FRACTIONATION OF THE HEMICELLULOSES OF THE LEAVES OF *Polygonum weyrichii* AND CHARACTERIZATION OF THE STRUCTURE OF THE ARABINOXYLOGLUCAN ISOLATED

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The results are given of investigations of the polysaccharide complex of the leaves of *Polygonum weyrichii*. The complex was isolated by alkaline extraction from the leaves. The water-soluble fraction of the alkali-soluble hemicelluloses has been characterized. It consists of an arabinoxyloglucan. Information is given on the isolation and characterization of the composition and properties of the xyloglucan. Structural features of the biopolymer have been investigated.

D-Xylose is a component part of a number of polysaccharides. Its combination with D-glucuronic acid and L-arabinose leads to the formation in lignified tissues of glucurono- and arabinoglucuronoxylans [1] in which the longest chain of the polymer is constructed of xylose residues and the side chains of glucuronic and 4-O-methylglucuronic acid and arabinose residues. It has been shown that xylose can form the side chains of glucans similar to cellulose [2].

Xyloglucans are present in plant tissues, accompanying cellulose and, apparently, associated with it [2].

We give the results of the fractionation of the hemicelluloses (HMCs) isolated from the leaves of *Polygonum weyrichii* Fr. Schmidt and of a study of the primary structure of the xyloglucans. The leaves of the herb *P. weyrichii* were collected in June, 1980, in the Main Botanical Garden of the Moldavian SSR (Kishinev). The results of an analysis of its chemical composition have been given previously [3]. The HMCs were extracted with a 6% solution of potassium hydroxide from leaves that had previously been treated with ether; the yield was 4%. Using the methods of gas–liquid and paper chromatography in parallel, glucuronic acid, galactose, arabinose, xylose, and glucose in a percentage ratio of 12:4:8:47.4:28.6 were detected in a hydrolysate. The further fractionation of the HMCs was carried out by the following scheme: An alkaline solution was acidified with acetic acid to pH 4.5 and the resulting precipitate I was separated by centrifugation and was not studied further because of its high (more than 20%) protein content and its low solubility. The centrifugation residue was treated with ethanol and the resulting precipitate II was dissolved in hot water. The insoluble fraction III was discarded (yield less than 1%). From the solution, ethanol precipitated polysaccharide IV, which was subjected to separation on DEAE-cellulose in the acetate form. As a result, four fractions were isolated — A, B, C, and D — the monosaccharide compositions of which are given below (% ratios):

Fraction	Eluent	Uronic acids	Galactose	Glucose	Arabinose	Xylose
A	Water	3,5	11,4	40,2	8,5	36,4
B	5 M buffer	9,5	4,7	11,8	23,8	53,2
C	0.1 N NaOH	4.5	Tr.	19,5	10,9	64,1
D	0.5 N NaOH	4,5	Tr.	44,5	11,2	40,8

In view of the capacity of certain polysaccharides for being strongly sorbed on a cellulose support [2], fraction A with the lowest uronic acid content was subjected to separation on a column filled with cellulose powder. Water and solutions of alkalis of various concentrations were used as the eluents. Three polysaccharide fractions were obtained the monomeric compositions of which were as follows (% ratio):

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Eluent	Sub-fraction	Yield of the fraction %	Uronic Acids	Galactose	Glucose	Arabinose	Xylose
Water	A_1	15.0	10.7	8.8	12.4	19.0	42.5
0.1 N NaOH	A_2	12.2	9.1	3.8	36.3	10.2	40.6
0.5 N NaOH	A_3	62.8	—	Tr.	59.4	9.6	31.0

Fractionation on a cellulose column of the polysaccharide present in fraction A led to the separation of the acidic components from the neutral ones. Two acid fractions A_1 and A_2 were obtained in minor amounts, and fraction A_3 proved to be predominating. Fraction A_1 contained a galactoarabinoglucuronoxylan, as was shown by its monomeric composition. Fraction A_2 had a similar nature, but differed from A_1 by a larger amount of glucose residues and a smaller amount of arabinose and galactose residues. Fraction A_3 contained an arabinoxyloglucan (AXG). A hydrolysate of this fraction contained glucose and xylose and a very small amount of arabinose. The AXG was homogeneous under the conditions of gel chromatography (Sephadexes G-100 and G-200) and of electrophoresis. In its absorption spectrum there were no peaks characteristic for aromatic compounds in the 200-400 nm region, which shows the absence of lignin.

The specific optical activity was +41° and was within the range characteristic for the majority of such biopolymeric substances [2].

A specific property of the xyloglucans (XGs) is their capacity for interacting with an iodine reagent to form a colored compound. The reaction was carried out by Kooiman's method [4]. Analysis showed that the absorption maximum of the iodine complex or the xyloglucan of the leaves of *Polygonum weyrichii* was located at 640 nm; analogous complexes of the xyloglucans of white mustard and tamarind give absorption maxima at 650 and 640 nm, respectively [4].

The products of the acetolysis of the AXG and, in particular, cellobiose octaacetate, were isolated and characterized by the method of Eda and Karto [2]. Cellobiose was found as a degradation product, which indicates the presence of a cellulose-like basis of the AXG macromolecule constructed of D-glucopyranose residues linked by 1 → 4 glycosidic bonds.

The structure of the AXG was characterized with the aid of the methylation method (Hakomori [5]), and then the formolysis of the polymer was carried out. The degradation products were identified by paper chromatography and, in parallel, by gas–liquid chromatography after they had been converted into the corresponding polyol acetates. It was established that they methylated AXG contained residues of 2,3,5-tri-O-methyl-L-arabinose, 2,3,4-tri-O-methyl-D-xylose, 3,4-di-O-methyl-D-xylose, 2,3,4,6-tetra-O-methyl-glucose, 2,3,6-tri-O-methyl-D-glucose, 2,3-di-O-methyl-D-glucose, and 3-mono-O-methyl-D-xylose, their molar ratio being 8:10:10:1:24:14:6. The methylation results showed a branched nature of the structure macromolecules of the AXG. Its main chain — its core — is a cellulose-like polyglucoside chain constructed of D-glucose residues linked by 1 → 4-β bonds. Each third or fourth glucose residue of the core bears a branch at C_6. The side chains are formed by xylopyranose and arabinofuranose residues linked by 1 → 2 glycosidic bonds. Consequently, the AXG contains the fragments

$$\beta\text{DGlp}\,[4\beta\text{DGlp1}];\quad \left[\begin{matrix}4\beta\text{DGlp1}\,\vert^{6}_{1}\\ \alpha\text{DXylp}\end{matrix}\right];\quad \left[\begin{matrix}4\beta\text{DGlp1}\,\vert^{6}_{1}\\ \alpha\text{DXylp}\,\vert^{2}_{1}\\ \text{LAraf}\end{matrix}\right];\quad \text{and}\quad 4\beta\text{DGlp.}$$

EXPERIMENTAL

Chromatography. Paper chromatography was performed with the solvent systems: 1) butan-1-ol–pyridine–water (6:4:3) and 2) pyridine–butan-1-ol–benzene–water (3:5:1:3). The revealing agent was aniline phthalate.

Gas–liquid chromatography was performed on a Chrom-4 chromatograph with an FID using a column 1200 mm long with t_{ev} = 220° and t_{col} = 140-200°C; the carrier gas was helium.

Electrophoresis of the AXG on paper 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 electrophoretogram had been developed, a single spot was found at the point of deposition.

The gel filtration of the AXG was carried out on a column filled with Sephadex G-100 or G-200. A 2% solution of the xyloglucan in 0.5 N NaOH was deposited on the column. Elution was carried out with 0.5 N NaOH. In both cases, fractionation yielded a single clear peak.

Isolation of the HMCs. The dried and comminuted leaves of *Polygonum weyrichii* were defatted with ether and treated repeatedly with water at 80°C, the pectin substances being eliminated by heating with 0.5% ammonium oxalate solution. The residue was extracted with 6% KOH solution as described previously [3].

Fractionation of the HMCs. The HMCs were extracted with water at 100°C for 2 h, and the insoluble part was removed by centrifugation. The water-soluble HMCs (0.5 g) were deposited on a column of DEAE-cellulose in the acetate form (5× 40 cm) and eluted in steps with water, 5 M acetate buffer, pH 6, and 0.1 and 0.5 N NaOH. The yields of the fractions were monitored by the anthrone method. The fraction eluted by water was precipitated with ethanol, which gave 40 mg of material. On acid hydrolysis, this yielded uronic acids, galactose, glucose, arabinose, and xylose, in a percentage ratio of 3.5:11.4:40.2:8.5:36.4.

Purification of the AXG. The polysaccharide (40 mg) was dissolved in water and passed through a column of cellulose, 2 × 40 cm. The fraction eluted by water contained uronic acids, galactose, glucose, arabinose, and xylose, in a percentage ratio of 10.7:8.8:12.4:19.0:42.5, and was not investigated further. The fraction eluted by 0.5 N NaOH was acidified with acetic acid and dialyzed, giving 24 mg of material. In the products of its acid hydrolysis, arabinose, xylose, and glucose were detected in a percentage ratio of 9.6:31.0:59.4.

Iodine Reaction according to Kooiman. A 0.5% solution of iodine in 1% aqueous KI (0.5 ml) was added to an aqueous solution of the polysaccharide containing 0.05-0.3 mg of AXG (1 ml). A color developed in the course of 60-90 min. The spectrum was recorded in the 500-700 nm region.

Acetolysis of the AXG was performed by the method of Eda and Karto [2]. For this purpose, a mixture of 2 ml of glacial acetic acid, 2 ml of acetic anhydride, and 0.2 ml of sulfuric acid was added at 0°C to 10 mg of the xyloglucan. The mixture was kept at 40°C for three days and then at room temperature for two days. The solution was poured into ice water and extracted with chloroform, and the products were acetylated. The mixture of acetates was dissolved in dry methanol (2 ml) and was deacetylated by the addition of 1 M methanolic solution of sodium methanolate (2 ml). After deionization, cellobiose was detected by the PC method using solvent 1.

Acid Hydrolysis of the AXG. The AXG was treated with 72% H_2SO_4 at 20°C for 2 h, and then the acid was diluted to 3% and the mixture was heated in a sealed tube at 100°C for 1 h. The hydrolysate was neutralized with $BaCO_3$, deionized with Dowex-50, and concentrated, and the monosaccharide composition was determined by PC and GLC. Mixture 2 was used as the mobile solvent.

The AXG was methylated by Hakomori's method in a dimethyl sulfoxide solution of the methylsulfinyl carbanion and methyl iodide. The completeness of methylation was checked by TLC on Al_2O_3, and also by IR spectroscopy. The methylated AXG was purified by a passage of a solution of it in toluene–ethanol (9:1) through a column filled with alumina using toluene–ethanol (9:1) as the eluent.

The methylated AXG was hydrolyzed with 2 M trifluoroacetic acid in sealed tubes at 120°C for 60 min. The formolysis of the methylated AXG with formic acid was carried out in parallel, and it was then hydrolyzed with 2.5% H_2SO_4. The hydrolysate was investigated by PC and GLC, and the methylated sugars were determined quantitatively by the GLC method.

Separation of the Monomethylxyloses. To establish the position of the methyl group (C_2 or C_3), the monomethylxyloses were separated by chromatography on paper impregnated with borax.

SUMMARY

Alkali-soluble hemicelluloses have been isolated from the leaves of *Polygonum weyrichii* Fr. Schmidt. The presence of an arabinoxyloglucan (AXG) in them has been established. The polysaccharide is branched. Its main chain is constructed of glucose residues linked by β-1 → 4 bonds, and the side chains are formed by xylopyranose and arabinofuranose residues.

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CARBOHYDRATES OF *Symphytum asperum*

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It has been shown that the carbohydrates of *Symphytum asperum* L. — a promising fodder plant — include water-soluble polysaccharides, pectin substances, protopectin, hemicelluloses, a glucomannan, and cellulose. The quantitative amounts of the polysaccharide fractions in the raw material have been established and their characteristics are given. The protopectins of the leaves and stems of *Symphytum asperum* L. have been characterized. Their macromolecules are based on a fragment constructed of D-galacturonic acid residues linked by α-glycosidic bonds.

Symphytum asperum L. (prickly comfrey) is a perennial plant of the family Boraginaceae which is distinguished by valuable biological characteristics and a high crop yield and is promising as a fodder and foodstuff.

The Boraginaceae contain alkaloids, vitamins, polyphenols, and other physiologically active compounds and have long been used in folk medicine [1, 2]. We have shown previously [3] that the comfrey contains a considerable amount of protein and ash substances, free sugars, and polysaccharides. The free sugars are represented by sucrose, glucose, and fructose, and accumulate mainly in the stems.

TABLE 1. Amounts of Polysaccharides in the Leaves and Stems of *Symphytum asperum* L. (% on the absolutely dry weight of the substance) and Their Monosaccharide Compositions

Extractant	Object of investigation	Amt. of polysaccharide	Monosaccharide (molar ratios)								
			UA	Gal	Glc	Man	Ara	Xyl	Rha	Rib	unidentified sugar
Water, 20°C	Leaves	4.01	6.0	1 0	0,9	—	2,1	0,5	1,1	1,0	Tr..
	Stems	2,47	6.9	3 9	1 1	—	4 3	1,9	0.9	1,2	Tr .
Water, 90°C	Leaves	0,33	3,6	5.2	1.8	—	4.3	1,6	5 6	2,0	1.9
	Stems	0,81	1.5	7.8	1,0	—	7,3	1,4	3,3	0,4	Tr.
0.5% ammonium oxalate	Leaves	1,27	10,0	1,1	0,7	—	0,9	Tr.	1,7	0,8	0,3
	Stems	3,69	11,2	1,0	0,5	—	1,1	0,5	1,9	0,9	0 5
10% KOH	Leaves HMC A	5,39	—	6,0	24,0	—	13,0	15,0	Tr.	Tr..	—
	Leaves HMC B	3,63	1.4	1,1	1,6	—	1,2	14,0	Tr.	Tr.	—
	Stems HMC B	7,68	4,2	0,6	1 8	—	0,5	7,5	Tr.	Tr	—
18% NaOH + 4% H_3BO_3	Leaves	1 06	—	—	0,3	28,3	—	12 5	—	—	Tr ·
	Stems	1,23	—	—	1,3	15,2	—	5,6	—	—	Tr..
Residue	Leaves	5,09	—	—	19,5	4,7	—	2,0	—	—	—
	Stems	12,45	—	—	10,0	—	—	1,8	—	—	—

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