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STRUCTURE OF A GLUCURONOXYLAN OF THE STEMS OF *Symphytum asperum*

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The results are given of an investigation of the structure of a glucuronoxylan of the stems of *Symphytum asperum* Ler. The xylan was isolated by alkaline extraction and was purified by reprecipitation via the copper complex. The polysaccharide was homogeneous according to the results of gel filtration and electrophoresis. It was shown by hydrolysis, periodate oxidation, methylation, oxidation with chromium trioxide and IR and ^{13}C NMR spectroscopy that the macromolecules were based on a β-(1→4)-polyxyloside chain having side chains at the second carbon atoms in the form of 4-O-Me-D-glucuronic acid. To each side chain there were not less than 12 xylose residues.

Continuing a study of the polysaccharides of Symphytum asperum Ler. (prickly comfrey) [1], we have investigated the structure of a glucuronoxylan from the stems of this plant.

The xylan was isolated by alkaline extraction and was purified by two reprecipitations via the copper complex. Its homogeneity was shown by electrophoresis and by gel chromatography on Sephadex G-200. On the gel-filtration elution curve, the protein component coincided with the carbohydrate component, which indicates the possibility of a chemical bond between them.

The preparation isolated was characterized by a high polysaccharide content and a minor amount of impurities of noncarbohydrate nature: ash 1.2%; nitrogen 0.32%.

Complete acid hydrolysis showed the presence in the polysaccharide of D-oxylose, D-glucuronic acid and 4-O-methylglucuronic acid residues.

The negative specific optical activity, $[\alpha]_D^{20}$ −68.0, and the results of IR spectroscopy permitted the assumption of the presence of a β-bond between the monomeric residues in the macromolecule of the glucuronoxylan.

To determine the configuration of the glycosidic bonds we used the method of oxidation with chromium trioxide, which is based on the different behaviors of completely acetylated α- and β-hexopyranosides in relation to CrO_3 [2]. Under the action of chromium trioxide on the acetates of polysaccharides, only β-glycosidic bonds undergo oxidation. The completeness of oxidation was checked by IR spectroscopy from the absence of absorption bands of hydroxy groups, while there were well-defined absorption band at 1240 and 1750 cm^{-1} in the spectrum. Analysis of the oxidation products of the xylan by PC and GLC showed that the reaction mixture contained no monosaccharides, which indicates the presence of β-glycosidic bonds between the xylose residues.

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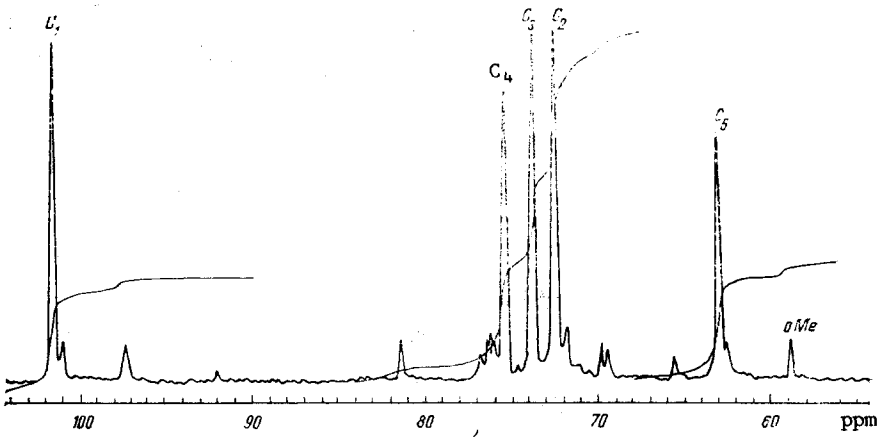


Fig. 1. ^{13}C NMR spectra of the glucuronoxylan of the stems of Symphytum asperum.

The structural features of the polysaccharide under study were established by comparing the results of periodate oxidation, Smith degradation, and methylation.

Complete oxidation of the polysaccharide was achieved 72 h after the beginning of the experiment with the consumption of about 1 mole of $NaIO_4$ per anhydrohexose unit. At the same time, 0.22 mole of formic acid was liberated, which shows the branched nature of the structure of the macromolecule.

The reduction of the polyaldehydroxylan with sodim tetrahydroborate to the polyol and its subsequent hydrolysis led to the formation of two components: xylosylglycerol and glycerol. Consequently, the xylose residues were bound by 1→4-glycosidic bonds and the side chains in the main polyxyloside chain were present at carbon atom 2. The results were obtained in harmony with those of methylation.

The xylan was methylated by Hakomori's method [3]. The completeness of methylation was confirmed by TLC on Al_2O_3 plates and IR spectroscopy from the absence of absorption bands of OH groups in the 3300-3360 cm^{-1} region. In a hydrolysate of the methylated xylan 2,3-di-O-methylxylose was identified by GLC as the main component, while 2,3,4-tri-O-methylxylose and 3-mono-O-methylxylose were detected in small amounts together with traces of methylated uronic acid. The molar ratio of 3-O-methylxylose and 2,3-di-O-methylxylose was 1:12. The 4-O-methylglucuronic acid residues were the terminal groups of the branches, as was confirmed by the presence of 2,3,4-tri-O-methyl-D-glucuronic acid.

The results of the chemical investigation of the structure of the xylan were confirmed by those of ^{13}C NMR spectroscopy.* To interpret the signals in the spectrum use was made of the results obtained previously from an analysis of the ^{13}C NMR spectra of xylans and xylooligosaccharides [4, 5]. The chemical shifts of the signals in the ^{13}C NMR spectra of the xylan from S. asperum are given below:

4-O-Substituted xylan residues	$C_{(1)}$	$C_{(2)}$	$C_{(3)}$	$C_{(4)}$	$C_{(5)}$	C_1 of 2-O-Substituted residues
p.D 14	102.75	73.6	74.85	76.9	63.9	100.9

The value of the chemical shifts of the signals indicated that the xylose residues were present in the pyranose form, but for 1→4-bound monosaccharides this conclusion cannot be made unambiguously on the basis of the results of methylation.

On comparing the results of the chemical investigation and those of ^{13}C NMR spectroscopy, the plan of the glucuronoxylan from the stems of S. asperum can be represented in the following way:

$$\begin{array}{l} Xyl\,p1\left(\xrightarrow{\beta} 4Xyl\,p1\right)_{11}\xrightarrow{\beta} 4Xyl\,p\beta OH \\ \quad 2 \\ \quad \uparrow \\ \quad 1\,GlcpA\text{-}4\text{-}O\text{-}Me \end{array}$$

*The recording and interpretation of the ^{13}C NMR spectra of the polysaccharide were performed by A. S. Shashkov.

EXPERIMENTAL

Isolation of the Xylan. The comminuted stems of prickly comfrey were defatted with diethyl ether and the free sugars were removed by boiling with 82% ethanol. The residue was extracted with 10% potassium hydroxide in a current of nitrogen for 72 h. The alkaline solution of HMCs was rapidly cooled in an ice bath and the HMC A was precipitated by acidification with 50% acetic acid to pH 5.0. The precipitate was separated off by centrifugation, and the HMC B was precipitated from the supernatant with ethanol. The "crude" HMC B was reprecipitated twice via the copper complex to constant composition.

Paper electrophoresis was performed in borate buffer, pH 11.2, at a current strength of 15-20 mA, voltage 500 V, time 6 h.

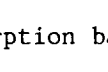
Gel fitration was performed in a 1 × 32 cm column filled with Sephadex G-200. The gel was prepared by the method given in [6].

The hydrolysis of the xylan was performed with 2 N H_2SO_4 in sealed tubes at 100°C for 16 h. The monosaccharides were identified by PC and GLC. A Chrom chromatograph with a flame-ionization detector was used for GLC. 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. The temperature was programmed at 2°C per minute in the range of 140-220°C. The monosaccharides were analyzed in the form of the corresponding alditol acetates [7].

The periodate oxidation of the xylan was performed with a 0.3 M solution of $NaIO_4$ at room temperature for 72 h, by which time the consumption of sodium periodate had reached constancy. The consumption of $NaIO_4$ was determined iodometrically and the amount of formic acid produced was determined by titration with a 0.02 M solution of NaOH.

Smith Degradation. The completely oxidized xylan was dialyzed and was reduced with sodium tetrachloride for 12 h. The polyol so obtained was hydrolyzed with 0.1 N HCl at 100°C for 6 h. The degradation products – xylosylglycerol and glycerol – were detected by PC in the following solvent systems: 1) butan-1-ol-benzene-pyridine-water (5:1:3:3), and 2) 3% ammonia solution –butan-1-ol (40:90) in the presence of markers.

Methylation was performed in dimethyl sulfoxide with a solution of methylsulfinyl carbanion and methyl iodide by Hakomori's method. The formolysis of the methylated xylan was performed with 90% HCOOH at 100°C for 1 h, and the product was hydrolyzed with 0.25 M H_2SO_4 for 14 h. The hydrolysates were studied by the PC and GLC methods.

Oxidation with CrO_3. In the form of a dispersion in formamide, 0.15 g of the polysaccharide was acetylated with a mixture of freshly distilled pyridine and acetic anhydride and pyridine (1:1) at room temperature for 52 h. The reaction mixture was poured into ice water and the precipitate was separated off by centrifugation and it 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 but had well-defined absorption bands of the  group (1240 and 1750 cm^{-1}).

After the dissolution of 0.6 g of CrO_3 in 12 ml of glacial CH_3COOH, 0.13 g of the acetylated polysaccharide was added 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 dry 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).

The ^{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. The solvent was DMSO-d_6 or D_2O, pD 14. The chemical shift δ_{DMSO} = +39.5 ppm. Methanol was used as the standard for the aqueous solutions (δ_{MeOH} = 502 ppm). For the solutions in DMSO we also recorded the spectra under the conditions of retaining the spin–spin coupling of carbon with protons in order to determine the $^1J_{^{13}C_1-^1H_1}$ SSCCs.

SUMMARY

The xylan of the stems of Symphytum asperum Ler. belongs to the glucoronoxylan group and

the structure of its macromolecule is similar to that of the glucuronoxylans of leguminous herbs studied previously. It consists of xylopyranose residues linked to one another by 1→4 bonds. 4-O Methyl-D-glucuronic acid residues are attached in the form of side chains at the C-2 position of some of the xylopyranose residues; to each side chain corresponds not less than 12 xylose residues.

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POLYSACCHARIDES FROM THE INFLORESCENCES OF Matricaria matricarioides

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Fractionation of the polysacchride complex of the inflorescences of pineapple weed has given a galacturonan and a pectic acid. The structures of the galacturonan and pectic acid have been studied by the methods of enzymatic hydrolysis, periodate oxidation, and exhaustive methylation. It has been established that the galacturonan is a linear polysaccharide consisting of D-galacturonic acid residues in the pyranose form with α-1→4-bonds. The main polysaccharide chain of the pectic acid consists of D-galacturonic acid residues in the pyranose form, D-galactose, L-arbinose, and D-xylose residues are covalently bound to carbon atoms 2 or 3 of the main chain of the polysaccharide.

Earlier [1, 2], a water-soluble polysaccharide complex possessing pronounced biological activity [3,4] was isolated from inflorescence of Matricaria matricarioides (pineapple weed, family Asteraceae).

We have studied the structure of the galacturonan and the pectic acid obtained on the fractionation of the initial polysaccharide complex [2].

After the enzymatic hydrolysis of the galacturonan, mono-, di-, tri-, and tetragalacturonic acids were detected [5].

On periodate oxidation [6], the galacturonan consumed 1 mole of sodium metaperiodate per anhydro unit. From the oxidation products was isolated a products was isolated a polyaldehyde with water), which was subjected to further oxidation with 25% nitric acid [7] and was hydrolyzed. The hydrolysis products were investigated by the PC method in system 4. Oxalic and tartaric acids were identified in comparison with authentic samples. The isolation of the tartaric acid in the crystalline state with mp 167-169°C (a mixed melting point with an authentic sample of tartaric acid gave no depression) showed that grouping involving the second and third carbon atoms had been oxidized by the periodic acid. This is possible in the case of a pyranose ring with 1→4-bonds between the galacturonic acid residues. The presence of the pyranose ring was also shown by the resistance of the substance to acid hydrolysis and by its IR spectra with characteristic absorption bands in the 1000-1100 cm^{-1} region.

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