolyzate of sargassan with a statement of the residence times of the individual components of the mixture. It can be seen that gas-liquid chromatography gives a fairly good separation of the peaks. This permits the quantitative determination of the monosaccharide composition of sargassan and its derivatives (Table 3).

TABLE 1

Polysaccharide	Content, %		
	neutral monosaccharide [2]	uronic acids [3]	sulfate groups
Sargassan (I)	53,4	26,4	13,2
Desulfated I (II)	57,6	31,6	4,7
Degraded I (III)	53,4	23,8	14,7
Desulfated II (IV)	62,7	30,2	8,1

The total content of neutral monosaccharides determined by GLC agrees with the figures obtained by the phenol–sulfuric acid method. The high results for the degraded polysaccharides (III and IV) are due to the aldehydes formed in the hydrolysis of these compounds.

In order to determine the nature of the changes in the quantitative composition of any particular monosaccharide residue, the results were recalculated to the ash-free glucuronoglycan.

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TABLE 2

Monosaccharide	K_1*	K_2†	Mixture 1			Mixture 2		
			taken	calc. by K_1	calc. by K_2	taken	calc. by K_1	calc. by K_2
			%					
Fucose	0,47	0,61	19,8	19,9	15,7	21,7	21,6	18,6
Xylose	0,22	0,45	19,9	22,1	10,9	21,7	22,7	12,8
Mannose	0,28	0,38	20,0	18,5	15,6	22,2	21,0	17,6
Galactose	0,41	0,41	20,2	19,7	17,2	21,2	22,7	22,8
Glucuronic acid	—	—	0,0	—	—	12,2	—	—

*K_1 is a calibration coefficient determined from mixtures kept under hydrolysis conditions.

†K_2 is a calibration coefficient determined on mixtures not subjected to treatment.

TABLE 3

Polysaccharide	Amount, %								Tot. amt. of neutral monosaccharides, %	
	fucose		xylose		mannose		galactose			
	1†	2‡	1	2	1	2	1	2	GLC	PSM*
I	17,9	21,8	10,8	13,0	13,1	16,0	13,4	16,4	55,2	53,4
II	17,5	18,3	12,5	13,4	14,2	15,3	13,3	14,3	57,5	57,6
III	11,7	16,1	7,4	10,1	16,9	23,2	13,5	18,5	49,5	53,4
IV	9,0	11,1	7,2	8,9	29,5	36,5	9,6	6,0	50,6	62,7

* PSM) Phenol–sulfuric acid method.

†1) Determined by GLC.

‡2) Calculated on the ash-free glucuronoglycan.

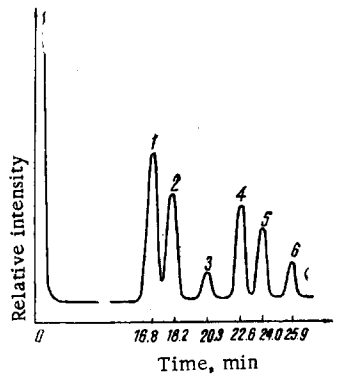

Fig. 1. Chromatogram of a hydrolyzate of sargassan (aldononitrile acetate derivatives): peaks: 1) rhamnose; 2) fucose; 3) xylose; 4) mannose; 5,6) galactose.

A comparative study of the Smith degradation products of natural and desulfated sargassan makes it possible to draw several conclusions concerning the structure of the polysaccharide under investigation. The initial and the desulfated sargassan have similar quantitative monosaccharide compositions (see Table 3). Consequently, the degradation of sargassan on desulfation is insignificant. Among the low-molecular-weight Smith degradation products of both polysaccharides we found glycerol, glyceraldehyde, propylene glycol, and glycolaldehyde. In desulfated sargassan the amount of propylene glycol had increased. The polysaccharide contained residues of fucose obviously included in the polysaccharide chain by 1,2-bonds and bearing sulfo groups in position 3 or 4, as was confirmed by comparing the quantitative monosaccharide composition of the degraded and initial polysaccharides, the former of which was obtained by Smith degradation (see Table 3). Desulfation increased the percentage of oxidized fucose residues.

From the accumulation of mannose residues in the degraded polysaccharides it may be considered that they are not oxidized either in the initial or in the partially desulfated polysaccharide. From the amount of sulfate groups in the polysaccharide (IV) it is clear that part of the mannose residues are not sulfated, and these mannose residues are not decomposed on periodate oxidation; consequently, they may be centers of branching or be bound by 1,3-bonds.

The desulfation of sargassan leads to a considerable cleavage of the galactose residues. It follows from this that sulfate groups in the galactose residues prevent their oxidation. After the removal of the sulfate groups, the galactose residues are oxidized by periodate. This is possible if the galactose residues are connected in the polysaccharide chain by 1,2-bonds and have the sulfate group in position 3 or 4, or by 1,6-bonds with the sulfate group in position 3.

EXPERIMENTAL

The polysaccharides were analyzed on a Tswett-2 chromatograph [OKBA (Experimental Design Bureau for Automation) Dzerzhinsk] with a double system of columns and a flame-ionization detector. The spiral stainless-steel coils were filled with 10% (w/w) of poly(tetramethylene succinate) on Chromosorb W, AW, DMCS (45-60 mesh). The rate of flow of the carrier gas (nitrogen) was 60 ml/min, that of hydrogen 60 ml/min, and that of air 300 ml/min. The temperature of the evaporator was 280°C, and that of the thermostat was raised from 100 to 225°C at 5°C/min. The aldononitrile acetates were obtained as described in the literature [4]. The samples of polysaccharides (I, III, and IV) were prepared as described previously [5]. The polysaccharides were desulfated by a known method [6]. The content of sulfate groups was determined by the usual method [7].

SUMMARY

1. The gas-liquid chromatography of the monosaccharides in the form of the aldononitrile acetates has permitted the analysis of polysaccharides containing sulfate groups and uronic acid residues.

2. The Smith degradation of natural and of partially desulfated sargassan has been studied by the GLC method. Conclusions concerning the structure of sargassan have been drawn from the results obtained.

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