STRUCTURE OF THE XYLAN OF Onobrychis viciifolia

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The xylans of herbs have been little studied [1-3]. In the present paper we give the characteristics of the structure of the xylan of the stems of Onobrychis viciifolia (common sainfoin). The stems contained (%): moisture, 10; readily hydrolyzable polysaccharides (RHP), 34.08; difficultly hydrolyzable polysaccharides (DHP), 41.27; lignin, 16.01; ash substances, 5.86; and total nitrogen, 1.54. The monosaccharide composition of a hydrolyzate of the RHP of the stems is given below:

Component	Percentage of the absolutely dry weight	Proportion, %
Uronic acids	3.66	10.32
Galactose	1.85	5.24
Glucose	3.62	10.22
Arabinose	5.02	14.30
Xylose	21.20	59.92

A hydrolyzate of the DHP contained (%): glucose, 22.60; xylose, 10.00; and mannose, 9.70. Thus, the composition of the DHP of sanfoin differs considerably from the DHP of wood and of wheat straw by the presence of large amounts of mannose and xylose. A hydrolyzate of the xylan isolated was found to contain only xylose and uronic acids, which characterizes this polysaccharide as a glucuronoxylan. Its composition is identical with that of the xylans of broad-leaved trees and differs from the araboglucuronoxylars of coniferous trees and the stems of cereals.

Periodate oxidation of sanfoin xylan took place at a greater rate than that of the analogous polysaccharides isolated from other types of raw material and led to the rapid overoxidation of the xylan.

Lowering the concentration of the oxidizing agent reduced the rate of oxidation somewhat. Complete oxidation was achieved in two days, and then a rapid increase in the consumption of periodate with the accumulation of formic acid took place (Table 1).

TABLE 1

Time of oxidation, days	Consumption of $NaIO_4$ per mole of pentose residue		Amount of HCOOH liberated on oxidation	
	0.3 M solution	0.1 M solution	by a 0.3 M solution of $NaIO_4$	by a 0.1 M solution of $NaIO_4$
One	1,12	0,85	0,18	0,12
Two	1,37	0,98	0,31	0,14
Three	1,68	1,33	0,48	0,26
Four	2,51	—	0,71	—

When the process was performed at lower temperatures, the rate of oxidation again fell, but in these cases no stability of the process was achieved even in the presence of sodium hyposulfite which, according to some statements, almost excludes the process of "overoxidation." Complete oxidation was achieved in 2 h with the use of a 0.3 M solution of periodate and in two days with the use of a 0.1 M solution. The consumption of periodate in this time amounted to 0.98 mole per mole of pentose residue. The number of moles of formic acid formed from the macromolecule of the xylan (DP 129) was 18. Of

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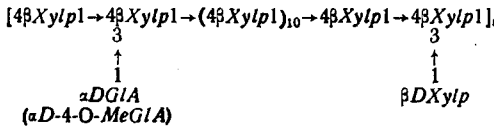
these, 3 moles were produced by terminal groups and 15 moles from side chains. This does not take into account 4-O-methylglucuronic acid present in the side chains but not liberating formic acid on oxidation.

From the results obtained in a study of the Smith decomposition of the xylan, the ratio between the number of unbranched and branched xylopyranose residues was 4:1, which correlates well with the results of methylation.

The results of periodate oxidation, Smith degradation, and methylation showed that the structure of the glucuronoxylan of the herb sainfoin has the following form:



EXPERIMENTAL

Isolation of the Xylan. The xylan was isolated from comminuted stems of Onobrychis viciifolia of the 1971 crop gathered in the variety section of the Odessa agricultural institute.

The stems were previously separated from the leaves and flowers. Then they were treated repeatedly with water at 40°C. The residue after this treatment (300 g) was extracted with a 6% solution of KOH (1.5 liter), and the xylan was obtained by the method of Dudkin et al. [4]. The xylan was purified via the copper complex. The purity of the xylan was determined chromatographically after hydrolysis.

Hydrolysis of the Xylan. The xylan was hydrolyzed with 2% HCl under reflux in the boiling-water bath for 4 h. The carbohydrate composition of the hydrolyzate was determined by paper chromatography. Xylose and uronic acids were found.

Characteristics of the Xylan. The xylan investigated was electrophoretically homogeneous. The molecular weight determined viscosimetrically was 17,000 and the degree of polymerization 129; $[\alpha]_D^{20}$ −70° (c 1.6%, KOH. The readily hydrolyzable part of the xylan of sainfoin amounted to 89.0%. The RHP contained uronic acids and xylose in a ratio of 9:88 (% of the absolutely dry weight).

Distribution of the Uronic Acids. The unrevealed sections of the chromatograms, corresponding to the total uronic acids [solvent: butanol−benzene−pyridine−water (5 : 1 : 3 : 3)] were cut out and stitched to a clean sheet of chromatographic paper, and a second mobile phase in which the uronic acids have different mobilities [ethyl acetate−pyridine−water (10 : 4 : 3)] was passed. After separation and the treatment of the chromatogram with revealing agents, four spots were found corresponding to D-glucuronic acid (R_{xyl} 1.15), aldotetrauronic acid (R_{xyl} 0.45), 4-O-methyl-D-glucuronic acid (R_{xyl} 1.15), and aldobiuronic acid (R_{xyl} 0.91) were found. The acids were isolated in the crystalline state and the D-glucuronic acid was identified by comparison with an authentic sample, the 4-O-methyl-D-glucuronic acid from its angle of rotation, α_D^{20} −18°, and the aldobiuronic acid after hydrolysis with 10% HCl in methanol for 4 h. The xylose and D-glucuronic acid were found in a ratio of 1:1.

Periodate Oxidation. The xylan was oxidized with a 0.3 M solution of sodium periodate at room temperature for 4 h.

Smith Degradation. The oxidized and dialyzed xylan (0.5 g) was reduced with sodium tetrahydroborate (0.17 g). The resulting polyol was hydrolyzed with 0.2 N HCl for 6 h at room temperature. By paper chromatography, the hydrolysis products were found to contain glycerol and xylosylglycerol. The composition of the latter was determined by its hydrolysis with 5% HCl for 6 h. The hydrolyzate contained xylose and glycerol in a ratio of 1:1.

Methylation. The reaction was performed by two methods: by Haworth's method in tetrahydrofuran with solid alkali and dimethyl sulfate, and by Hakamori's method in dimethyl sulfoxide with a solution of the methylsulfinylcarbanion and methyl iodide. By the first method, complete methylation was achieved after nine treatments of the glucuronoxylan with the methylating reagents, and by the second method after three methylations. The completeness of methylation was determined by thin-layer chromatography on plates with Al_2O_3 and also by IR spectroscopy from the constancy of the absorption band in the 2910 cm^{-1} region that is characteristic for the $-OCH_3$ group.

Hydrolysis of the Methylated Xylan. The methylated product was subjected to formolysis with 90% HCOOH for 1 h at 100°C, and was then hydrolyzed with 0.25 M H_2SO_4 for 14 h at the same temperature. The hydrolyzate obtained was neutralized with $BaCO_3$ solution, centrifuged, and evaporated to small volume.

Analysis of the products of the hydrolysis of the methylated polysaccharide performed by paper chromatography showed that it contained mono-, di-, and trimethylxyloses (2 : 12 : 1, respectively) and a methylated uronic acid.

Separation of the Monomethylxyloses. To determine the position of the methyl group (2 or 3) on the monomethylxylose, it was chromatographed on paper impregnated with borax, upon which the monomethylxyloses have different mobilities. 3-O-Methylxylose was found. Consequently, the side chains are attached to carbon atom 2.

SUMMARY

1. Among the polysaccharides of Onobrychis viciifolia has been found a glucuronoxylan the main chain of which is constructed of β-D-xylopyranose units linked by the 1 → 4 carbon atoms. In the one-unit side chains there are residues of D-glucuronic and 4-O-methyl-D-glucuronic acids and also of β-D-xylopyranose attached to the main chain through the second carbon atom.

2. The xylan of common sainfoin is characterized by ease of oxidation by periodate. Complete oxidation is achieved by the action of 0.3 M sodium periodate at room temperature in 2 h.

3. The carbohydrate composition of the xylan of the stems differs from the xylan of the stems of cereals (wheat) and is similar to the xylans of broad-leaved trees.

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