THE STRUCTURE OF THE XYLAN FROM COTTON-PLANT STEMS

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UDC 547.917

We have previously shown that the xylan from cotton-plant stems has a molecular weight of 8300 ± 500 (DP 65-67), $[\alpha]_D$ +79° [1]. The present paper gives the results of a study of the structure of this xylan.

The complete acid hydrolysis of the xylan formed xylose (74.4%), glucose (11.6%), and glucuronic acid (12.3%). The xylose that we obtained from the hydrolyzate of cotton-plant stems had mp 140-141°C (from ethanol) and $[\alpha]_D$ −32° (c 0.8; water); phenylosazone had mp 158-159°C, $[\alpha]_D$ +20° (c 0.5; methanol). Consequently, the xylan from cotton-plant stems consists mainly of L-xylose, and it is the first time that this has been found in natural xylans.

To determine the structure of the main chain of the xylan, hydrolysis was performed under mild conditions, when only the side chains of the polysaccharides are split off and the bonds of the main chain are hardly affected [2]. In the hydrolyzate obtained in this way we identified L-xylose, D-glucose, and D-glucuronic acid, which apparently form the side chains of the xylan. After the mild hydrolysis, the residue of the xylan contained only L-xylose.

Oxidation of the xylan with periodic acid gave 14 moles of formic acid from 1 mole of xylan, which corresponds to 11 branching points [3] in the polysaccharide molecule.

The following are the main substances obtained from the products of the oxidative cleavage of xylan by Barry's method [4]: glyoxal di(phenylhydrazone) (I), formed from oxidized terminal nonreducing xylopyranoses; glycerose phenylosazone (II), formed from 1→4-linked xylopyranose units; xylose phenylosazone (III) formed from xylopyranose units substituted in position 2 or 3 and not having undergone oxidation; and a phenylhydrazone which we have not identified, with mp 215°C (IV). The molar ratio of the products obtained – (I), (II), (III), and (IV) – was 5 : 51 : 13 : 7.

The formation of glycerose phenylosazone as the main product of the Barry decomposition shows that the basic chain of the polysaccharide contains 1→4-glycosidic linkages. The positive rotation of the xylan constructed from L-xylopyranose units is possible only if β-glycosidic bonds are present [5]. Consequently, the main chain of cotton-plant-stem xylan is that of a 1→4-β-L-xylopyranoside. The amount of xylose osazone formed on oxidative degradation shows the presence of twelve branching points in the xylan molecule, which agrees with the number of branching points calculated from the yield of formic acid on the periodate oxidation of the xylan.

On the basis of the results of a study of the products of the hydrolysis and oxidative cleavage of the xylan from cotton-plant stems, it has been established that the main chain of the polysaccharide consists of 6-7 β-L-anhydroxylose units and has two branching points at which the side chains are attached in position 2 or 3. The side chains consist of D-glucose and D-glucuronic acid residues.

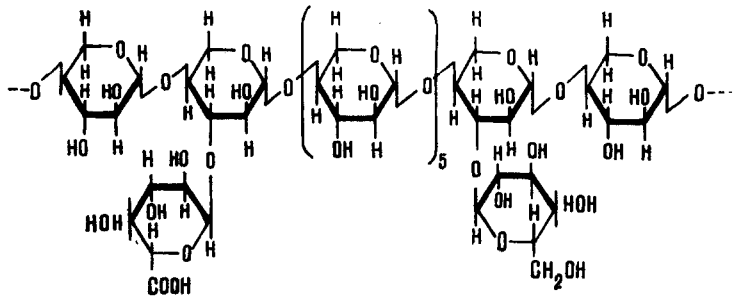
Thus, the following structure may be proposed for cotton-plant-stem xylan (see scheme on next page).

The type of bonds between the main and the side chains is assumed by analogy with the structure of the xylans from other plants [6].

EXPERIMENTAL

The chromatographic analysis was performed on Whatman No. 1 paper. The chromatograms were of the radial and ascending types, with an exposure time of 48 h. The following systems of solvents were used:

V. I. Lenin Tashkent State University. Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 305-308, May-June, 1973. Original article submitted July 3, 1972.



1) ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4), and 2) toluene–ethanol–water (270 : 30 : 1). The chromogenic agents were aniline phthalate and ammoniacal silver solution.

Hydrolysis of the Xylan. A mixture of 1 g of the xylan and 100 ml of concentrated formic acid was heated at 100°C for 16 h. The unhydrolyzed part was separated off on the centrifuge (0.01 g). The formic acid was distilled off in vacuum at 45°C, and the dry residue was heated with 100 ml of 0.5% sulfuric acid for 5 h. The hydrolyzate was neutralized with barium hydroxide to pH 6. The barium sulfate was filtered off and the residue was washed with hot water. The filtrate and the wash waters were combined and concentrated in vacuum at 45°C to small volume. The addition of methanol (two volumes) to this syrup gave a precipitate of the barium salt of a uronic acid. The precipitate was separated off, washed with methanol, and dried (yield 0.2 g).

The amount of reducing substances in the neutral hydrolyzate after the separation of the barium uronate was determined by the ebulliostatic method [7]. The amount of reducing substances formed was 98.2% (on the weight of the dry residue from the neutral hydrolyzate).

Isolation of L-Xylose. The glucose was removed from the hydrolyzate by fermenting the neutral hydrolyzate with yeast of the "Leningrad" strain. The solution after fermentation was purified by boiling with activated carbon and was evaporated in vacuum to dryness. The dry residue was recrystallized from acetic acid and then from ethanol. This gave L-xylose with mp 140–141°C, $[\alpha]_D$ −32° (c 0.8; water), phenylosazone with mp 158–159°C, $[\alpha]_D$ +20° (c 0.5; methanol).

Mild Hydrolysis of the Xylan. A mixture of 1 g of the xylan and 100 ml of 30% formic acid was boiled for 1 h. The residue (degraded xylan) was separated on the centrifuge and washed with water. The filtrate and the wash waters were combined and the solvent was eliminated completely in vacuum. The residue obtained was chromatographed in system 1. An aldobiuronic acid, xylose, glucose, and a small amount of oligosaccharides were detected on the chromatograms. After inversion (1.0% hydrochloric acid, 100°C, 5 h), xylose, glucose, and an aldobiuronic acid were found.

Hydrolysis of the Degraded Xylan. The residue after mild hydrolysis in 100 ml of 30% formic acid was heated until it had dissolved completely (24 h). The acid was removed from the hydrolyzate in the same way as in the preceding experiment. After inversion, only L-xylose was found on a chromatogram of the hydrolyzate (system 1).

Periodate Oxidation of the Xylan. A solution of 4 g of the xylan in 100 ml of hot water was cooled to 5°C, and a cold solution of 3.8 g of periodic acid in 25 ml of water was added. The mixture was left to oxidize in the refrigerator. After every 24 h, the consumption of periodate was determined on an aliquot [8]. Oxidation was complete after 120 h. The consumption of periodate was 0.5 mole per anhydropentose unit. After the end of oxidation, the amount of formic acid formed was determined in an aliquot by titration with 0.01 N caustic soda in the presence of Methylene Red. The yield of formic acid was 0.076 g (0.00165 mole).

Treatment of the Oxidized Xylan with Phenylhydrazine. A solution of the oxidized xylan was brought to pH 6 with barium carbonate. The precipitate, consisting of barium iodate and periodate, was filtered off and washed with cold water. The filtrate and the wash waters were combined, and 80 ml of the solution obtained was treated with 10 ml of freshly distilled phenylhydrazine in 24 ml of 10% acetic acid. This gave 2.2 g of a yellow precipitate.

Degradation of the Oxidized Xylan Derivative. A mixture of the yellow precipitate (2.0 g), ethanol (60 ml), phenylhydrazine (7 ml), glacial acetic acid (9 ml), and water (30 ml) was boiled for 4 h. Then the reaction mixture was evaporated in vacuum to small volume. On cooling, a precipitate of the di(phenylhydrazone) of glyoxal precipitated with mp 165–166°C (from benzene). Yield 1.39 g. On dilution with water,

the filtrate gave a resinous precipitate (2.73 g).

Separation of the Products of the Cleavage of the Oxidized Xylan. The resinous residue was triturated with alumina and a small volume of benzene and was transferred to a column containing 100 g of alumina. The column was washed successively with benzene, benzene–ether (1:1), ether, ethanol, and ethanol–water (9:1), and 20-ml fractions were collected (monitoring by radial paper chromatography in system 2). The following fractions were obtained:

Fractions 1-5 (benzene): glyoxal di(phenylhydrazone), mp 165°C (from benzene), R_f 0.94, yield 0.13 g.
Fractions 6-10 (benzene): mixture of glyoxal di(phenylhydrazone) with R_f 0.94 and glycerose phenylosazone with R_f 0.76;
Fractions 11-15 [benzene–ether (1:1)]: glycerose phenylosazone, mp 129-130°C [from benzene–petroleum ether (1:1)], R_f 0.76, yield 1.37 g;
Fractions 16-18 [benzene–ether (1:1)]: mixture of unidentified substances;
Fractions 19-25 [benzene–ether (1:1)]: N-acetylphenylhydrazine, mp 128°C (from petroleum ether), R_f 0.75, yield 0.32 g;
Fractions 26-35 (ether): mixture of products;
Fractions 36-41 (ethanol): mixture of products;
Fractions 42-60 (ethanol): xylose phenylosazone, mp 158-159°C [ethanol–water (1:1)], R_f 0.47, yield 0.345 g;
Fractions 61-67 [ethanol–water (9:1)]: mixture of products; and
Fractions 67-75 [ethanol–water (9:1)]: product with mp 215°C, R_f 0.44, yield 0.19 g.

SUMMARY

The results of a study of the products of the hydrolysis and oxidative cleavage according to Barry of cotton-plant-stem xylan has shown that its main chain consists of 1→4-β-L-anhydroxylopyranose units. Each elementary link of the xylan contains 6-7 xylopyranose units and has two side chains formed by residues of D-glucose and D-glucuronic acid, which are attached in position 2 or 3 of the xylose units of the main chain.

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