ARABINOGLUCURONOXYLANS OF THE STEMS OF *Fagopyrum sagittatum* AND *Polygonum weyrichii*

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The primary structures of the arabinoglucuronoxylans of the stems of common buckwheat and *Polygonum weyrichii* have been established. It has been shown that the polysaccharides differ by their degrees of branching and also by the nature of the addition of certain side chains.

The structure of hemicelluloses of plants of the family Polygonaceae has been studied little [1]. This paper gives the results of an investigation of the structure of the xylans of the stems of plants belonging to two genera of this family, *Fagopyrum* and *Polygonum*: *Fagopyrum sagittatum* Gilib (common buckwheat), a widespread and economically important grain crop, and *Polygonum weyrichii* Fr. Schmidt — a promising fodder plant.

Arabinoglucuronoxylans are predominating among the hemicelluloses of the stems of buck wheat and *P. weyrichii*. The polymers were extracted with a dilute solution of potassium hydroxide and were purified by reprecipitation via the copper complexes. The homogeneity of the polysaccharide preparations was shown by electrophoresis and gel chromatography on Sephadexes.

The monosaccharide composition of the xylans was as follows (%):

Xylan of the stem of	D-Glucuronic and 4-O-methyl-D-glucuronic acids	L-Arabinose	D-Xylose
Buckwheat	13,9	3,2	82,9
Polygonum weyrichii	16,3	3,5	80,2

The polysaccharides had a complex monomeric composition and differed from the xylans of a number of other annual plants by their high content of uronic acid in combination with a small amount of arabinose. The degree of polymerization of the arabinoglucuronoxylans of the stems of the buckwheat and *P. weyrichii* were, respectively, 164 and 137: $[\alpha]_D^{27}$ −60.7 and −50.2°.

The polysaccharides were methylated and the methyl ethers obtained were subjected to hydrolytic degradation and the composition of the hydrolysates was determined by the GLC method (molar ratios):

Monosaccharide	Xylan of the stems of	
	Buckwheat	P. weyrichii
2-O-Methyl-D-xylose	17	9
3-O-Methyl-D-xylose	39	22
2,3-Di-O-methyl-D-xylose	51	74
2,3,4-Tri-O-methyl-D-xylose	29	5
2,3,5-Tri-O-methyl-L-arabinose	5	5
Methylated uronic acids	23	22

The predominance in the hydrolysates of the methylated products of 2,3-di-O-methylxylose gives grounds for assuming that the main chains of the macromolecules were constructed from xylopyranose residues linked with one another in the 1 → 4 manner. The presence of 2-O-methylxylose and of 3-O-methylxylose shows that some of these residues have side chains terminated by nonreducing xylopyranose, arabinopyranose, and urionic acid residues at the third or second carbon atom. This is shown by the presence in the hydrolysates of the methylated xylans of 2,3,4-tri-O-methylxylose, 2,3,5-tri-O-methylarabinose, and methylated uronic acid, the total amount of which correlates with the total amount of 2-O-methylxylose and 3-O-methylxylose.

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In parallel, we transformed the xylans into their polyaldehyde derivatives followed by reduction with sodium tetrahydroborate to high-molecular-weight polyols. In the hydrolysates of the product investigated we detected glycerol and glycerol xyloside, which agrees with the results of the investigation of the polysaccharides by the methylation method.

Additional information on the nature of the side chains was obtained from the results of the partial hydrolytic degradation of the biopolymers. The fragmentation of the macromolecules under the action of a hydrolyzing agent gave oligosaccharides which were then separated into acidic and neutral components.

In the acid fractions of the oligomers we detected fragments with degrees of polymerization of from 2 to 10. An aldobiouronic acid predominated. An appropriate analysis of its methyl derivatives showed that it consisted of α-D-glucuronopyranosyl-(1 → 2)-D-xylose:

$$\alpha\text{-}D\text{-GlA1} \rightarrow 2D\text{-Xyl.}$$

The configuration of the glycosidic bond in this compound was determined by Klyne's method [2].

In the neutral fraction of the oligomers from the xylan of the buckwheat stems we identified compounds with degrees of polymerization of 2, 4, and 6 constructed of D-xylanopyranose residues. The degree of their polymerization was calculated from the change in their reducing power in the process of hydrolysis, and also by French's method [3]. The structure of the xylooligomers was established by the methods of methylation and partial hydrolysis. The configuration of the glycosidic bond was determined by Klyne's method [2]. The composition of the hydrolysates of the methylated products are given below (molar ratios):

Monosaccharide	Xylobiose	Xylotetraose	Xylohexaose
2,3,4-Tri-O-methyl-D-xylose	1	1	2
2,3-Di-O-methyl-D-xylose	1	3	3
3-O-Methyl-D-xylose	—	—	1

Consequently, the oligomers with degrees of polymerization of 2 and 4 are β-D-xylopyranosyl-(1 → 4)-D-xylose (I) and β-D-xylopyranosyl-(1 → 4)-β-D-xylopyranosyl-(1 → 4)-β-D-xylopyranosyl-(1 → 4)-D-xylose (II):

$$\beta\text{-}D\text{-Xylp1} \rightarrow 4D\text{-Xyl (I)};\ \beta\text{-}D\text{-Xylp1}\left[\rightarrow 4\beta\text{-}D\text{-Xylp1}\right]_2\rightarrow 4D\text{-Xyl (II)}.$$

The presence in the hydrolysate of the methylated xylohexaose of a monomethyl derivative shows the branched nature of the compound. By subjecting the methylated oligomer to preliminary reduction of the aldehyde group, we obtained a product in the hydrolysate of which no 3-O-methylxylose was detected, which shows the attachment of the side chain to the reducing end of the oligosaccharide and permits the following structure to be ascribed to it:

$$\beta\text{-}D\text{-Xylp1}\left[\rightarrow 4\beta\text{-}D\text{-Xylp1}\right]_m\rightarrow 4D\text{-Xyl} \overset{2}{\leftarrow} {}^{1}\left[\beta\text{-}D\text{-Xylp}\right]_n \overset{4}{\leftarrow} {}^{1}\beta\text{-}D\text{-Xylp}$$

$$m + n = 3$$

No partial hydrolytic cleavage of the xylohexaose in an alkaline medium was observed, which confirms the absence of 1 → 3 bond between the xylopyranose residues in this compound.

The arabinoglycuronoxylans of the stems of buckwheat and *P. weyrichii* are cleaved by xylanase. A greater degree of hydrolysis was observed for the xylan of the buckwheat stems.

The combination of the results obtained permits us to consider that the following fragments are characteristic for the macromolecules of the xylans studied:

$$\left[\begin{array}{c}\to 4\beta\text{-}D\text{-Xyl}p1\to\\ 3\\ \uparrow\\ 1\\ (\beta\text{-}D\text{-Xyl}p)_{m_1}\\ 4\\ \uparrow\\ 1\\ \beta\text{-}D\text{-Xyl}p\end{array}\right]_{12},\quad \left[\begin{array}{c}\to 4\beta\text{-}D\text{-Xyl}p1\to\\ 3\\ \uparrow\\ 1\\ (\beta\text{-}D\text{-Xyl}p)_{n_1}\\ 4\\ \uparrow\\ 1\\ \alpha\text{-}L\text{-Ara}f\end{array}\right]_{5},\quad \left[\begin{array}{c}\to 4\beta\text{-}D\text{-Xyl}p1\to\\ 2\\ \uparrow\\ 1\\ (\beta\text{-}D\text{-Xyl}p)_{p_1}\\ 4\\ \uparrow\\ 1\\ \beta\text{-}D\text{-Xyl}p\end{array}\right]_{16},\quad \left[\begin{array}{c}\to 4\beta\text{-}D\text{-Xyl}p1\to\\ 2\\ \uparrow\\ 1\\ \alpha\text{-}D\text{-GlA}\end{array}\right]_{23},$$

$$\beta\text{-}D\text{-Xyl}p1\left[\to 4\beta\text{-}D\text{-Xyl}p1\to\right]_{q_1};\quad 12m_1 + 5n_1 + 16p_1 + q_1 = 51$$

Xylan of the Buckwheat Stems

$$\left[\begin{array}{c}\to 4\beta\text{-}D\text{-Xyl}p1\to\\ 3\\ \uparrow\\ 1\\ (\beta\text{-}D\text{-Xyl}p)_{m_2}\\ 4\\ \uparrow\\ 1\\ \beta\text{-}D\text{-Xyl}p\end{array}\right]_{4},\quad \left[\begin{array}{c}\to 4\beta\text{-}D\text{-Xyl}p1\to\\ 3\\ \uparrow\\ 1\\ (\beta\text{-}D\text{-Xyl}p)_{n_2}\\ 4\\ \uparrow\\ 1\\ \alpha\text{-}L\text{-Ara}f\end{array}\right]_{5},\quad \left[\begin{array}{c}\to 4\beta\text{-}D\text{-Xyl}p1\to\\ 2\\ \uparrow\\ 1\\ \alpha\text{-}D\text{-GlA}\end{array}\right]_{22},\quad 4m_2+5n_2+p_2=74$$

$$\beta\text{-}D\text{-Xyl}p1\left[\to 4\beta\text{-}D\text{-Xyl}p1\to\right]_{p_2};$$

Xylans of the Stems of P. weyrichii

There is a number of common features in the structures of the polysaccharides studied. Thus, the core of the polymers is a β-D-polyxylosidic chain constructed in the 1 → 4 manner. The arabinoglucuronoxylans have side chains connected to the main chain by 1 → 3 bonds and the same alternating xylose and arabinose residues, and also single-unit-side-chains in the form of uronic acids connected with the xylopyranose units at the position of the second carbon atom. In the xylan of the buckwheat stems, unlike that of the polymer of the same type from *P. weyrichii* stems, a number of xylose residues is attached to the main chain by a 1 → 2 bond. This polysaccharide is characterized by a higher degree of branching.

EXPERIMENTAL

Descending PC was carried out in the following systems: 1) butan-1-ol—pyridine—water—benzene (5:3:3:1); 2) butan-1-ol—acetic acid—water (4:1:5); 3) ethyl acetate—pyridine—water (10:4:3); 4) pyridine—ethyl acetate—acetic acid—water (5:5:1:3); and 5) butan-1-ol—ethanol—water (5:4:1).

The following reagents were used to indicate the spots: 1) aniline hydrogen phthalate; 2) an alkaline solution of silver nitrate; 3) periodate—potassium permanganate—benzidine.

The samples were subjected to GLC in a Chrom 4 instrument with a flame-ionization detector using a steel column 1.2 m long with Chromaton NAW-DMCS as solid support and 5% of XE-60 as the stationary liquid phase at column temperatures of 140-200°C with helium as the carrier gas.

Isolation of the Xylans. The stems of common buckwheat and of *P. weyrichii* were comminuted and treated successively with ether, 82% ethanol, and 0.5% ammonium oxalate. The xylans were extracted with 6% potassium hydroxide solution in an atmosphere of nitrogen for 72 h [4]. The yields of hemicelluloses were 11.5% and 9.3% of the mass of the stems of the buckwheat and *P. weyrichii*. After purification by precipitation via the copper complex, the yield of xylans from the buckwheat stem was 8% and from the *P. weyrichii* stems 6.5% on the weight of the initial material. The products isolated from the stems of the buckwheat and *P. weyrichii* contained 99.2% and 98.7% of polysaccharide, 0.2% and 0.4% of mineral matter, 1.3% and 1.9% of OCH_3, and 4.5% and 5.1% of COOH, respectively. Methoxy groups were determined by the method of Vieboch and Schwappach [5], and carboxy groups by carboxylation [5].

Hydrolysis of the Xylans. A mixture of 0.5 g of one of the xylans and 25 ml of 2 N sulfuric acid was heated for 7 h. Then it was neutralized with barium carbonate and filtered, the filtrate was studied by PC (systems 1 and 2; revealing agents 1 and 2). Reduction of the uronic acids as described by Sviridov et al. [6] led to the formation of glycose and 4-O-methylglucose. The absolute configurations of the monosaccharides were determined from their optical rotations. The quantitative determination of the carbohydrates eluted from the sec-

tions of the chromatograms not treated with the revealing agents was carried out by the phenol–sulfuric acid method.

Parts of the hydrolysates in the form of polyol acetates were studied by GLC.

Gel chromatography of the polysaccharides was performed on Sephadexes G-100 and G-200 [6].

Electrophoresis was performed on a EFA-1 instrument in borate buffer, pH 11, at a potential gradient of 15-18 V/cm and a current strength of 15-20 mA for 6 h. Revealing agent 3.

Periodate Oxidation and Smith Degradation. A mixture of 0.5 g of one of the xylans and 15 ml of 0.3 M sodium periodate solution was left in the dark at room temperature. At intervals, the excess of sodium periodate in 0.5-ml aliquots was determined with a solution of sodium thiosulfate. The consumption of sodium periodate amounted to 0.92 mole/pentose residue for the buckwheat stem xylan and 0.94 mole/pentose residue for the xylan of the stem of *P. weyrichii* over two days, and it changed no further.

The oxidized products were dialyzed and reduced with sodium tetrahydroborate. The polyols were hydrolyzed with 0.2 N hydrochloric acid at 100°C for 5 h, and the hydroysates in the form of polyol acetates were studied by GLC. In the products of the cleavage of the xylans of the buckwheat and *P. weyrichii* stems xylose and glycerol were detected in molar ratios of 28:25 and 21:49, respectively. On milder hydrolysis of the polyols (0.5 N hydrochloric acid at 20°C, 8 h) glycerol and glycerol xyloside (which, on hydrolysis, was split into xylose and glycerol in a molar ratio of 1:1) were detected in their hydrolysates by PC (system 1, revealing agents 1 and 3).

Methylation of the Xylans. A 0.3-g sample of xylan was methylated by Hakomori's method [8]. Completeness of methylation was checked from the absence of the absorption band of a hydroxyl in the IR spectra of the products by TLC on alumina (toluene–ethanol (9:1) system; revealing agent sulfuric acid). The yield of methyl ether from the xylan of the stem of the buckwheat was 0.21 g and from that of the *P. weyrichii* 0.91 g, and their OCH_3 contents were 39.2% and 28.9%, respectively.

The enzymatic hydrolysis of the polysaccharides was carried out with the preparation ksilonigrin P10x in acetate buffer (pH 4.2) at 40°C. The endo- and exoxylanase activities of the enzyme amounted to 450 and 293 units/g, respectively. The greatest depth of hydrolysis was observed on the fifth day. It amounted to 75.1% and 54.0%, respectively, for the xylans of the stems of the buckwheat and of *P. weyrichii*.

Partial Hydrolysis of the Xylans. Over 30 min, 1 g of each of the xylans was dissolved at –16°C in concentrated hydrochloric acid, and the solution was kept at 0°C for 1.5 h [7]. Then it was neutralized with barium carbonate and filtered. The acid fractions was separated from the neutral fractions with the aid of Dowex 2 × 8 ion-exchange resin in the acid form. The acidic oligosaccharides were investigated by PC (system 4, revealing agent 1). The compounds detected were characterized by their degrees of polymerization, which were determined from their chromatographic mobilities [4].

The aldobiouronic acid was eluted from the sections of the chromatograms that had not been treated with revealing agents and was purified by rechromatography this yielded 0.31 g of a compound from the hydrolysate of the xylan of the buckwheat stems and 0.028 g from the products of the cleavage of the xylan of the *P. weyrichii* stems. When both disaccharides were hydrolyzed with 10% HCl in methanol for 4 h, they split into D-xylose and D-glucuronic acid in a molar ratio of 1:1. The methylation and subsequent hydolysis of the aldobiouronic acid led to the formation of a methylated D-glucuronic acid and 3,4-di-O-methylxylose in a molar ratio of 1:1.

The neutral oligomers obtained on the partial acid hydrolysis of the buckwheat xylan were separated by PC (system 1, revealing agents 1 and 2). Seven oligomeric compounds, xylose, and arabinose were detected. The individual oligomers were separated by preparative PC and purified by rechromatography. This gave 0.062 g of a xylobiose, 0.023 g of a xylotetraose, and 0.041 g of a xylohexaose. Hydrolysis of the oligomer was carried out with 2 N sulfuric acid in the boiling water bath. Only oxylose was found in the hydrolysates of all three compounds.

The partial hydrolysis of the xylohexaose was carried out with a 0.2 M solution of barium hydroxide at 22°C for 72 h. No cleavage of the oligomer was observed under these conditions.

Methylation of the oligomers was performed by Hakomori's method [8].

The hydrolysis of the methylated products was carried out by their successive treatment with formic and sulfuric acids [7]. The hydrolysates were studied by PC (system 5, revealing agent 1). Part of each of the hydrolysates was reduced with sodium tetrahydroborate, acetylated, and studied by GLC.

SUMMARY

The primary structures of the arabinoglucuronoxylans of the stems of common buckwheat and of *Polygonum weyrichii* have been established. It has been shown that the polysaccharides differ by their degree of branching and also by the nature of the attachment of some of the side chains.

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PHOSPHOLIPIDS OF THE GRAPE

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In the berries of the cultivated grape vine *Vitis vinifera* L. nine phospholipid fractions have been identified, the main ones being phosphatidylcholines and phosphatidylethanolamines. The composition and position distribution of the fatty acids has been studied.

The influence of phospholipids (PLs) on the properties of the products of the processing of grapes and, in particular, on the stability [1], the organoleptic properties [2], and the direction of redox processes in wines [3] is a matter of doubt at the present time. Nevertheless, the PLs of the grape have scarcely been studied in the chemical respect [4].

The present paper gives the results of an investigation of the chemical composition and structural features of the PLs of the grape and its component parts (flesh, skin, seeds).

The extraction and purification of the lipids was carried out from the component parts and whole fruit of the cultivated grapevine *Vitis vinifera* L., of the Pinot Gris variety by a modified Bligh–Dyer method [5], and the PLs were isolated by column chromatography on silica gel and were subfractionated by two-dimensional TLC in systems 1 (first direction) and 2 (second direction) [6].

According to the experimental results, the amounts of PLs in the total lipids was small, amounting to 9.7% for the seeds, 6.6% for the flesh, and 2.3% for the skins.

Nine phosphorus-containing spots were detected with the following R_f values (in the second direction): 0.75 (diphosphatidylglycerols — DPGs); 0.61 (phosphatidic acids — PAs); 0.57 (phosphatidylethanolamines — PEs); 0.44 (phosphatidylglycerols — PGs); 0.31 (phosphatidyl-

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