L-Arabino-(4-*O*-methyl-D-glucurono)-D-xylan from the straw of the wheat Orchon (*Triticum bulgary* Z.)

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An L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan has been isolated from the straw of the wheat Orchon (*Triticum bulgary* Z.) by extraction with sodium hydroxide. The polysaccharide is composed of L-arabinofuranose, 4-*O*-methyl-D-glucopyranuronic acid, and D-xylopyranose in the approximate mole fraction ratio 1.2 : 1.2 : 10. The results of both chemical analyses and ^{13}C NMR measurements have shown that the polymer has a linear backbone of β-(1 → 4)-linked D-xylopyranose residues. Every fourth D-xylopyranose unit, on the average, is substituted either by a monomeric unit of 4-*O*-methyl-α-D-glucopyranuronic acid at C-2 position or by a monomeric unit of α-L-arabinofuranose at C-3. The polysaccharide has essentially the same basic structural features as those found for L-arabino-(4-*O*-methyl-D-glucurono)-D-xylans isolated from wheat straw of other varieties.

Из соломы пшеницы сорта Орхон (*Triticum bulgary* Z.) посредством экстракции гидроокисью натрия был выделен L-арабино-(4-*O*-метил-D-глюкуроно)-D-ксилан. Этот полисахарид содержит L-арабинофуранозу, 4-*O*-метил-D-глюкопирануроновую кислоту и D-ксилопиранозу в приблизительном молярном соотношении 1,2 : 1,2 : 10. Результаты химического анализа и измерений посредством ^{13}C ЯМР спектроскопии показали, что данный полимер состоит из линейной цепи β-(1 → 4) связанных единиц D-ксилопиранозы. В среднем каждая четвертая единица D-ксилопиранозы замещена в положении C-2 мономерной 4-*O*-метил-α-D-глюкопирануроновой кислотой или в положении C-3 мономерной единицей α-L-арабинофуранозы. Полисахарид обладает в принципе подобными основными структурными чертами, как L-арабино-(4-*O*-метил-D-глюкуроно)-D-ксиланы, выделенные из соломы пшеницы других сортов.

Wheat of variety Orchon (*Triticum bulgary* Z.) belongs to the most important cereals grown in Mongolia. The wheat straw is used there partially for feeding purposes and floor covering in stables but most of it represents an agricultural waste material not utilized properly thus far. In order to utilize effectively the straw in biotechnological processes, its chemical composition was studied and

the main emphasis was put on investigation of structure and properties of the hemicellulosic portion.

Wheat straw contains ≈ 40 % of cellulose and 23—28 % of hemicelluloses composed mainly of L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan [1]. In this paper, structural features of a polysaccharide isolated from the straw of the wheat Orchon (*Triticum bulgary* Z.) by extraction with 10 % aqueous solution of sodium hydroxide are discussed. The results of methylation analysis are correlated with ^{13}C NMR data.

The straw was crushed and sieved and subjected to several analyses the results of which are given in Tables 1 and 2. It is evident from Table 1 that the main components of the straw are the so-called acid-resistant polysaccharides, which represent apparently cellulose, then acid-labile polysaccharides, which may comprise hemicelluloses, and lignin. The values presented, as well as ash and nitrogen contents are in agreement with the data in literature [1]. D--Glucopyranose was present almost exclusively in the hydrolysate of acid-resistant polysaccharides and D-xylopyranose prevailed in the hydrolysate of acid-labile polysaccharides. It can be judged, therefore, that cellulose was the main component in the former case and in the latter were the polysaccharides of D-xylan type.

Of amino acids, glutamic acid, proline, lysine, and phenylalanine prevailed in the straw analyzed (Table 2). The overall composition and proportion of individual amino acids is typical for proteins occurring in plants.

Extraction of the straw with aqueous solution of sodium hydroxide containing sodium hypochlorite and subsequent precipitation of the extract in ethanol yielded an L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan with the physicochemical properties listed in Table 3.

The polysaccharide was methylated by the *Hakomori* [2] method, followed by

Table 1

Characterization of the straw from the wheat Orchon (*Triticum bulgary* Z.)

	w_i/%*
Humidity	4.8
Ash	5.9
Nitrogen	0.9
Substances soluble in light petroleum	0.7
Substances soluble in ethanol	3.2
Acid-labile polysaccharides	25.1
Acid-resistant polysaccharides	40.2
Lignin	19.2

* On absolutely dry straw.

Table 2

Amino acid composition in the straw of the wheat Orchon (*Triticum bulgary* Z.)

Amino acid	w_i/%*
Aspartic acid	0.26
Serine	0.12
Threonine	0.11
Glutamic acid	0.49
Proline	0.36
Glycine	0.14
Alanine	0.20
Valine	0.15
Methionine	0.04
Isoleucine	0.10
Leucine	0.21
Tyrosine	0.14
Phenylalanine	0.28
Histidine	0.06
Lysine	0.31
Arginine	0.09

* On absolutely dry material.

Table 3

Physicochemical data for L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan from the straw of the wheat Orchon (*Triticum bulgary* Z.)

$[\alpha]_D$	−82°
Relative molecular mass $\bar{M}_{n,r}$	19 039
w(Uronic acid residues)	14.6 %
w(Methoxyl)	2.3 %
n(D-Xyl : 4-*O*-Me-D-GlcA)	8.4
n(D-Xyl : L-Ara)	8.3

two *Purdie* [3] methylations. After hydrolysis of the permethylated polymer, the partially methylated saccharides were converted into the corresponding alditol acetates and identified [4] by GLC—mass spectrometry (Table 4).

The ^{13}C NMR shifts for the L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan are given in Table 5. Assignments of the individual signals were accomplished on the basis of well known rules [5, 6] valid for the ^{13}C chemical shifts of *O*-alkylated saccharides and by comparison with the ^{13}C spectral data of methyl β-D-xylopyranoside, methyl 4-*O*-methyl-α-D-glucopyranosiduronic acid, and methyl α-L--arabinofuranoside [5—8]. Furthermore, the results of methylation analysis of the polysaccharide were considered and also the fact that shifts produced by

Table 4

Methylated saccharides from the hydrolysate of the methylated L-arabino-(4-*O*-methyl-D--glucurono)-D-xylan

Saccharide derivative	*x*/mole %
2,3,5-Me_3-Ara[a]	10.8
2,3,4-Me_3-Xyl	0.8
2,3-Me_2-Xyl	66.0
2-Me-Xyl	11.2
3-Me-Xyl	11.2

a) 2,3,5-Me_3-Ara = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methyl-L-arabinitol, *etc.*

O-methylation and *O*-glycosylation, having the same direction, may differ [5] in the order of their magnitude. In this way, most signals of the spectrum (Fig. 1) could be attributed to the corresponding carbon atoms of the L-arabino-(4-*O*--methyl-D-glucurono)-D-xylan.

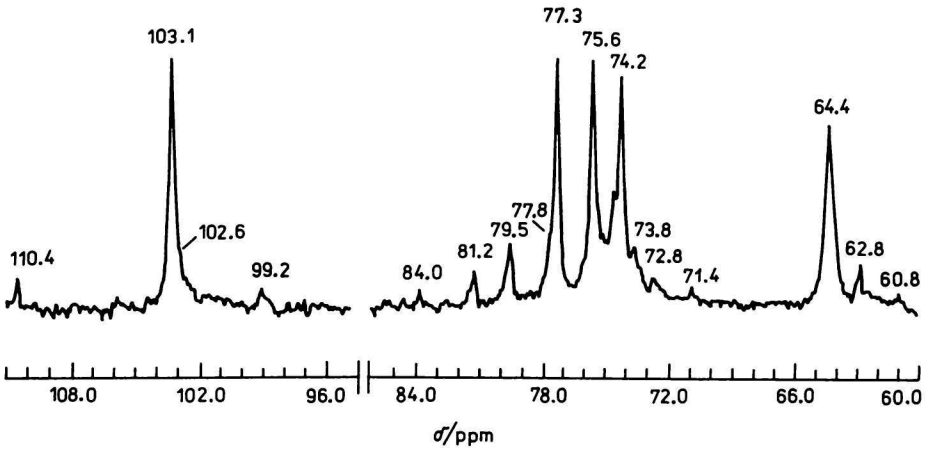


Fig. 1. ^{13}C NMR spectrum of L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan (ϱ(in D_2O) = = 10 g dm^{-3}).

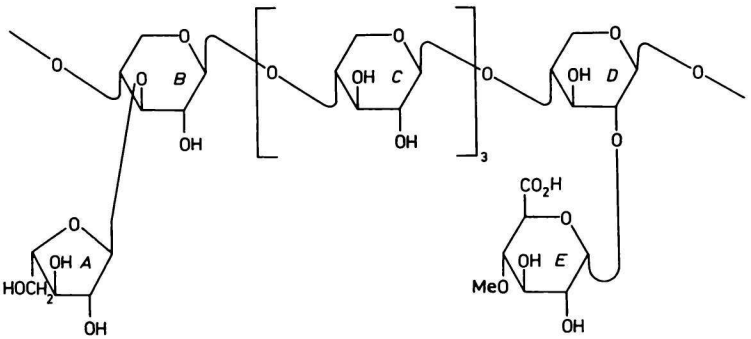
It has been known from literature [5] that the integrated intensities of signals in the ^{13}C NMR spectrum of polysaccharides permit conclusions on the relative proportion of constituent saccharides in the polymer investigated. Integration of the spectrum of L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan revealed the ratio of C-3 signals of D-xylopyranoses to those of C-3 substituted D-xylopyranoses to be $\approx 7:1$. Similarly, the ratio of C-2 signals of D-xylopyranoses and substituted D-xylopyranoses was $\approx 7:1$. This indicates that the polysaccharide

main chain contains approximately an equal number of D-xylopyranosyl residues which are the sites of branching at C-2 or C-3, and also the fact that every fourth D-xylopyranose unit, on the average, is involved in branching. The results are in agreement with the data of methylation analysis (Table 4).

Monitoring by GLC revealed that hydrolysis with dilute oxalic acid resulted in a selective cleavage of most of the L-arabinofuranosyl residues in the polysaccharide. In ^{13}C NMR spectrum of the modified polysaccharide, a noticeable decrease was observed in the intensity of the signal at $\delta = 79.5$ ppm, which was assigned to C-3 of D-xylopyranoses substituted at these carbon atoms. Thus, it has been proved unambiguously in this way that monomeric side chains of L-arabinofuranosyl residues are linked to C-3 of D-xylopyranosyl units in the main chain. It can be judged, therefore, that 4-*O*-methyl-D-glucopyranosyluronic acid groups are attached to C-2 of D-xylopyranosyl units.

The results of both chemical analyses and ^{13}C NMR measurements have shown that the L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan backbone is composed of β-(1 → 4)-linked D-xylopyranose units of which each fourth, on the average, is substituted either at C-2 position by a monomeric 4-*O*-methyl-α-D-glucopyranosyluronic acid residue or at C-3 by a monomeric α-L-arabinofuranosyl unit.

From the results presented, a structure depicted in Scheme 1 may be proposed though other variations are also possible.



Scheme 1

In the past, L-arabino-(4-*O*-methyl-D-glucurono)-D-xylans present in straw of various wheat varieties were investigated [1, 9, 10] in considerable detail. The structural features of these polysaccharides were discussed mainly on the basis of methylation analysis data and periodate oxidation studies on the original polymers. Variations were reported in the proportions of L-arabinofuranosyl, D-glucopyranosyluronic acid, and 4-*O*-methyl-D-glucopyranosyluronic acid

Table 5

^{13}C NMR data for the L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan from the straw of the wheat Orchon (*Triticum bulgary* Z.)

Saccharide residues[a]	Chemical shifts δ/ppm					
	C-1	C-2	C-3	C-4	C-5	MeO
A	110.4	81.2	77.3	84.0	62.8	—
B	103.1	74.2	79.5	77.3	64.4	—
C	103.1	74.2	75.6	77.3	64.4	—
D	102.6	77.8	75.6	77.3	64.4	—
E	99.2	72.8	73.8	81.2	71.4	60.8

a) For location of *A*—*E* see Scheme 1.

groups. In the work of *Aspinall* and *Meek* [9], methylation analysis of L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan indicated branching of the D-xylan backbone. In another paper [11], this possibility was also discussed on the basis of the analysis of xylooligosaccharides obtained on autohydrolysis of the polysaccharide in water at 120 °C. In some wheat L-arabino-(4-*O*-methyl-D-glucurono)-D-xylans, the uronic acid groups were concluded to be attached to C-3 atoms of D-xylopyranoses [12—14]. These incorrect interpretations arose from the fact that there was no adequate method available to correlate the data obtained by methylation analysis, where any unsubstituted hydroxyl group of D-xylopyranoses left unmethylated could be wrongly suspected to be a potential site of branching. In our work, the results of chemical methods were compared with ^{13}C NMR data in investigations of the structure of the polysaccharide. As in both ways similar information on the structure of the polysaccharide under study was obtained, the approach presented appears to be very effective for elucidation of the structures of most polysaccharides, if suitable model compounds or literature data are available.

The isolated L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan has essentially the same structural features as L-arabino-(4-*O*-methyl-D-glucurono)-D-xylans originating from wheat straw of other varieties [1]. Differences are apparent only in structural details, as in the degree of branching at C-2 and C-3 of D-xylopyranosyl units, the ratio of L-arabinofuranose and 4-*O*-methyl-D-glucopyranuronic acid, and the relative molecular mass. These structural variations together with chemical composition of the original material may depend on many factors, as the soil composition, climate, vegetation period, fertilizers and isolation procedures applied, *etc.*

Experimental

Straw sample was of Mongolian origin from wheat variety Orchon (*Triticum bulgary* Z.), obtained from the Institute of Chemistry, Ulan Bator, in 1985. Its characterization is given in Table 1. The nitrogen content and lignin were determined by Kjehldal and modified Klason [15] methods, respectively. Both acid-resistant and acid-labile polysaccharides were determined according to *Obolenskaya et al.* [16]. The amino acid composition (Table 2) was established after hydrolysis of straw with 6 M-HCl at 100 °C for 24 h.

The polysaccharide was hydrolyzed with 72 % sulfuric acid at 0 °C for 1 h and after dilution with water to 5 % acid, it was hydrolyzed further at 100 °C for 7 h. The uronic acid content in the polysaccharide was determined by titration with 0.1 M-KOH. Analysis of methoxyl groups was performed according to *Vieböck* and *Brecher* [17] for the polysaccharide in H^+ form.

Optical rotation was measured with a Perkin—Elmer Model 141 polarimeter on a 0.5 % aqueous solution of polysaccharide at 20 °C. Identification of the saccharides and their quantitation were accomplished after conversion to the corresponding alditol trifluoroacetates [18] by GLC. A Hewlett—Packard Model 5711A chromatograph was applied equipped with a column (200 cm × 0.3 cm) of 3 % of OV-225 on 0.147—0.175 mm Chromosorb W (AW-DMCS), at a programmed temperature range of 120 °C (4 min) to 170 °C at 2 °C min^{-1}. GLC—mass spectrometry of alditol acetates [4] of methylated saccharides was performed with a JMS-D 100 (Jeol) spectrometer, using a column (200 cm × 0.3 cm) packed with 0.124—0.147 mm Supelcoport coated with 3 % of SP 2340. The inlet helium pressure was 101.3 kPa at a programmed temperature range of 180 °C (4 min) to 220 °C at 2 °C min^{-1}. FT—^{13}C NMR spectra of the polysaccharide solutions (ϱ (in D_2O) = 10 g dm^{-3}) were monitored at 23 °C with a Bruker AM-300 spectrometer using inverse gated decoupling. The spectral width was 17 kHz; pulse delay 1.5 s; data points 32 k; pulse width 12 μs (60°). Chemical shifts were measured relative to methanol (δ = 50.15 ppm from Me_4Si) as the internal standard. The average relative molecular mass $\bar{M}_{n,r}$ of L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan was estimated osmometrically, using a Knauer Membrane osmometer and Zweischicht-Membrane (Knauer) as the membrane after equilibration with 0.1 M-NaCl at 30 °C.

Isolation of L-*arabino-(4-O-methyl-*D-*glucurono)-*D-*xylan*

To a crushed and sieved straw (200 g, particle size 2.4—3.5 mm), 10 % aqueous solution of sodium hydroxide (2.5 dm^3) and 15 % aqueous solution of sodium hypochlorite (740 cm^3) were added. The suspension was stirred at 40—45 °C for 2 h. The extract was then filtered through a fiber-glass cloth and the solid residue was washed with 10 % aqueous solution of sodium hydroxide (2.5 dm^3). The combined extracts were poured slowly with stirring into 96 % ethanol (17.2 dm^3). The precipitate, which sedimented quickly, was decanted several times with 75 % ethanol, neutralized with a dilute solution of acetic acid, and finally washed with 75 % ethanol to remove the residual salts.

The material was then suspended in water and a polysaccharide was recovered by lyophilization in the yield of 24.2 %. Its physicochemical data are given in Table 3.

Methylation analysis of L-*arabino-(4-O-methyl*-D-*glucurono)*-D-*xylan*

The polysaccharide (40 mg) was solubilized in dry dimethyl sulfoxide (5 cm^3) and methylated with methyl iodide (5 cm^3) in the presence of methylsulfinyl carbanion [2]. The solution was then poured into water (30 cm^3), dialyzed 48 h in distilled water, and evaporated. The sirupy residue was dissolved in methyl iodide (15 cm^3), silver oxide (100 mg) was added [3], and the mixture was stirred and boiled under reflux for 24 h. The procedure was repeated once. The fully methylated polysaccharide (30 mg) was hydrolyzed with 2 M trifluoroacetic acid (5 cm^3) in a sealed tube at 105 °C for 24 h and the partially methylated saccharides were conventionally converted into the corresponding alditol acetates and quantitatively analyzed [4] by GLC—mass spectrometry. The methylated saccharides detected are listed in Table 4.

Hydrolysis of L-*arabino-(4-O-methyl*-D-*glucurono)*-D-*xylan with oxalic acid*

The polysaccharide (90 mg) was hydrolyzed with 4×10^{-2} M oxalic acid (30 cm^3) at 100 °C for 3 h. Only L-arabinose was identified in the hydrolysate by PC. After dialysis, the polysaccharide was recovered by lyophilization. D-Xylose and traces of L-arabinose were determined in the hydrolysate of modified polysaccharide by GLC.

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