STRUCTURE OF THE XYLAN FROM THE STEMS OF THE COTTON PLANT

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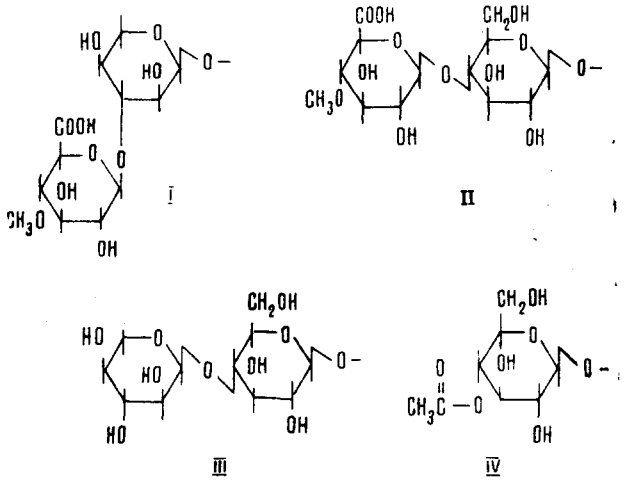
The xylan from the stems of the cotton plant is a 1→4-β-L-anhydroxylopyranoside. Its basic link consists of six to seven 1→4-β-L-anhydroxylose units and has two points of branching [1]. The present paper gives information on the structure of the side chains and the positions of their attachment obtained by a study of the products of the hydrolysis of the completely methylated polysaccharide. Methylation was performed by Hakomori's method [2]. The substances shown in Table 1 were found in the hydrolyzate of the methylated xylan by paper chromatography.

The main product in the hydrolyzate of the methylated xylan was 2,3-di-O-methyl-L-xylose, which confirms the β-1→4 bond in the main chain of the polysaccharide. The amount of 2-O-methyl-L-xylose agrees fairly well with the number of branching points determined from the yield of formic acid on periodate oxidation [1]. The formation of 2-O-methyl-L-xylose shows that the side chains of the molecule are attached to the main chain in the C-3 positions of the xylose units.

The presence of 2,4-di-O-methyl-L-xylose and of 2,3,6-tri-O-methyl-L-xylose among the hydrolysis products and also the fact that all the glucose and a considerable amount of the xylose is split off under mild conditions of hydrolysis, under which the β-1→4 bond is not hydrolyzed, indicates that these two sugars are present in the side chains.

On the basis of the facts given, the most probable structures I, II, III, and IV can be proposed for the side chains of the xylan:



The choice between I and II was made on the basis of the following facts. The aldobiuronic acid isolated from the xylan hydrolyzate was reduced to a disaccharide, the hydrolysis of which gave equal amounts of 4-O-methyl-D-glucose and L-xylose. Thus, the aldobiuronic acid has the structure I, and the glucose is present in a side chain having structure III. Furthermore, 2,3,6-tri-O-methyl-D-glucose can be formed from the terminal glucose units acetylated in the C-4 [4] position. The presence of acetyl groups in the xylan

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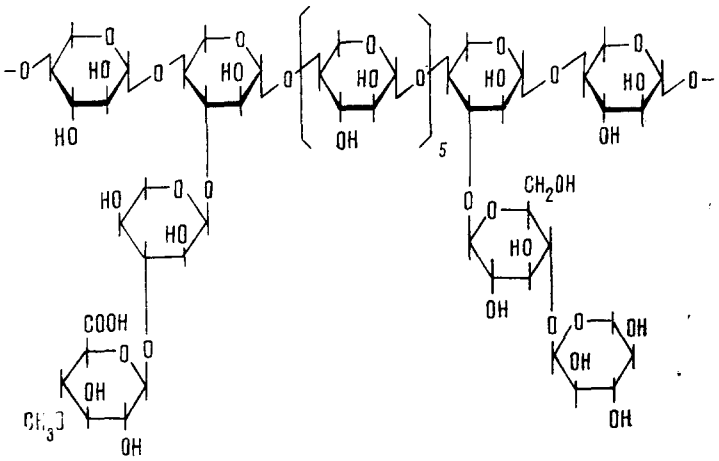
was confirmed by IR spectroscopy (strong band at 1250 cm^{-1}), and the mild conditions of methylation exclude the splitting off of these groups.

The results of the study of the hydrolysis of the completely methylated xylan, and also those obtained previously [1], permit the following most probable structure to be proposed for it:



EXPERIMENTAL

Paper chromatography was performed with Whatman No. 1 paper and the following systems: 1) butan-1-ol–ethanol–water–0.1% ammonia (40 : 10 : 49 : 1); 2) ethyl acetate–pyridine–water (5 : 1 : 5); and 3) ethyl acetate–acetic acid–formic acid–water (18 : 9 : 4 : 1). The spots were revealed with aniline phthalate.

The quantitative determination of the sugars and their methylated derivatives was performed by paper chromatography with a type DFÉ-10 densitometer. The IR spectrum of the methylated xylan was taken in dimethyl sulfoxide on a UR-10 instrument.

Methylation of the Xylan. 1) Preparation of the methylsulfinyl anion. A centrifuge tube containing 0.52 g of a 50% emulsion of sodium hydride in mineral oil was sealed completely with a rubber stopper. The test tube was evacuated several times and filled with argon through the needle of a syringe. To eliminate the mineral oil, the contents of the tube were washed three times with dry petroleum ether. The solvent was added to the tube and removed by means of the syringe. After the removal of the mineral oil, the residue was dried in vacuum, the tube was again filled with argon, and 4.8 ml of absolutely dry dimethyl sulfoxide was added. The hydrogen which vigorously evolved was released through the needle of the syringe. Then the mixture was heated at 50°C with constant stirring for 80 min. A transparent greenish solution of the dimethylsulfinyl anion was obtained.

2) Methylation. The xylan (0.2 g) which had been dried over P_2O_5 in vacuum was dissolved in 12 ml of absolute dimethyl sulfoxide. With the aid of a syringe, the solution was rapidly transferred to the tube containing the dimethylsulfinyl anion, and the mixture was cooled to 20°C. A viscous gel formed immediately, and then this gradually liquefied. The mixture was kept at room temperature for 10 h. After this, with the syringe, 10 ml of dry methyl iodide was added to the ice-water-cooled mixture, and the solution was kept at room temperature for another 3 h. Then it was poured into 200 ml of water and extracted with chloroform. The chloroform extract was washed twice with water. The chloroform was distilled off to dryness, toluene was added to the residue, and this was again distilled off to dryness.

The final residue was a viscous yellowish syrup with a yield of 0.137 g. The IR spectrum of this product lacked bands characteristic for hydroxy groups.

Hydrolysis of the Methylated Xylan. A mixture of 1 g of the methylated xylan and 5 ml of 90% formic acid was heated at 100°C for 1 h and was then cooled and evaporated in vacuum at 40°C to a syrup. The syrup was dissolved in 5 ml of 0.25 M sulfuric acid and heated at 100°C for 14 h. The hydrolyzate was neutralized with barium carbonate, the precipitate was separated off on a centrifuge and was carefully washed with water and ethanol. The combined solutions were evaporated in vacuum at a temperature not exceeding 40°C. The solution so obtained was chromatographed in systems 1 and 2 (the results are given in Table 1). The methylated sugars were identified in comparison with authentic samples and by the comparison of their R_g values with those given in the literature.

TABLE 1. Composition of the Hydrolysis Products of the Methylated Xylan

Methylated sugars	R_g values in systems		Number of moles per mole of xylan
	1	2	
2,3,4-Tri-O-methylxylose	0.91	0.96	9
2,3,6-Tri-O-methylglucose	0.83	0.85	8
2,3-Di-O-methylxylose	0.74	0.81	67
2,4-Di-O-methyl xylose	0.66	0.68	8
2-O-Methylxylose	0.38	0.62	14
O-Methylglucuronic acid I	0.18	0.32	3
O-Methylglucuronic acid II	0.13	0.2	5

Note. R_g was determined relative to 2,3,4,6-tetra-O-methylglucose.

Reduction of the Aldobiuronic Acid [5]. The barium salt (50 mg) of the aldobiuronic acid isolated from the hydrolyzate of cotton-plant stem xylan [1] was boiled with 4% hydrogen chloride in methanol for 4 h. Then the solution was neutralized with silver carbonate. The precipitate was separated off, and the solution was treated with 150 mg of sodium tetrahydroborate and was left at 5°C for 20 h. The residual hydride was decomposed with glacial acetic acid (12-15 drops), and the acid solution was treated first with Amberlite IR-120 and then with IR-4B. The solution was filtered, and the filtrate was evaporated to dryness in vacuum. Then the dry residue was treated several times with methanol and was again evaporated to eliminate borate ions.

Hydrolysis of the Reduced Aldobiuronic Acid. The residue obtained in the preceding experiment was heated with 1 N sulfuric acid at 100°C for 5 h. The hydrolyzate was neutralized with barium hydroxide, the precipitate was separated off, and the filtrate was evaporated in vacuum to a syrup. The syrup was chromatographed on paper in system 3. Xylose and 4-O-methylxylose in molar ratios (1:1) were found.

Quantitative Determination of the Sugars and Their Methylated Derivatives. A solution of the mixture of sugars under investigation with a definite concentration (about 0.5%) was prepared. Then a known amount of the solution (by weight or by volume) was deposited on paper, the amount being such that after chromatography the diameter of the spot was not more than 5-6 mm. The chromatograms were treated with aniline phthalate to reveal the spots, and to stabilize the colors of the spots they were kept at room temperature for a day. Then the diffusion density of the colors of the spots was measured on the densitometer. To calculate the amount of sugars the densitometer was calibrated with chromatograms of solutions of xylose and glucose of known concentration.

Since the amount of 2,3-di-O-methyl-L-xylose in the hydrolyzates was much greater than the amounts of the other methylated monoses, it was determined on separate chromatograms with a smaller amount of the substance being analyzed.

SUMMARY

1. The structure of the aldobiuronic acid isolated from the xylan of cotton-plant stems has been established by its reduction and the hydrolysis of the reduced product.

2. From a study of the products of the hydrolysis of the methylated xylan from cotton-plant stems the positions of attachment of the side chains in the molecule of the xylan and their structure have been determined, and a most probable structure for the xylan molecule has been put forward.

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