THE STRUCTURE OF THE XYLAN OF THE WOOD OF Platanus orientalis

M. S. Dudkin, G. V. Tatarkina, and N. G. Shkantova

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The raw material for the extraction of the xylan was plane-tree sawdust defatted with a mixture of ethanol and ether and freed from water-soluble sugars. The xylan was extracted by the alkali method, purified by reprecipitation via the copper complex, and dried over phosphorus pentoxide to constant weight. According to the results of electrophoresis, the xylan obtained was homogeneous. Its analysis is given in Table 1.

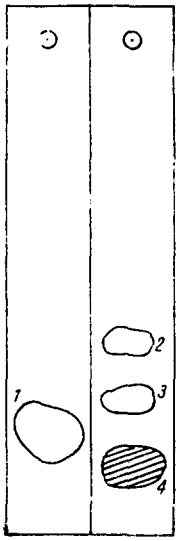

Fig. 1. Chromatogram of the uronic acids of the plane xylan: 1) xylose (model); 2) D-glucuronic acid; 3) aldobiuronic acid; 4) 4-O-methyl-D-glucuronic acid.

As acid hydrolysis of the polysaccharide showed, there was one uronic acid residue to 12-13 xylopyranose units. Based on a degree of polymerization of 222, the xylan macromolecule contains about 206 xylose residues and 16 uronic acid residues.

To determine the nature of the uronic acids present in xylan we used their separation in two solvent systems. A hydrolyzate of the xylan contained three acids: 4-O-methylglucuronic acid (the main component), and small amounts of glucuronic acid and an aldobiuronic acid with R_X 1.05, 0.84, and 0.93, respectively (Fig. 1). Absorption bands in the IR spectrum of the xylan (Fig. 2a) in the 3600 cm^{-1} region correspond to the stretching vibrations of secondary hydroxy groups in the free state. In the range from 3400 to 3300 cm^{-1} there is a broad absorption band corresponding to intermolecular hydrogen bonds in the polymer. Absorption bands at 1200-1040 cm^{-1} relate to the stretching vibrations of $-OH$ groups, at 1245 cm^{-1} to the bands of deformation vibrations of free $-OH$ groups, and at 1460-1360 cm^{-1} to those of bound $-OH$ groups, while at 890 cm^{-1} there is an absorption band characterizing the existance of a β linkage between the xylopyranose residues.

The structure of the xylan was determined by the method of periodate oxidation, Smith degradation, and methylation. The xylan macromolecule was oxidized completely by periodate in four days (Table 2).

It follows from the degree of polymerization of the xylan molecule that its complete oxidation liberates seven moles of formic acid, three

TABLE 1

Monosaccharide	Amt., %	Mol.-wt. of arbit. unit	$[\alpha]_D^{20}$ deg	Functional groups, % on absolutely dry weight	
				–COOH	$-OCH_3$
Xylose	90	29300	–64	2,65	2,50
Uronic acids	10	—	—	—	—

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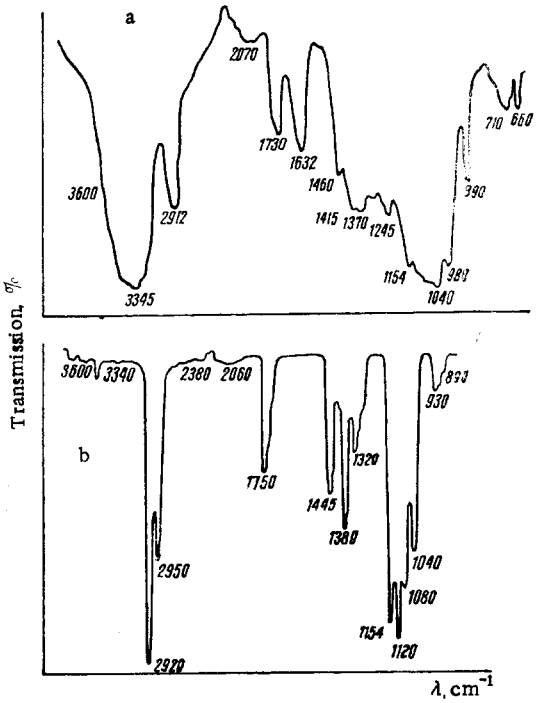

Fig. 2. IR spectra of the plane xylan (a) and its methylated derivative (b).

TABLE 2

Oxidation	Time, h					
	24	48	72	96	120	144
$NaIO_4$ Moles/pentose residue	0,720	0,760	0,790	0,830	0,840	0,830
HCOOH Moles/pentose residue	0,024	0,027	0,030	0,031	0,030	0,035

moles being formed from the terminal groups. In this case, only glucuronic acid forms formic acid. Consequently, of the 16 uronic acid residues four are in the form of D-glucuronic acid and the others in the form of 4-O-methyl-D-glucuronic acid.

When the resulting polyaldehyde was reduced and hydrolyzed, glycerol, xylose, and a small amount of an alcohol identified by its R_f value as 2-O-methylerythritol were detected. In addition, on a chromatograph a spot with a very low R_f value was found, presumably that of a xylosylglycerol. In its hydrolyzate we found xylose and glycerol (1 : 1). The total amount of glycerol in the hydrolyzate of the polyalcohol shows that there is one branching point for every eight xylopyranose units. To determine the positions of the points of branching and their nature, the xylan was methylated by Hakomori's method [1] until the absorption maxima in the IR spectra in the methoxy-group region (2920 cm^{-1}) and in the hydroxy-group region (3350 cm^{-1}) had a constant ratio (Fig. 2b).

The methylated product was hydrolyzed by a published method [2]. The hydrolyzate was found to contain 2-methylxylose, 2,3-dimethylxylose, 2,3,4-trimethylxylose (2 : 10 : 1) and methylated uronic acids. The trimethylxylose in the hydrolyzate shows the presence of terminal xylose in the side chains.

EXPERIMENTAL

The investigation was performed with sawdust from Platanus orientalis after the elimination of water-soluble and ether-soluble substances from it.

Isolation and Purification of 4-O-Methylglucuronoxylan. The sawdust was extracted with 6% KOH solution in an atmosphere of nitrogen for 72 h. The extract was filtered from the solid residue and acidified with glacial acetic acid to pH 5, after which the hemicelluloses were removed by the addition of a threefold amount of ethanol. The resulting material was purified by threefold reprecipitation via the copper complex.

Hydrolysis of the Xylan. The xylan was hydrolyzed with 2% HCl for 4 h in the boiling-water bath under reflux. The carbohydrate composition of the hydrolyzate was determined by quantitative paper chromatography using the solvent benzene–butan-1-ol–pyridine–water (1 : 5 : 3 : 3) and aniline phthalate was the revealing agent. The amounts of xylose and uronic acids found were 90% and 10%, respectively.

Separation and Identification of the Uronic Acids. The uronic acids were separated successively in two solvent systems: butan-1-ol–benzene–pyridine–water (5 : 1 : 3 : 3) and ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4). The revealing agent was aniline phthalate. 4-O-Methylglucuronic acid, glucuronic acid, and an aldobiuronic acid were detected on chromatograms. The amount of glucuronic acid (≈ 1%) was determined colorimetrically using a calibration curve. The composition of the aldobiuronic acid was determined after the hydrolysis of its eluate from the chromatograms; xylose and glucuronic acid were obtained in a molar ratio of 1 : 1.

The periodate oxidation of the xylan was performed with 0.3 M aqueous solution of $NaIO_4$ in the dark with periodic shaking until the consumption of sodium periodate ceased. After four days the polyaldehyde

was reduced to a polyalcohol and this was hydrolyzed [3] with 0.5 N HCl at room temperature for a day. The resulting hydrolyzate was studied chromatographically in the ethanol–butanol–water (1 : 4 : 5) system. The chromatogram showed traces of xylose and glycerol, a spot in the upper part of the chromatogram presumably being due to a xylosylglycerol. All these substances were determined quantitatively.

Determination of Xylosylglycerol. The compound of the suggested structure was eluted from the untreated parts of the chromatograms with water, the solution was evaporated in vacuum, and the residue was hydrolyzed with 4% HCl for 4 h. Chromatograms showed the presence of xylose (revealed with aniline phthalate) and glycerol (revealed with benzidine) in a molar ratio of approximately 1 : 1.

Determination of the Xylose. The solution under investigation was chromatographed in the benzene–butanol–pyridine–water system. After the spots had been shown up, the corresponding sections of the chromatograms were out out and eluted with a mixture of ethanol, water, and concentrated HCl (445 ml + 50 ml + 5 ml) for 2 h. The xylose was determined quantitatively on an FÉK-M colorimeter, using model calibration curves.

Determination of the Glycerol. The untreated parts of the chromatograms corresponding to glycerol were eluted with water, the eluates were oxidized with sodium periodate (0.3 M solution), amount of formic acid formed was determined by titration with 0.01 N NaOH, and from this the amount of glycerol was deduced [4].

Methylation of the Xylan. A sample of the xylan (0.5 g) was dissolved with gentle heating in dimethyl sulfoxide (75 ml). In parallel, a solution of the methylsulfinyl carbanion was prepared with a small amount of sodium hydride at 40°C, stirring being continued until a clear solution had been obtained (about 1.5 h). The solution of xylan was added to this solution after cooling, and the resulting mixture was left overnight. On the following day, methyl iodide (25 ml) was added and after another day the methylated product was isolated by dialysis.

Hydrolysis of the Methylated Xylan. A sample of the methylated polysaccharide (0.1 g) was covered with 90% HCOOH, and the mixture was maintained at 100°C for 1 h, after which it was hydrolyzed with 0.25 M H_2SO_4 in the boiling water bath for 14 h.

Determination of the Methylated Monoses. The amount of methylated monoses in the hydrolyzate was determined by paper chromatography in the ethanol–butanol–water system. 2-O-Methyl-D-xylose, 2,3-di-O-methyl-D-xylose, and 2,3,4-tri-O-methyl-D-xylose were found; a spot with a low R_f value was probably formed by methylated uronic acids. The amount of methylated monoses was determined iodometrically [5].

CONCLUSIONS

It has been established that the xylan molecule is constructed of 16 repeating units, these consisting basically of an unbranched chain of 12 β-xylopyranose residues joined by 1 → 4 bonds.

To the main polyxyloside chain are attached single xylopyranose residues in the 1 → 3 position and 4-O-methylglucuronic acid residues in the 1 → 2 position. The presence of tri-O-methylxylose in a hydrolyzate of the fully methylated xylan shows the presence of small amounts of xylose in the branches of the xylan macromolecule.

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