ISOLATION AND CHARACTERIZATION OF THE XYLANS OF THE COTTON PLANT

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The husk of cotton seeds has long been used as a raw material for the hydrolysis industry. It has been shown previously (Scientific-Research Institute of the Chemistry and Technology of Cotton Cellulose, Tashkent) that the stems of the cotton plant are also suitable for this purpose.

Both the husk of the seeds and also the stems of the plant are rich in pentosans [1-6], but the chemical structures of these polysaccharides have not been studied.

The present paper gives the results of the isolation of the xylans from the stems, valves, and seed husk of the cotton plant, and also the properties of these substances. The investigation was performed with samples of cotton plant of variety 108-F of Gossypium hirsutum (family Malvaceae) from the experimental part of the Botanical Garden of Tashkent State University. The xylans from the plant organs were isolated in the following way: First the plant raw material was exhaustively extracted with water, with the removal of the water-soluble polysaccharides; then the residue was extracted with 7% caustic soda at room temperature (xylan A) and upon heating by a boiling-water bath (xylan B). The xylans were precipitated from the alkaline solutions with Fehling's solution. Their yields (in %) are given below:

Plant organs	Xylan A	Xylan B
Stems	8.9	7.1
Boll valves	6.9	4.8
Seed husk	13.2	8.7

The xylans isolated were purified by reprecipitation from aqueous solutions with ethanol and were demineralized by dialysis against distilled water. Xylans A were studied further.

To obtain monodisperse preparations of the xylans we used the precipitation of the polysaccharides from aqueous solutions with ethanol of different concentrations [7] (Fig. 1). As can be seen from Fig. 1, the bulk of the xylans was precipitated in a fairly narrow range of ethanol concentrations. The fourth and fifth fractions of the xylans were used for the subsequent experiments.

The homogeneity of these samples of xylans was checked by gel filtration on Sephadex G-75 [8]. The results of this process confirmed their homogeneity (Fig. 2).

The results of the chromatography of hydrolyzates of the initial xylans and of the third, fourth, and fifth fractions obtained in the ethanol fractionation, and also of the fourth and fifth fractions obtained in gel filtration showed that they were completely identical (Fig. 3). The xylans isolated in this way had the form of slightly yellowish powders which dissolved slowly in hot water; they were optically active (Table 1).

The molecular weights of the xylans were determined viscosimetrically and from the yield of formic acid on periodate oxidation.

As Table 1 shows, the xylans of each organ of the cotton plant differ from each other, both in molecular weight and in optical activity.

As is well known [9], only levorotatory xylans have been isolated from the plant. We have established that dextrorotatory xylans are present in the stems and in the boll valves of the cotton plant Gossypium hirsutum. However, the xylan from the seed husk rotates to the left. This is the first time that the two optical isomers of xylan have been found in the same plant.

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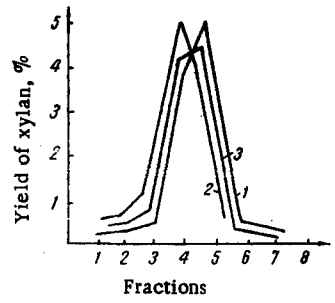

Fig. 1

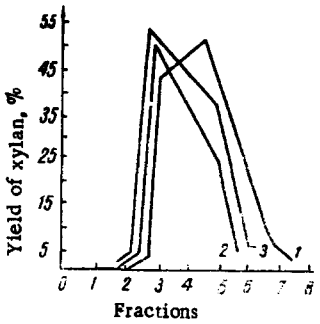

Fig. 2

Fig. 1. Results of the fractionation of cotton xylans by precipitation with ethanol of increasing concentration: 1) xylan from the stems; 2) xylan from the boll valves; 3) xylan from the seed husk.

Fig. 2. Results of the gel filtration of the cotton xylans on Sephadex G-75 (symbols as for Fig. 1).

TABLE 1

Plant organs	Method of determining molecular weights				$[\alpha]_D$, deg
	viscosimetric		oxidation		
	mol. wt.	DP	mol. wt.	DP	
Stems	8 300±500	64–67	7 900±500	60–65	+79
Boll valves	15 800±300	120–122	15 300±400	116–119	+68
Seed husks	11 600±300	87–90	11 900±300	90–93	−23,7

EXPERIMENTAL

Chromatographic analysis was performed on Whatman No. 1 paper in the solvent system ethyl acetate–acetic acid–formic acid–water (18 : 3 : 1 : 4), with aniline phthalate as the chromogenic agent. Radial chromatograms were made with an exposure time of 12 h, being run twice. The hydrolyzate was evaporated at a temperature not exceeding 45°C.

Extraction of the Plants with Water. The comminuted defatted plant material (1 kg) was exhaustively extracted with water at the boil.

Extraction of the Plant with 7% Caustic Soda. The residual plant raw material after extraction with water was extracted at room temperature with 7% caustic soda solution until extraction was complete (as determined by reaction with Fehling's solution). Then extraction was continued with heating in the boiling-water bath until it was again complete.

Isolation of the Xylan from the Alkaline Solutions. The alkaline extracts obtained at room temperature were combined and acidified with hydrochloric acid to a weakly acid reaction. Methanol (two volumes) was added to the solution. The precipitate that deposited was separated off, washed with methanol, and dissolved in 5% caustic soda solution. Fehling's solution was added in small portions to the alkaline solution until the formation of a precipitate ceased. The precipitate was filtered off, washed with water, and ground in a mortar with a small amount of dilute hydrochloric acid (1 : 1) with ice cooling. After the decomposition of the copper complex, the xylan was precipitated by the addition of two volumes of methanol. The precipitate was filtered off and was washed with cold water to neutrality and then with methanol. For purification, the xylan was repeatedly reprecipitated from aqueous solution with methanol. The purified xylan was demineralized by dialysis against distilled water, precipitated with methanol, filtered off, washed with the alcohol and with ether, and dried over phosphorus pentoxide (xylan A).

The xylan from the alkaline solutions obtained by extraction with heating in the boiling-water bath was isolated in the same way (xylan B).

Fractionation of the Xylans by Precipitation with Increasing Concentrations of Ethanol. A solution of 10 g of xylan in 1 liter of hot water was left to stand for 12 h. The small amount of precipitate that formed was separated off in the centrifuge, washed with ethanol and ether, dried over phosphorus pentoxide,

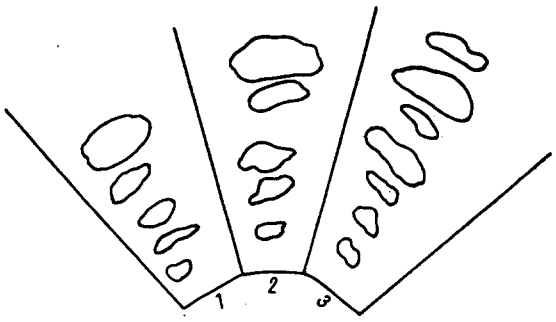

Fig. 3. Chromatograms of hydrolyzates of cotton xylans (symbols as in Fig. 1).

and weighed. The residual solution was then treated with 100 ml of ethanol, and the precipitate was separated off. The operation was repeated until no further precipitate formed on the addition of ethanol.

Gel Filtration of the Xylans on a Column of Sephadex. A solution of 0.2 g of xylan in 200 ml of hot water was passed through a column of Sephadex G-75 (25× 60 mm). The polysaccharide was eluted with water, 100-ml fractions being collected. The fractions were evaporated in vacuum to small volume and were precipitated with methanol. The precipitate that deposited was separated off in a centrifuge, washed with methanol and with ether, dried, and weighed. The first three fractions of the eluate contained only a very small amount of xylan, and therefore the total yield of the three fractions was determined. For the same reason, the total yield of xylan from the fifth to eighth fractions was determined.

Hydrolysis of the Xylan. A 50-mg (or, for the fractions from the Sephadex, 25-mg) fraction of the xylan in 20 ml of 1 N sulfuric acid was heated at 100°C for 10 h. Then the hydrolyzate was neutralized with barium hydroxide. The precipitate was filtered off and carefully washed with water. The filtrate and the wash water were combined and evaporated to a syrup. The syrup was chromatographed.

Determination of the Molecular Weight of the Xylan. A. Viscosimetrically. For this purpose, a solution of xylan in Schweitzer's reagent with a concentration of 0.001% was prepared. The characteristic viscosity was determined in an Ostwald viscometer. The molecular weight was calculated by means of Staudinger's equation [10]. The constant K for xylan is $5 \cdot 10^{-4}$ [11].

B. Periodate Oxidation Method. A sample of xylan (50-70 mg) was dissolved in water, 30 mg of sodium tetrahydroborate was added, and the mixture was left for 48 h. The excess of tetrahydroborate was decomposed with acetic acid, bringing the pH to 5.5. Then the solution was cooled to 5°C, 5 ml of 0.5 M solution of sodium metaperiodate was added, the volume of the solution was made up to 25 ml, and it was left for oxidation in the dark at 5°C. After every 12 h, the consumption of periodate (in an individual experiment) was determined by titration with sodium arsenite [12].

The oxidation of the xylan was continued until the concentration of periodate in the reaction mixture had become constant.

To determine the amount of formic acid, an aliquot of the reaction mixture (2 ml) was dissolved in 20 ml of water, and 0.5 ml of ethylene glycol was added dropwise to decompose the excess of periodate. The formic acid was titrated with 0.01 N caustic soda in the presence of Methylene Red.

The number of units in the polysaccharide molecule was calculated by means of the formula given by Stepanenko [13]. The specific rotation of the xylan was measured in 2% caustic soda solution (c 0.1%) after the solution had been kept for a day.

SUMMARY

1. The specific rotations and molecular weights of the xylans isolated from the stems, boll valves, and seed husks of the cotton plant Gossypium hirsutum (family Malvaceae) have been determined.

2. The homogeneity of the xylan preparations has been shown by their fractionation by precipitation from aqueous solutions with increasing concentrations of ethanol and by gel filtration on Sephadex G-75.

3. Dextrorotatory xylans have been isolated from the plants and the simultaneous presence of the optical isomers of the xylans in the one plant has been established for the first time.

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