

2. The ester nature of the bond of the sulfuric acid residues with the primary and secondary carbon atoms of the galactose residues has been shown.

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#### FRACTIONATION OF THE POLYSACCHARIDES OF *Chara aculeolata*

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The structure of the polysaccharides of *Chara* algae has been little studied [1, 2]. Continuing investigations in this field, we have fractionated these polymers.

The polysaccharides were isolated by the successive extraction of the alga with various solvents, and their amounts in the raw material were determined. To characterize the products isolated we studied the monomeric compositions of hydrolyzates by various methods of chromatography, enzymatic hydrolysis, and spectrophotometry. In selecting the conditions for fractionation we started from the results obtained in a determination of the overall chemical composition of *Chara aculeolata* [3].

The scheme for fractionating the alga consisted of the following stages: isolation of the water-soluble substances, isolation of the substances soluble in ammonium oxalate, and isolation of the substances soluble in alkali.

The results of the fractionation are given in Tables 1-3. When the alga was treated with various solvents, more than 50% of the dry matter of the alga passed into solution, about 22% of it consisting of carbohydrate-containing polymers.

The amount of water-soluble polysaccharide (WSP) in the alga was 3.5%. In its hydrolyzate glucose predominated and the other monomers were present in practically equal amounts, with the exception of rhamnose (see Table 3).

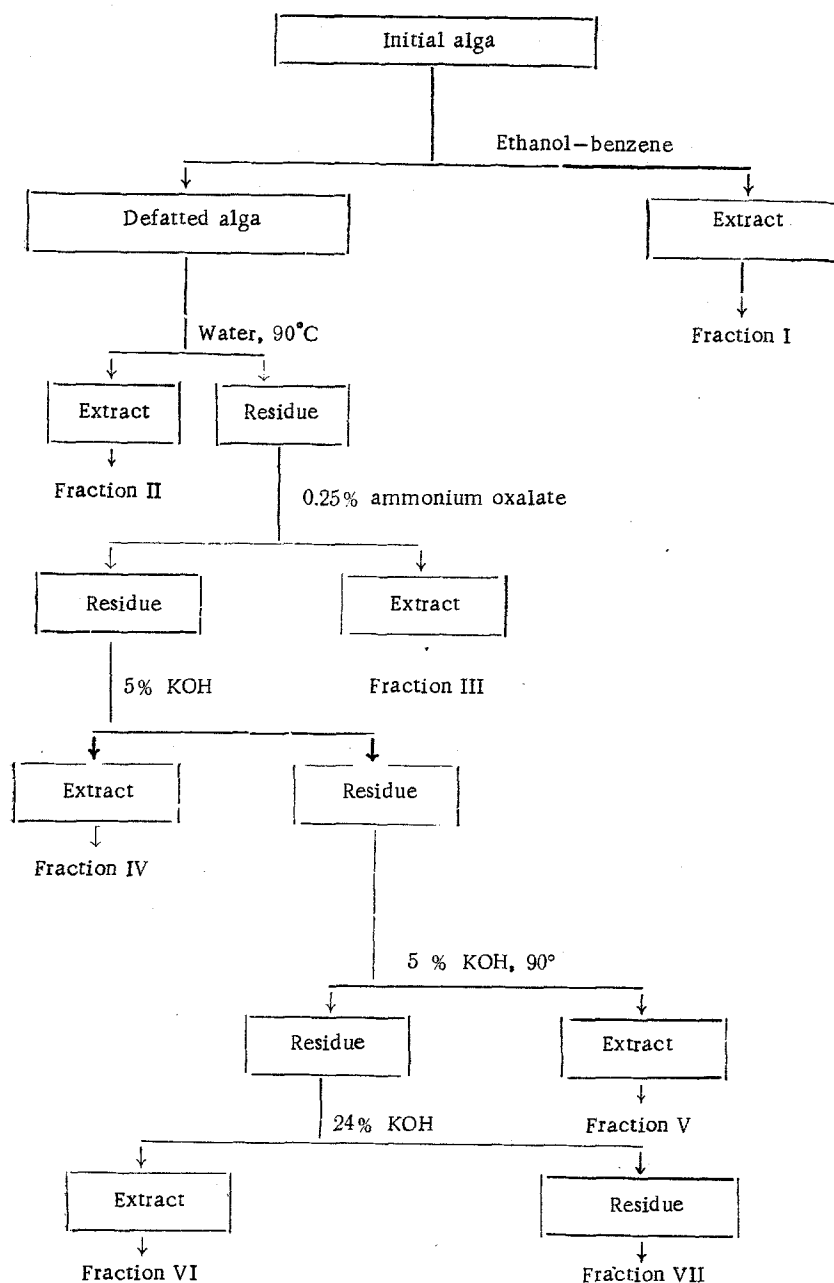
The fractionation of the total WSPs on Sephadexes C-75, 100, and 150 showed that they contained two fractions with different molecular weights differing in their monomeric composition. The polymer with the lower molecular weight had a neutral character and that of higher molecular weight an acid character.

The neutral polysaccharide was a starch-like substance, as was shown by: a) a positive reaction with iodine; b) the specific nature of the UV spectrum of its iodine complex; and c) a high degree of attackability by amylase.

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Scheme of the fractionation of *Chara aculeolata*

The acidic polysaccharide was esterified with sulfuric acid residues and had a more complex monomeric composition. In addition to uronic acids it included residues of galactose, glucose, arabinose, xylose, and rhamnose.

The fraction isolated by ammonium oxalate amounted to more than 17% of the weight of the dry matter on the alga and contained the largest amount of carbohydrates (see Table 2). When the polysaccharide extracted by this solvent was hydrolyzed, considerable amounts of uronic acids were formed. The identification of the uronic acids by the PC method, and also their reduction to galactose, showed that they consisted solely of D-galacturonic acid. Its high proportion (>50%) in the polysaccharide isolated permits the assumption that this polymer belongs to the class of pectic substances.

Alkali-soluble polysaccharides are present in the algae in considerably smaller amounts. The fractions isolated by 5% KOH solution under various temperature conditions were practically identical in monosaccharide composition. The polymers extracted by 24% KOH solution contained smaller amounts of arabinose and galactose (see Table 3). Each of these polysaccharides gives a single peak on the gel filtration curve. Repeated reprecipitation of

TABLE 1. Dry Matter Contents of the Algal Fractions

Extractant	Weight of the fraction, g	Amount of fraction according to exptl. results	Corrected amount of the fraction, %
I. Ethanol-benzene (1:1)	0,4	1,33	1,38
II. Water, 90°C	4,2	14,00	14,48
III. Ammonium oxalate, 90°C	5,2	17,33	17,48
IV. Potassium hydroxide, 5%, 20°C	2,5	8,33	8,62
V. Potassium hydroxide, 5%, 90°C	2,3	7,66	7,93
VI. Potassium hydroxide, 24%, 20°C	0,6	2,00	2,07
VII. Residue	13,8	46,00	47,59
Total	29,0	96,65	100

TABLE 2. Amounts of Polysaccharides in the Fractions of the Alga

Extract	Amount of polysaccharides, %		Amount of nitrogen in polysaccharide, %
	in raw material	in fraction	
Water	3,5	24,1	1,6
Potassium hydroxide			
5%, 20°	1,1	1,39	0,3
5%, 90°	1,3	16,7	0,3
24%, 20°	0,5	24,1	0,5
Ammonium oxalate, 90%	5,0	28,5	0,4
Residue after extraction	29,7	62,2	0,7

TABLE 3. Monosaccharide Compositions of Hydrolyzates of Fractions of the Alga

Extractant	Monosaccharide (molar ratios)					
	uronic acids	galactose	glucose	arabinose	xylose	rhamnose
Water	0,8	1,1	1,5	1,0	0,9	Tr.
Ammonium oxalate	5,0	1,3	1,0	1,2	1,3	Tr.
KOH, 5%, 20°	0,3	0,5	1,2	0,5	0,8	0,2
5%, 90°	0,2	0,4	1,3	0,8	0,8	0,3
24%, 20°	0,5	Tr.	1,0	Tr.	0,7	Tr.

the alkali-soluble polysaccharides with ethanol followed by amylolysis enabled a polymer to be obtained in the hydrolyzate of which galacturonic acid, xylose, and glucose were found.

The residue of the alga obtained after extraction consisted mainly of carbohydrate-containing substances. The characteristic violet coloration arising when this product was treated with zinc chloride/iodine, its resistance to hydrolysis, and the predominance of glucose in a hydrolyzate showed its cellulose-like nature. We have studied the structure of this polysaccharide previously [4].

Thus, fractionation has shown that the *Chara* alga contains a polysaccharide of the starch type, hemicelluloses, cellulose, an acidic sulfated heteropolysaccharide, and a polygalacturonide of complex structure.

#### EXPERIMENTAL

Fractionation of the Polysaccharides. The comminuted algae (30 g) were extracted in a Soxhlet apparatus with a mixture of methanol and benzene (1:3). The defatted algae were treated with hot water (90°C) with stirring. The volume of aqueous extract was 3000 ml. The residue from the algae after extraction under the same condition was treated with a 0.25% solution of ammonium oxalate in 0.25% oxalic acid. The volume of extract was 3000 ml.

The solid product obtained was treated with 5% KOH at 20 and 90°C. The volume of each extract was 2000 ml. Then it was extracted with 24% KOH at 20°C for 3 h. The volume of this extract was 500 ml. The completeness of each extraction was checked by the anthrone method.

The polysaccharides of each fraction were hydrolyzed with 2% HCl for 4 h in the boiling water bath under reflux. Hydrolysis was performed in parallel with 2 N H<sub>2</sub>SO<sub>4</sub> in sealed tubes at 100°C for 8-10 h. The monomeric compositions of the hydrolyzates were investigated by PC using as the mobile solvent butanol-pyridine-water-benzene (5:3:3:1).

Identification of the Galacturonic Acid. The uronic acid isolated from the hydrolyzate by preparative PC was chromatographed on paper in the ethyl acetate-acetic acid-formic acid-water (18:3:1:4) system in comparison with markers. Galacturonic acid was found. In parallel, the same uronic acid was reduced with sodium tetrahydroborate. The reduced products was chromatographed in the butanol-pyridine-water-benzene (5:3:3:1) system, and galactose was found.

Sulfo groups were determined by the method of Peat et al. [5].

The amylolysis of the polysaccharides to remove starch was performed as described by Kozhina and Mamatova [6].

Gel filtration of the polysaccharides was performed on Sephadexes C-75, 100, and 150. The fractions were monitored by the anthrone method.

#### SUMMARY

The carbohydrate complex of the Chara alga *Chara aculeolata* Kütz is peculiar and includes polysaccharides of the types of starch, hemicelluloses, and cellulose and also acid polymers of two types, one of which is esterified with sulfuric acid residues while the other contains D-galacturonic acid as the main component.

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