STRUCTURE OF THE ARABINOGLUCURONOXYLAN OF Poa pratensis

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We have previously described the structure of a 4-O-methylglucuronoxylan from Onobrychis viciifolia (common sainfoin). In the present paper we give the results of a study of the structure of an arabinoglucuronoxylan of another fodder herb Poa pratensis (Kentucky bluegrass).

In the stems of this plant we found (%): moisture 9.0; readily hydrolyzable polysaccharides (RHPs) 25.40; difficultly hydrolyzable polysaccharides (DHPs) 27.46; lignin (ash-free) 16.71; ash substances 7.37; total nitrogen 1.9.

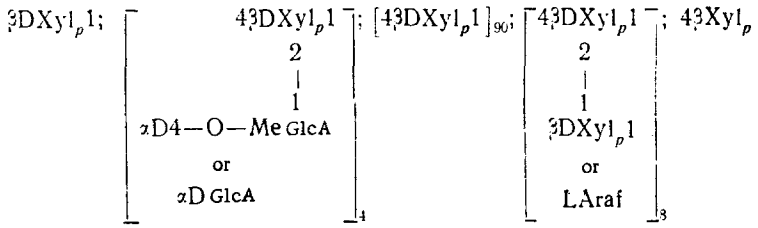
Below we give the monosaccharide composition of a hydrolysate of the RHPs:

Monosaccharides	Percentage by weight	Percentage ratio
Uronic acids	1.12	3.92
Galactose	0.91	3.17
Glucose	1.45	5.06
Arabinose	4.96	17.34
Xylose	20.50	70.51

It can be seen from the figures given that the raw material contains xylan, with other polysaccharides in smaller amounts. The DHPs contain mainly glucose.

The xylan was isolated by alkaline extraction and purified by the usual method. Its homogeneity was shown electrophoretically, and chromatographic analysis of a hydrolyzate demonstrated the presence in the substance of only D-xylose, L-arabinose, D-glucuronic acid, and 4-O-methyl-D-glucuronic acid.

The structure of the arabinoglucuronoxylan was established by the parallel use of a series of methods: periodate oxidation, Smith degradation, methylation, and IR spectroscopy. The complete oxidation of the polysaccharide by sodium periodate was achieved in five days. The consumption of periodate per anhydro unit was 0.97-0.99 mole. The reduction of the polyaldehyde derivative of the xylan with sodium tetrahydroborate to a polyol and its subsequent mild hydrolysis led to the formation of xylose and glycerol in a ratio of 1:11. This shows that there is one xylose unit bearing branching for every 11 xylose residues in the linear chain of the polymer. The results of methylation and the formation on the hydrolysis of the methylated xylan of 3-O-methylxylopyranose, 2,3-di-O-methylxylopyranose, and 2,3,4-tri-O-methylxylopyranose in a ratio of 4:33:3 confirmed the results of periodate oxidation. Hence the structure of the arabinoglucuronoxylan of the stems of Kentucky bluegrass can be represented schematically in the following way:



EXPERIMENTAL

Preparation of the Raw Material. The bluegrass stems were comminuted and sieved through a sieve having apertures with a diameter of 1 mm. The raw material was treated with water at 40°C to eliminate

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free sugars and water-soluble polysaccharides and then with a 0.5% aqueous solution of ammonium oxalate. The amount of pectin substances isolated was 0.4% on the weight of the sample taken.

Isolation of the Xylan. The residue after treatment with water (300 g) was extracted with 6% KOH solution (1.5 liter). The xylan was obtained and purified as described previously [1]. The purity of the xylan was determined chromatographically.

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

Electrophoresis was performed in borate buffer, pH 10.8, at a current strength of 20 A and a voltage of 420 V for 5 h. After staining, a single spot was found which had moved only slightly relative to the starting line, showing the homogeneity of the preparation. The molecular weight was 15,000 c.u., $[\alpha]_D^{20} -73°$. Composition of the xylan (%): RHPs 87.68; DHPs 2.34; lignin 5.08; ash 0.81; $-OCH_3$ 1.80; monosaccharide composition of a hydrolyzate (%): xylose 87.50, arabinose 5.56, and uronic acids 3.36.

Separation of the Uronic Acids. Unstained sections of the total uronic acids [solvent: butanol–benzene–pyridine–water (5 : 1 : 3 : 3)] were cut out and stitched to a new sheet of chromatographic paper and were then run in the solvent ethyl acetate–pyridine–water (10 : 4 : 3). After separation, treatment with aniline phthalate showed the presence of D-glucuronic acid, an aldotetrauronic acid, an aldobiuronic acid, and 4-O-methyl-D-glucuronic acid.

Periodate Oxidation. The xylan was oxidized with a 0.3 M solution of sodium periodate at room temperature until the consumption of sodium periodate was constant (4-5 days).

Smith Degradation. The oxidized xylan was reduced with sodium tetrahydroborate to a polyol and this was then hydrolyzed with 0.2 N HCl with heating to 100°C for 5 h. Among the hydrolysis products we found xylose and glycerol. The amount of xylose was determined with the aid of a photoelectric colorimeter and the amount of glycerol by paper chromatography [2].

The methylation of the xylan under investigation was performed by Hakomori's method [4]. Complete methylation was achieved after three treatments with the methylating reagent. The end of methylation was checked on plates of Al_2O_3 from the absence of free sugars after hydrolysis and through the IR spectra from the disappearance of the absorption bands in the 3340 cm^{-1} region that characterized the presence of free hydroxy groups in the methylated xylan.

Hydrolysis of the Methylated Polysaccharide and Its Characterization. To cleave the methylated xylan, formolysis was performed with 90% HCOOH at 100°C for 1 h, followed by hydrolysis with 0.25 M H_2SO_4 for 14 h. The hydrolyzate was found to contain methylated uronic acids and 2(3)-mono-, 2,3-di-, and 2,3,4-trimethylxyloses. The presence of 2,3,4-trimethylxylose showed that a small amount of the xylose is present in a branched part of the chain. In view of the fact that the mobility of trimethyl derivatives of arabinose and xylose in the solvent used are the same, we performed the demethylation of these compounds and found arabinose and xylose.

The results of a quantitative determination of the methylated sugars by the iodometric method showed that the 2(3)-monomethylxylose, 2,3-dimethylxylose, and 2,3,4-trimethylhexose are present in them in a ratio of 4 : 33 : 3.

SUMMARY

The xylan of the stems of Poa pratensis belongs to the arabinoglucuronoxylans. It is constructed of D-xylopyranose residues connected to one another by 1→4 bonds. The branches contain residues of glucuronic acid and its 4-O-methyl ether, L-arabinose, and D-xylose.

LITERATURE CITED

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