STRUCTURE OF A GLUCURONOXYLAN FROM ALFALFA STEMS

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The results are given of a study of the primary structure of a xylan from the stems of alfalfa, Medicago sativa. The structural features of the xylan were established by comparing the results of chemical and physical methods of investigation: methylation, periodate oxidation, acid and enzymatic hydrolysis, oxidation with CrO$_3$, and IR and NMR spectroscopy. It was found that the macromolecule is based on a linear chain constructed of D-xylopyranose residues, and side chains constructed of 4-O-methylglucuronic acid residues are attached to the second carbon atoms.

In the generation of hemicelluloses in a plant tissue various polysaccharides are formed: xylans, galactans, mannans, and glucans [1]. Coniferous wood, cereal stems, and the surface layers of grains [2] contain arabinoglucuronoxylans. The wood of broad-leaved trees and the stems of leguminous plants contain glucuronoxylans, and the seeds of cereals and other plants contain arabinoxylans. Xylans with different structures are formed simultaneously in one and the same plant tissue. For example, in maize cobs [3] a glucuronoxylan, a glucuronoarabinoxylan, an arabinoxylan, and a monomethylglucuronogalactoarabinoxylan have been found, and in the husks of oats, millet, and rice [4] an arabinoglucuronoxylan, an arabinoxylan, and an arabinoglucoxylan.

The structures of xylans of various types have been described in a number of publications [5, 6]. Aspinall isolated a 4-O-methylglucuronorhamnoxylan from alfalfa by means of an aqueous solution of potassium hydroxide [7]. In view of the fact that alfalfa belongs to the leguminous plants for which the presence of glucuronoxylans has been shown previously [6] as a chemotaxonomic characteristic, we assumed the presence of this polysaccharide in alfalfa, also.

The xylan was isolated from alfalfa stems by alkaline extraction and was purified as described previously [5]. In an electrophoretic study of the xylan it migrated towards the anode. No separation into fractions was observed. The elution curve from the gel filtration of the polysaccharide had a single narrow symmetrical peak, which showed the homogeneity of the xylan with respect to molecular mass. The results obtained indicated its homogeneity. The preparation isolated was characterized by a high content of polysaccharide and a small amount of impurities of noncarbohydrate nature.

D-Xylose and D-glucuronic acid were found in an acid hydrolysate of the polysaccharides. Rechromatography of the uronic acids showed the presence of D-glucuronic, 4-O-methylglucuronic, an aldobiuronic, and an aldotriuronic acid. Xylans with such a composition have been isolated previously from a number of leguminous plants [6]. Acid hydrolysis under mild conditions showed that the unhydrolyzable part of the polysaccharide contained only D-xylose residues and, therefore, that the core of the polysaccharide molecule was a polyxyloside chain.

The negative specific optical rotation $[\alpha]_D^{20}$ −72.8° (C = 0.1%; solvent 2% NaOH), the results of the oxidation of the polysaccharide with chromium trioxide, CrO$_3$ (monosaccharides were absent from the reaction mixture), and those of IR spectroscopy (presence of an absorption band at 890 cm^{-1}) and of ^{13}C NMR spectroscopy (SSCC, $^1J_{^{13}C_1-^1H_1}$ = 161.8 Hz) showed the presence of a β-bond between the monomeric residues in the glucuronoxylan macromolecule. D-Glucuronic acid residues were attached to the main chain by α-bonds (SSCC $^1J_{^{13}C_1-^1H_1}$ = 175.7 Hz).

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The structural characteristics of the polysaccharides under investigation were established by comparing the results of periodate oxidation, Smith degradation, and methylation.

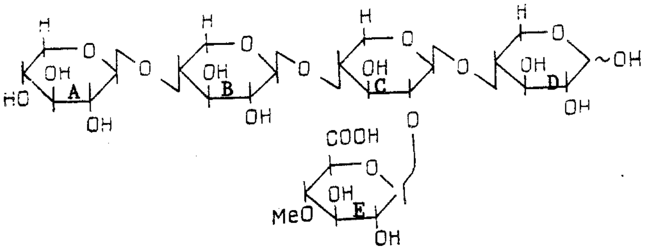
The oxidation of the polysaccharide by sodium periodate was complete after 72 h, when the consumption of periodate was 1 mole per anhydropentose unit. The results of periodate oxidation showed the branched structure of the polysaccharide. The oxidation of the xylan yielded 11.2 moles of formic acid, which corresponds to six branching points.

Reduction of the xylan polyaldehyde with sodium tetrahydroborate to a polyol and its subsequent hydrolysis led to the formation of two components, a xylosylglycerol and glycerol in a molar ratio of 1:10. The formation of glycerol was possible only in the case of 1 → 4 bonds between the carbohydrate residues. The results obtained agreed with those of methylation, which was carried out by Hakomori's method. In a hydrolysate of the permethylate, 3-O-methylxylose, 2,3-di-O-methylxylose, 2,3,4-tri-O-methylxylose, and methylated uronic acids were detected. The composition of the products indicated that the main chain of the polysaccharide was constructed of xylose residues linked by 1 → 4 bonds and the side chains were present at the second carbon atoms. The molar ratio of 2,3-di-O-methylxylose and 3-O-methylxylose was 10.4:1, which agreed with the results of Smith degradation.

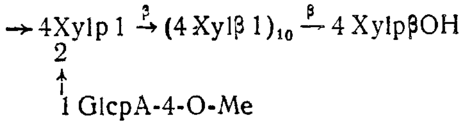
In parallel with an evaluation of the results of the chemical investigation, to investigate the structure of the polysaccharides we made use of ^{13}C NMR spectroscopy. The spectra were taken in two solvents — D_2O and DMSO. The interpretation of the signals in the spectrum was based on results obtained previously from analyses of ^{13}C NMR spectra of xylans and xylooligosaccharides [8]. Below we give the assignment of the signals of the carbon atoms in the ^{13}C NMR spectrum of the alfalfa xylan,* ppm:

Atom	Monosaccharide residue				
	A	B	C	D	E
C-1	101.1	101.6	101.0	93.7	97.3
C-2	72.6	72.6	77.0	73.6	71.8
C-3	76.3	73.9	72.7	73.9	72.0
C-4	69.4	75.3	75.3	75.3	81.4
C-5	65.6	63.1	63.1	63.0	69.6

Designation of the fragments



Having compared the results of the chemical investigation and those obtained by ^{13}C NMR spectroscopy, a scheme of the structure of the glucuronoxylan of alfalfa stems can be represented in the following way:



$$\begin{array}{l}\rightarrow 4\mathrm{Xylp}\,1 \xrightarrow{\beta} (4\,\mathrm{Xyl}\beta\,1)_{10} \xrightarrow{\beta} 4\,\mathrm{Xylp}\beta\mathrm{OH} \\ \quad 2 \\ \quad \uparrow \\ \quad 1\,\mathrm{GlcpA\text{-}4\text{-}O\text{-}Me}\end{array}$$

EXPERIMENTAL

Gel Filtration. A solution (0.8%) of the polysaccharide was added to a 1 × 40 cm column filled with Sephadex G-150. Fractions with a volume of 2 ml each were collected and were analyzed by the anthrone method.

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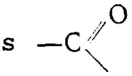
Paper electrophoresis was carried out in borate buffer at a current strength of 15-20 mA and a voltage of 500 V for 6 h.

The xylan was hydrolyzed with 2 N sulfuric acid at 100°C. The monosaccharides were identified by PC and GLC. For GLC a Chrom chromatograph with flame-ionization detector was used. The stationary phase was 5% of XE-60 on Chromaton N-AW-DMCS (0.2-0.25 mm) in steel columns (3 × 1200 mm). The rate of flow of carrier gas (helium) was 35 ml/min. Programming of the temperature at 2°C per minute in the interval of 140-220°C. The monosaccharides were analyzed in the form of alditol acetates.

The periodate oxidation of the xylan was carried out with a 0.3 M solution at room temperature until the consumption of periodate ceased (72 h). The consumption of $NaIO_4$ was determined iodometrically and the amount of formic acid by titration with a 0.02 M solution of NaOH.

Smith Degradation. The completely oxidized xylan was dialyzed and was reduced with sodium tetrahydroborate. The resulting polyol was hydrolyzed with 0.1 N HCl at 100°C for 6 h. The decomposition products – a xylosylglycerol and glycerol – were detected by PC in the solvent systems: 1) butanol–benzene–pyridine–water (5:1:1:3) and 2) 3% ammonia solution–butan-1-ol (4:9) in the presence of markers.

Methylation was performed in dimethyl sulfoxide with a solution of the methylsulfinylmethyl carbanion and methyl iodide by Hakomori's method. Formolysis of the methylated xylan was carried out with 90% formic acid at 100°C for 1 h, and the product was then hydrolyzed with 0.25 M H_2SO_4 for 14 h. The hydrolysates were investigated by PC and GLC.

Oxidation by CrO_3. A dispersion of 0.2 g of the polysaccharide in formamide was acetylated with a mixture of freshly distilled pyridine and acetic anhydride (1:1) at room temperature for 56 h. Then the reaction mixture was poured into ice water and the precipitate was separated off by centrifugation, was washed with water and with methanol, and was dried over P_2O_5. The IR spectrum of the substance obtained lacked the absorption bands of hydroxy groups and contained well-defined absorption bands of carbonyl groups  $-\mathrm{C}\begin{smallmatrix}/\!\!/\mathrm{O}\\ \diagdown\end{smallmatrix}$ (1240 and 1750 cm^{-1}). The acetylated polysaccharide (0.15 g) was added to a solution of 0.6 g of CrO_3 in 12 ml of glacial acetic acid and the mixture was heated at 50°C for 4 h. Then it was poured into water and extracted with chloroform. The chloroform extracts were dried with anhydrous Na_2SO_4 and evaporated to dryness. The anhydrous residue was hydrolyzed with 1 N H_2SO_4 at 100°C for 16 h. The hydrolysate was analyzed by PC in the solvent butan-1-ol–pyridine–water–benzene (5:3:3:1).

^{13}C NMR spectra were taken on a Bruker-Physik WM-250 instrument with a working frequency for carbon of 62.9 MHz at 80°C.

SUMMARY

The stems of alfalfa, Medicago sativa, contain, together with a 4-O-methylglucuronorhamnoxylan, a 4-O-methylglucuronoxylan. Its molecule is branched. The main chain is constructed of β-D-xylopyranose residues linked by 1 → 4 bonds. 4-O-Methylglucuronic acid residues are attached at C-2 of the xylopyranose residues; there is one branch for not less than each 11 xylose residues.

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