THE STRUCTURE OF THE ARABOGLUCURONOXYLAN OF THE FRUIT HUSKS OF *Fagopyrum gaertn*

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The structure of the xylans of *Fagopyrum gaertn* (buckwheat) has scarcely been investigated. In the present paper we give the characteristics of the structure of the araboglucuronoxylan of the fruit husks removed from buckwheat kernels. The husks were found to consist of hemicelluloses (25.48%), cellulose (28.89%), and lignin (30.30%).

The xylan was extracted with a weak solution of alkali and was purified by reprecipitation via the copper complex. According to electrophoresis and gel filtration, the polysaccharide isolated was homogeneous and contained a single fraction. The following monosaccharides were found in a hydrolyzate of the xylan by paper partition chromatography: D-xylose (78.35%), L-arabinose (1.96%), and D-glucuronic and 4-O-methyl-D-glucuronic acids (19.69%). The molecular weight of the xylan was 25,700 carbon units, SP-194, $[\alpha]_D^{20}$—56.8°. The negative angle of rotation shows the presence of β bonds in the polymer molecule, which was confirmed by IR spectroscopy.

When the xylan was subjected to partial hydrolysis, the solution was found by chromatography to contain monosaccharides (D-xylose, L-arabinose, and uronic acids) and oligosaccharides (aldobiuronic and aldotriuronic acids). The part of the polysaccharide that was not hydrolyzed under mild conditions was constructed solely of D-xylose residues.

An idea of the structure of the xylan molecule was obtained by a comparison of the results of its methylation and periodate oxidation and those of the hydrolysis of the reduced polyaldehydoxylan.

The following were found in a hydrolyzate of the methylated xylan: 2-O-methyl-D-xylose, 3-O-methyl-D-xylose, 2,3-di-O-methyl-D-xylose, 2,3,4-tri-O-methyl-D-xylose, and 2,3,5-tri-O-methyl-L-arabinose in a molar ratio of 51:75:228:45:10, and methylated uronic acids. This shows that the chain of the polysaccharide is constructed of β-D-xylose residues connected through the 1 → 4 carbon atoms. In the side chains there are residues of uronic acids, xylose, and arabinose attached to the main chain at the second and third carbon atoms.

The oxidation of the polysaccharide by sodium periodate took place fairly rapidly and was practically complete in two days:

Time, days	Consumption, mole/pent. res.	Amount of HCOOH, mole/mole
1	0.82	—
2	0.97	0.18
3	1.00	0.19
4	1.00	0.19

The amount of formic acid liberated shows that each xylan molecule is oxidized at 34 monosaccharide residues present in the side chains. These result correlate with those of the analysis of the methylated sugars.

After the reduction of the polyaldehyde with sodium tetrahydroborate, a hydrolyzate of the polyol was found to contain glycerol and xylosylglycerol, which confirms the presence in the xylan of 1 → 4 bonds between the xylopyranose units, and also the branched nature of the polysaccharide. The results obtained showed that the following fragments are characteristic for the structure of the xylan of buckwheat husks:

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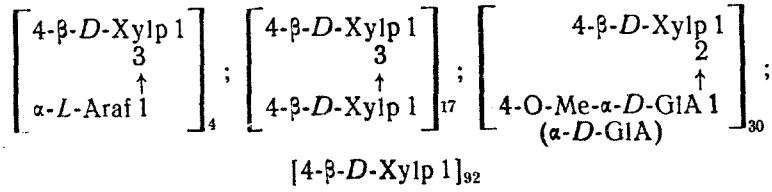

EXPERIMENTAL

Isolation of the Xylan. The xylan was isolated from the fruit husks of buckwheat of the variety Odesskaya, 1973 crop, grown in the Odessa oblast. The raw material was defatted with diethyl ether and was washed repeatedly with water at 40°C, and the pectin substances were eliminated by heating it with a 0.5% solution of $(NH_4)_2C_2O_4$. The residue was extracted with a 6% solution of KOH by the method described previously [1]. The xylan was purified by fourfold reprecipitation via the copper complex. The purity of the product obtained was determined chromatographically after hydrolysis.

Paper electrophoresis was performed in borate buffer, pH 11.2, at a current strength of 15 mA and a voltage of 500 V for 6 h. After the staining of the electrophoretogram, one spot close to the position of deposition was found.

Gel filtration was performed in a column filled with Sephadex G-100. A 5% solution of the xylan in 3% alkali was added to the column, and elution was performed with 3% KOH. The fractionation of the xylan gave a single sharp peak.

The hydrolysis of the xylan was performed with 2% HCl on the boiling water bath under reflux for 4 h. The carbohydrate composition of the hydrolyzate was determined by paper chromatography [solvent:butanol—benzene—pyridine—water (5:1:3:3)].

Separation of the Uronic Acids. The total uronic acids were separated by chromatography [solvent: ethyl acetate—pyridine—water (10:4:3)]. After staining, the chromatogram showed three spots corresponding to D-glucuronic acid (R_X = 0.62), 4-O-methyl-D-glucuronic acid (R_X = 1.15), and an aldobiuronic acid (R_X = 0.91). The aldobiuronic acid was hydrolyzed with 10% HCl in methanol for 4 h. Xylose and D-glucuronic acid were found in a ratio of 1:1.

The partial hydrolysis of the xylan was performed with 30% HCOOH on the water bath under reflux for 1 h [2]. The filtrate was separated and the carbohydrates that had passed into solution were hydrolyzed additionally with 1% HCl for 5 h. The carbohydrate part of the hydrolyzate was determined by paper chromatography. The unhydrolyzed residue was heated with 2% HCl on the water bath under reflux for 4 h. The hydrolyzate was investigated chromatographically.

The xylan was methylated by Hakomori's method in dimethyl sulfoxide with a solution of methylsulfinylcarbanion and methyl iodide. The completeness of methylation was checked by thin-layer chromatography on Al_2O_3 and also by IR spectroscopy. The methylated product was subjected to formolysis with 90% HCOOH at 100°C for 1 h and then with 0.25 M H_2SO_4 at the same temperature for 14 h. The hydrolyzate was investigated by paper chromatography, and the methylated sugars were determined quantitatively by iodometry [3].

Analysis of the hydrolyzate of the methylated xylan by the GLC method confirmed the results of the iodometric analysis.

Separation of the Monomethylxyloses. To determine the position of the methyl group (2 or 3), the monomethylxyloses were separated by chromatography on paper impregnated with borax.

Periodate Oxidation. The xylan was oxidized with a 0.3 M solution of sodium periodate at room temperature.

Smith Degradation. The oxidized xylan was dialyzed and was then reduced with sodium tetrahydroborate. The polyol obtained was hydrolyzed with 0.1 N HCl for 6 h. The xylosylglycerol found in the hydrolysis products was additionally hydrolyzed with 5% HCl for 6 h, giving xylose and glycerol in a ratio of 1:1.

SUMMARY

Buckwheat husks, like the surface layers of the grain of other cereals, contain an araboglucuronoxylan distinguished by the ratio of the monosaccharides of which it is composed.

The main chain of the araboglucuronoxylan isolated from buckwheat husks is constructed of residues of β-D-xylopyranose units connected at the position of the 1 → 4 carbon atoms. In side chains there are residues of α-D-glucuronic and 4-O-methyl-α-D-glucuronic acids, attached to the main chain at the position of the second carbon atom, and the residues of β-D-xylopyranose and α-L-arabopyranose attached to the main chain at the position of the third carbon atom.

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THE GLUCAN OF THE BLUE-GREEN ALGA *Microcystis aeruginosa*

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A number of representatives of the blue-green algae contain a branched glucan [1-3] diffusely deposited at the periphery of the cell in the chromatoplasm [4, 5].

From the freshwater blue-green alga *Microcystis aeruginosa* we have isolated a glucan containing no protein and consisting of D-glucose residues. The glucose was identified chromatographically in a hydrolyzate and by converting it into the osazone with mp 209°C.

With iodine, the polysaccharide forms a colored complex. UV spectrum: λ_{max} (in water) 480 nm, which is characteristic for glycogen. The complex of the glucan with protein does not give a coloration with iodine. After the treatment of the polysaccharide with α-amylase, D-glucose and maltose were identified in the hydrolyzate. In the IR spectrum (Fig. 1) there are characteristic absorption bands in the frequency regions 750 and 930 cm^{-1}, due to the vibrations of pyranose rings, and 844 cm^{-1} — deformation vibrations of C_1—H groups found for polysaccharides with α-1,4 bonds [6]. The absorption band of the hydroxy groups in the 3100-cm^{-1} region is shifted to low frequencies which is possibly caused by a high density of intermolecular hydrogen bonds.

On periodate oxidation, the following numbers of moles of periodate were consumed by one mole of D-glucose residues: after 24 h, 0.9; after 48 h, 0.98; after 72 h, 1.15; and after 96 h, 1.15, which shows the ready oxidizability of the polysaccharide and also the presence of several terminal groups in the molecule.

After the reduction of the oxidized polysaccharide with sodium tetrahydroborate and hydrolysis of the resulting polyol, glycerol and erythritol were found in the solution by chromatography. There was no free glucose.

Among the products of the hydrolysis of the completely methylated glucan the following were identified by the use of methods of paper, thin-layer, and gas—liquid chromatography in parallel (Fig. 2) 2,3,4,6-tetra-O-methyl-D-glucose, 2,3,6-tri-O-methyl-D-glucose, and 2,3-di-O-methyl-D-glucose in a ratio of 5:14:4. The reducing capacity of the polysaccharide was 4.34%, which corresponds to a molecular weight of 3740 and a degree of polymerization SP of 23. From the number of units of 2,3,4,6-tetra-O-methyl-D-glucose and the total number of glucose residues in the molecule it is obvious that there are five D-glucopyranose units to each terminal group.

Judging from the facts given, the glucan of the blue-green alga is constructed from α-D-glucose residues in the pyranose form connected by 1—4 bonds in the main chain and 1—6 bonds at the branching points.

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