A GLUCAN FROM Chara aculeolata

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In plant systematics, the green algae occupy an intermediate position between single-celled and higher plants. The structure of the polysaccharides from the cell walls has been studied little [1-4]. In connection with the necessity for solving the problem of the use of algae in the food industry and for the production of technical materials the study of the main component of the cell wall of this raw material – a glucan – is of interest.

The present paper gives the experimental results characterizing the difficultly hydrolyzable glucan of the seaweed Chara aculeolata Kütz, which is widely distributed in the Egorlytskii bay of the northwestern part of the Black Sea.

The polysaccharide was isolated by treatment with an ethanolic solution of nitric acid [5]. The product obtained was a solid with an ill-defined fibrous structure. The results of chromatography showed that the polysaccharide contained only glucose. A microchemical test for cellulose by the action of zinc chloride was positive. The glucan contained 2.38% of ash and 0.6% of nitrogen.

In an attempt to purify the glucan further, it was acetylated with acetic anhydride in the presence of acetic and sulfuric acids. The resulting product contained 1.0% of ash and 0.1% of nitrogen. However, in the acetylation process the glucan underwent degradation, and therefore the nonacetylated polysaccharide was used in the subsequent investigation.

The structure of the polysaccharide was determined by the parallel use of methods of methylation, periodate oxidation, Smith degradation, the isolation of an oligomer, and IR spectroscopy.

EXPERIMENTAL

Isolation of the Glucan. A solution of hydrochloric acid was added to the comminuted seaweed to free it from ash substances, and then the substances accompanying the glucan were eliminated by treatment with an ethanolic solution of nitric acid in accordance with Kürchner's method [5].

The polysaccharide was hydrolyzed with a solution of H_2SO_4 for 4 h. The carbohydrate composition of the hydrolyzate was determined by paper chromatography using the benzene–butan-1-ol–pyridine–water (1:5:3:3) solvent system with aniline phthalate as chromogenic agent. According to the results of the chromatography of the hydrolyzate, the glucan is constructed only of glucose residues. The glucose was additionally identified by its conversion into glucosazone (mp 210°C).

Methylation of the Glucan. The process was performed by a modification of Haworth's method [6]. For this purpose, a weighed sample of the polysaccharide was treated in tetrahydrofuran with dimethyl sulfate and solid caustic soda, the process being monitored by thin-layer chromatography on plates coated with alumina, and also by IR spectroscopy. Complete methylation was achieved after 26 treatments of the polysaccharide. After purification on a column of alumina, the methylated product obtained had $[\alpha]_D^{20} - 14.2°$ (c 1%; chloroform), which shows the presence of a β linkage in it. It was methylated with 5% hydrogen chloride in dry formate in sealed tubes at 100°C for 10 h.* The derivatives were identified by paper chromatography [solvent system: ethanol–butanol–water (1:5:4)].

*This evidently error-ridden sentence presumably relates to some process of hydrolysis or methanolysis – Translator.

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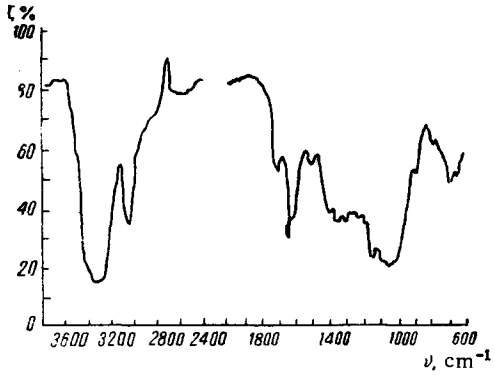

Fig. 1. IR spectrum of the glucan isolated from Chara aculéolata.

The results of thin-layer chromatography in layers of impregnated silica gel and of gas–liquid chromatography in comparison with markers permitted the identification in the hydrolysis products of the methylated glucan of 2,3,6-tri-O-methyl-D-glucopyranose and trace amounts of 2,3,4,6-tetra-O-methyl-D-glucopyranose, which shows the presence of a 1→4 linkage between the anhydro-D-glucose residues.

Periodate Oxidation of the Glucan. The glucan was dispersed in a solution of $NaIO_4$ (concentration 0.3 M) the mixture was shaken, and the consumption of periodate was determined after predetermined intervals of time. It amounted to 1.11 mole per link. After the end of oxidation, the polyaldehyde was separated from the reaction medium, washed with water, and dried, and its content of carbonyl groups was determined [7]. Then the polyaldehyde was treated with sodium tetrahydroborate until aldehyde groups were no longer present and the product was hydrolyzed with 0.1 N hydrochloric acid at 100°C for 10 h. Polyhydric alcohols were found by chromatography in the hydrolyzate after its neutralization, deionization, and evaporation.

The results of the chromatographic analysis showed that the hydrolyzate contained the polyol erythritol and trace amounts of glycerol. The appearance of erythritol is due to the transformation of D-glucopyranose units linked by 1→4 bonds; the glycerol was formed from the terminal groups.

Complete information on the sequence of monosaccharide residues in the chain and the determination of the configuration of the glycosidic linkages are possible as a result of partial degradation and the preparation of low oligomers.

The acetolysis of the polysaccharide under investigation formed cellobiose octaacetate with mp 222°C, $[\alpha]_D^{20}$ +39° (c 0.5%; chloroform) and cellobiose [8], which were identified by chromatography in comparison with a model solution, and by their specific rotations.

Consequently, the polysaccharide under investigation is analogous in the structure of its main chain to the cellulose of higher plants and is a linear polymer consisting of anhydro-D-glucose units with β-(1→4) linkages.

The presence of free hydroxy groups in the glucan was shown by acetylation [5]. The proportion of acetyl groups in the acetylglucan obtained was shown by its IR spectrum and also by the degree of substitution after its saponification with alkali. The acetyl glucan contained 60% of acetyl groups, which corresponds to their theoretical amount in cellulose triacetate.

The polysaccharide isolated was characterized by IR spectroscopy (Fig. 1). The spectra were taken on an IKS-14 spectrophotometer in the 4000-2000 and 2000-700 cm^{-1} regions. The results obtained showed that the glucan of Chara aculeolata, unlike the cellulose of higher plants is characterized by a diffuse type of spectrum. An absorption band in the 3250 cm^{-1} region corresponds to the vibrations of the main structural elements of the macromolecule (CHOH and CH_2OH groups), and the absence of absorption bands in the 3600 cm^{-1} region permits the conclusion that practically all the hydroxy groups are involved in intra- and intermolecular hydrogen bonds of a polymeric nature. An absorption band in the 890 cm^{-1} region corresponds to the vibrations of the β anomers of pyranose units.

SUMMARY

The difficultly hydrolyzable glucan from Chara aculeolata is similar to the cellulose of higher plants in the structure of its main chain, consisting of a linear polymer composed of D-glucopyranose units with a β-(1 $\rightarrow$4) linkage.

LITERATURE CITED

1. El. S. Amin, J. Chem. Soc., No. 2, 280 (1955).
2. D. W. M. Andersen and N. Y. King, J. Chem. Soc., No. 7, 2914 (1961).
3. E. L. V. Percival and A. G.Ross, J. Chem. Soc., 3041 (1949).
4. M. S. Dudkin, E. I. Kozarez, V. N. Chekoi, and I. V. Areshidze, Abstracts of the 7th International Symposium on the Chemistry of Natural Compounds [in Russian], Riga (1970), p. 366.
5. A. V. Obolenskaya, V. P. Shchegolev, et al., Practical Work in the Chemistry of Wood and Cellulose [in Russian], Moscow (1965), pp. 75, 378.
6. N. E. Kochetkov, Methods of Carbohydrate Chemistry [in Russian], Moscow (1967), p. 460.
7. V. A. Klimova, Fundamental Micromethods of Analyzing Organic Compounds [in Russian], Moscow (1967), p. 155.
8. L. Gattermann and H. Wieland, Laboratory Methods of Organic Chemistry, Macmillan, New York (1932).