XYLAN SYNTHESIS FROM URIDINE-DIPHOSPHATE-D-XYLOSE BY PARTICULATE PREPARATIONS FROM IMMATURE CORNCOBS*

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Syntheses of several plant polysaccharides involving *in vitro* glycosyl transfer from nucleotide sugars have now been reported using the enzymes present in particulate preparations from plant tissues. Such syntheses include starch from UDP-D-glucose¹ or ADP-D-glucose,² pectin from UDP-D-galacturonic acid,³ callose from UDP-D-glucose,⁴ and cellulose from GDP-D-glucose⁵ or UDP-D-glucose.⁶ Indirect evidence strongly suggests that UDP-D-xylose and UDP-L-arabinose are glycosyl sources in the synthesis of the xylan fractions of plant hemicellulose. Both UDP-D-xylose and UDP-L-arabinose have been found in plants,⁷ and enzymes catalyzing the conversion of UDP-D-glucuronic acid to UDP-D-xylose^{8, 9} and its subsequent epimerization to UDP-L-arabinose⁸ have been obtained in plant extracts. The actual transfer of xylose and arabinose from these sugar nucleotides to growing xylan polymer has not, however, been demonstrated.¹⁰ Although Pridham and Hassid¹¹ showed that corn shoot particulate preparations can transfer both D-xylose and L-arabinose from their UDP-compounds to a polymer, the possible polysaccharide formed was not characterized in detail.

Since germinating mung bean shoots are not particularly rich in xylan¹² but mature corncobs (*Zea mays*) are known to have an unusually high content of xylan,¹³ the immature, developing corncob was investigated as a source of xylan-synthesizing enzymes. The present paper reports the *in vitro* synthesis of xylan from UDP-D-xylose by particulate preparations from immature corncobs.

Materials and Methods.—Radioactive UDP-D-xylose-C¹⁴ of specific activity 106 $\mu\text{C}/\mu\text{mole}$ was kindly prepared by Dr. D. S. Feingold from UDP-D-glucuronic acid-C¹⁴ by the action of a wheat germ decarboxylase which was free from UDP-D-xylose epimerase.⁹ A hydrolysate of the UDP-D-xylose-C¹⁴ showed on paper chromatograms D-xylose-C¹⁴ but no detectable L-arabinose-C¹⁴. The UDP-D-glucuronic acid was prepared from α -D-glucose-C¹⁴-1-PO₄ and UTP by the sequential action of mung bean pyrophosphorylase¹⁴ and liver uridine diphosphoglucose dehydrogenase (Sigma). A mixture of UDP-D-xylose-C¹⁴ and UDP-L-arabinose-C¹⁴ (specific activity, 106 $\mu\text{C}/\mu\text{mole}$), designated as UDP-pentose-C¹⁴, was prepared by the action of crude wheat germ decarboxylase¹⁵ on UDP-D-glucuronic acid-C¹⁴. Water-insoluble xylan (Pfanstiel, Waukegan, Illinois) which contained 5% of arabinosyl residues was purified by dissolving in 2% (w/v) alkali and reprecipitating with acetic acid and ethanol (2 vol). Water-soluble arabinoxylan, containing 13% of arabinosyl residues, was prepared from wheat flour.¹⁶ Authentic β -1,4-xylodextrins, degree of polymerization (D.P.) = 2, 3, 4, and 5, respectively, were prepared by the partial hydrolysis of xylan with fuming HCl and subsequent fractionation of the hydrolysate on a charcoal-celite column. An aqueous solution of hemicellulase (Nutritional Biochemicals Corp.) was dialyzed at 2° before use. All other chemicals were obtained from commercial sources.

Corncob particulate preparations: Sweet corn plants, variety Captain Golden, were grown in a greenhouse and immature cobs collected immediately before use. They were harvested when the actual cob within the sheath was 2–3 in. long, which was at the time the silk had just emerged from the ear. After removal of the sheath and silk, 20–50 gm of the small cobs were broken into pieces, chilled to 2°, and ground in a mortar with sand and an equal weight of 0.1 M phosphate or Tris-HCl buffer (pH 7.5) containing 1% crystalline bovine serum albumin. The slurry was squeezed through cheesecloth, centrifuged at 1000 $\times g$ for 15 min to remove coarse debris and then at 30,000 $\times g$ for 30 min to sediment enzymically active particles. All operations were done

at 2–4°. The particles were resuspended in 0.3 ml of the same buffer per 10 gm of original cob tissue, and xylan synthetase activity was measured immediately. Unless otherwise indicated, standard enzyme mixtures contained 25 μ l of particulate preparation, 25 μ l of 0.1 *M* potassium phosphate buffer (pH 7.5), 1% of albumin, 0.4 *M* sucrose, and 1.3×10^{-4} μ mole of UDP-xylose-C¹⁴ (15,000 cpm; 1.1×10^6 cpm = 1 μ c) in a total volume of 51 μ l. After incubation for 5 min at 25° the enzymes were inactivated by heating for 2 min at 100° and the mixtures analyzed for radioactivity incorporated into polysaccharide. The pH values of duplicate mixtures were measured with a radiometer (Copenhagen) microelectrode.

Measurement of incorporated radioactivity: Water (1 ml) and 2–3 mg of the water-insoluble xylan were added to the inactivated mixture, and the well-mixed solution was centrifuged. The supernatant solution was carefully removed and any water-soluble polysaccharide recovered by precipitation with 2 vol of ethanol and washed with 80% (v/v) ethanol by centrifugation. The original water-insoluble residue was extracted twice at 37° with 1 ml of 5% (w/v) NaOH for 1 hr and centrifuged. The alkali supernatant solutions were added to the precipitate of water-soluble polysaccharide, and the combined solution, acidified with 0.3 ml of 50% (v/v) acetic acid, was kept for 6 hr at 2° and centrifuged. The polysaccharide was washed with 80% ethanol and dissolved in 0.3 ml of 2% alkali to give a final solution of xylan-C¹⁴. Radioactivity in this solution was measured by streaking 25- μ l portions of it on 1 $\times$ 3-cm strips of filter paper, neutralizing with 10–15 μ l of 50% acetic acid, and counting in Liquifluor (New England Nuclear Corp.) in toluene on a Tricarb scintillation counter.³ Counts of 300–400 cpm were found in the radioactive xylan solutions from boiled enzyme controls when they were extracted directly with warm alkali. These counts were largely eliminated by first washing the mixture with water to remove nucleotide sugars and recovering any water-soluble polymer from the washing. Less than 100 cpm of radioactivity was found in the 80% ethanol washings and alkali-extracted residue. The ethanolic supernatant solutions from the initial water extracts, however, contained 300–400 cpm which were immobile on electrophoresis in sodium borate buffer. These counts, which possibly represented polysaccharide soluble in water and 66% ethanol, were not further examined. Boiled enzyme and zero incubation time mixtures were run as controls with each enzyme preparation. They yielded xylan-C¹⁴ solutions containing 50–60 cpm, corrected for background, compared with usual incorporations of 1500–5000 cpm into xylan with active enzyme mixtures.

Analyses: Paper chromatograms were developed with one of the following solvents:¹⁷ (a), ethyl acetate–water–pyridine (2:2:1); (b), ethyl acetate–water–pyridine (10:4:3); (c), ethyl acetate–acetic acid–formic acid–water (9:1.5:0.5:0.2); and (d), ethyl acetate–*n*-propanol–water (5.7:3.2:1.3). Electrophoresis was performed in either 0.05 *M* sodium tetraborate (pH 9.2) or 0.008 *M* ammonium molybdate (pH 5.1). Sugars were located on papers with aniline hydrogen phosphate or silver nitrate spray reagents.¹⁷ Radioactive sugars were located by cutting the 2–3-cm-wide chromatogram or electrophoretogram strip into 0.5–1-cm-wide segments, and counting in Liquifluor in the Tricarb. The sugars were recovered from these segments by elution with water, after removal of counting fluid by washing with toluene followed by ether.

Protein in the particulate preparations and in the buffer, to correct for albumin, was measured by the Lowry method.¹⁸ For total hydrolyses of sugar polymers, the following strengths of hydrochloric or sulfuric acid at 100° were used for the times stated: sugar nucleotides, 0.1 *N*, 30 min; xylodextrins and xylodextrin alcohols, 1 *N*, 1 hr; and synthetic and plant xylan, 1 *N*, 2 hr. Arabinofuranoside links in xylans were hydrolyzed by heating the xylan at 100° for 30 min in 0.01 *N* acid, and xylodextrins were partially hydrolyzed to their lower homologues by similar treatment in 0.1 *N* acid for 30 min. Xylan was also partially hydrolyzed to xylodextrins by dissolving it at 0° in fuming HCl for 15 min, 25–50 mg of xylan per 1–2 ml of acid, and then diluting with water before removal of the acid. In all hydrolysates, acid was removed by treatment with resin (BioRad, AG-1-X8, carbonate form), but not, in the case of HCl, by evaporation under vacuum over solid NaOH. Individual oligosaccharides (0.5–2 mg) dissolved in 100 μ l of water were incubated with 2–4 mg sodium borohydride at room temperature overnight. After removal of the sodium ions with resin (BioRad, 50W-X8) and the borate with methanol, the sugar alcohols were purified by paper chromatography in solvent (c) prior to hydrolysis.

Results.—Preparation of active xylan-synthesizing particles: Provided that the immature cobs were 2–3 in. long, they were easily ground to give particulate prepara-

TABLE 1
EFFECT OF ALBUMIN AND SUCROSE ON THE RATE OF XYLAN SYNTHESIS

Preparation buffer	Additional Components in		Xylan Synthesized (cpm/mg protein)	
	Incubation buffer		Tris-HCl buffer	Phosphate buffer
None	None		640	2350
Albumin (1%)	Albumin (1%)		2330	3043
Albumin (1%)	Albumin (1%), sucrose (0.4 M)		4460	5767

Standard enzyme mixtures of a total volume of 51 μ l contained 25 μ l of particulate preparation (30 mg protein/ml), 25 μ l of 0.1 M buffer (pH 7.5) and 1.3×10^{-4} μ moles of UDP-D-xylose-C¹⁴ (15,000 cpm), and were incubated for 5 min at 25° and analyzed as described.

tions which readily incorporated the radioactive sugar from UDP-D-xylose-C¹⁴ into the polymer. Cobs that had reached greater maturity were more difficult to grind and gave preparations which appeared to be less active in xylan synthesis. The results listed in Table 1 show that although xylan synthetase activity was obtained in particles prepared in buffer only, using the conditions of Barber *et al.*,⁵ much more active preparations were obtained when the buffer also contained 1 per cent of albumin.³ The material removed from the homogenate at $1000 \times g$ showed little xylan synthetase activity, but the fraction which precipitated between $30,000$ and $100,000 \times g$ was about 35 per cent as active as the fraction obtained at $30,000 \times g$.

Composition of the particulate preparation: The preparations contained, corrected for albumin, 20–30 mg of protein per ml of suspension. After removal of soluble polysaccharides by successive extraction with hot ethanol, water, and 0.5 per cent ammonium oxalate, the hydrolysates prepared with 1 N HCl showed on chromatograms that were developed with solvent (a) intense spots corresponding to arabinose and glucose together with spots of much weaker intensity corresponding to xylose and galactose. In contrast, similar hydrolysates prepared from whole immature cobs showed intense spots identical with xylose and arabinose and much weaker spots of glucose and galactose.

Effect of various conditions on the xylan-synthesizing activity of the enzymes: Storage of the preparations, with or without albumin, at -20° for 2–5 days resulted in a loss of 70–80 per cent of the xylan-synthesizing activity; this loss was only reduced to 60 per cent by the presence of 0.4 M sucrose. The enzymes were therefore always used for xylan syntheses immediately after preparation; the results recorded in this paper were obtained with a number of preparations made over a period of several months.

Over the pH range 6.5–7.2, as measured in the final incubation mixtures, incorporation of radioactivity into xylan was virtually constant, but there was a rapid fall in the rate of synthesis above and below this pH range. In the standard enzyme mixtures prepared with buffer of an initial pH of 7.5, the actual pH of the mixtures was 7.0–7.1. As shown in Table 1, xylan synthesis in 0.1 M potassium phosphate gave higher results than in Tris-HCl, and the former buffer was used in most of the work.

When the standard enzyme mixtures contained equal amounts, 1.3×10^{-4} μ mole of UDP-D-xylose-C¹⁴ and UDP-pentose-C¹⁴, respectively, the xylan synthesis per mg of protein corresponded to an incorporation of 2900 cpm from the UDP-D-xylose-C¹⁴ and 3170 cpm from UDP-pentose-C¹⁴.

The incorporation of radioactivity into xylan by the particles increased steadily during 15–30 min incubation at 25°; the incorporation then slowed down and

FIG. 1.—Time course in the synthesis of xylan by corn cob particles. The enzyme mixture contained 200 μ l of particulate preparation (28 mg protein/ml) prepared in 0.1 *M* Tris-HCl buffer (pH 7.5) containing 1% of albumin, 200 μ l of the same buffer, and 26×10^{-4} μ moles of UDP-D-xylose- C^{14} (300,000 cpm). During incubation at 25°, 25- μ l portions were removed at intervals for measurement of synthesized xylan.

FIG. 2.—Effect of substrate concentration on xylan synthesis. Standard enzyme mixtures contained 25 μ l of particulate preparation (30 mg of protein per ml) in 0.1 *M* phosphate buffer (pH 7.5) containing 1% of albumin and 0.4 *M* sucrose, 25 μ l of the same buffer, 1.3×10^{-4} μ moles of UDP-D-xylose- C^{14} (15,000 cpm), and 50 or 100×10^{-4} μ moles of UDP-D-xylose in 1 μ l. After incubation for 5 min at 25°, labeled xylan measured. ●, Δ indicate results obtained from different preparations on separate occasions.

finally reached a constant value (Fig. 1). This type of result was obtained on several occasions with no sign of loss of incorporated radioactivity on further incubation. In typical large-scale standard enzyme mixtures containing 200 μ l of particulate preparation, 200 μ l of buffer, and 13×10^{-4} μ mole of UDP-D-xylose- C^{14} (150,000 cpm), 66.2 per cent (99,360 cpm) of the radioactivity was incorporated into xylan- C^{14} after 30 min incubation at 25°. The rate of xylan synthesis is also approximately linear with respect to enzyme concentration over a limited range. Thus, in standard enzyme mixtures diluted to 100 μ l and containing 10, 20, and 50 μ l of enzyme (containing 0.28, 0.56, and 1.40 mg protein, respectively), xylan synthesis corresponded to an incorporation, per mg of protein, of 3670, 3115, and 3020 cpm, respectively. When the concentration of UDP-D-xylose in standard enzyme mixtures was increased by adding unlabeled UDP-D-xylose, the rate of xylan synthesis, per mg of protein, was increased to a maximum of approximately 9×10^{-4} μ mole per 5 min in the presence of

TABLE 2
EFFECT OF ADDED COMPOUNDS ON THE RATE OF XILAN SYNTHESIS

Compound	Xylan Synthesized by Particulate Preparation (cpm/mg protein)	
	Buffer + albumin	Buffer + albumin + sucrose
None	2585	6574
UTP	1723	3087
UDP	845	—
UMP	399	774
Uridine	2409	—
ATP	3192	5004
AMP	—	3139
GTP	2931	8466
GMP	—	3529

Standard enzyme mixtures contained 25 μ l of particulate preparation (25–30 mg protein per ml) in 0.1 *M* potassium phosphate buffer, pH 7.5, albumin 1%, 25 μ l of the same buffer ($\pm$ sucrose, 0.8 *M*), UDP-D-xylose- C^{14} (1.3×10^{-4} μ mole; 15,000 cpm), and added compound (0.1 μ mole) in a total volume of 52 μ l, and were incubated at 25° for 5 min.

FIG. 3.—Distribution of radioactive sugars on a chromatogram developed in solvent (a) of a partial fuming HCl acid hydrolysate of synthetic xylan. Radioactivity measured by cutting the 3-cm-wide chromatogram strip into 0.5-cm-wide segments and counting in the Tricarb. Authentic sugars (xyl_2 — xyl_6 = β -1,4-xylohextrins of D.P. 2–6) on parallel strips located with aniline hydrogen phosphate.

Identification of synthetic xylan: Solubility properties: Successive extraction with cold water, hot water, and alkali dissolved the following percentages of the radioactive xylan: xylan synthesized from UDP-D-xylose- C^{14} , 15.0, 7.2, and 77.0 per cent; xylan synthesized from UDP-pentose- C^{14} , 20.6, 30.7, and 48.7 per cent. The presence of water-soluble wheat flour xylan as carrier in addition to the usual water-insoluble xylan only increased the radioactivity in the water-soluble fractions by 5–10 per cent. Acidification with 50 per cent acetic acid of alkaline solutions of xylan synthesized from UDP-D-xylose- C^{14} and UDP-pentose- C^{14} , respectively, precipitated 87 and 81 per cent of the incorporated radioactivity. All of the radioactive xylan was immobile on electrophoresis in sodium borate buffer, and 90 per cent of it was nondialyzable through Visking cellophane tubing.

Monosaccharide composition: Total acid hydrolysates of the synthetic xylans

TABLE 3

CHROMATOGRAPHIC MOBILITIES OF OLIGOSACCHARIDES FROM SYNTHETIC XILAN

Oligosaccharide	(a)	R_{xylose}	Values in Solvent	(d)	Proposed identity
Disaccharide-A	0.75	0.66	0.46	0.39	1,4-xylobiose
	(0.75)*	(0.66)	(0.46)	(0.39)	
Disaccharide-A alcohol			0.58		1,4-xylotriose
			(0.58)		
Trisaccharide-B	0.53		0.20	0.09	
	(0.53)		(0.20)	(0.09)	
Trisaccharide-B alcohol			0.25		1,4-xylotetraose
			(0.25)		
Trisaccharide-B-1	0.44		0.20	0.09	
	0.37				
Tetrasaccharide-C	(0.37)				1,4-xylopentaose
Pentasaccharide-D	0.18				
	(0.18)				

* In parentheses R_{xylose} values of authentic β -1,4-xylohextrins.

101.3×10^{-4} μmoles (19.2×10^{-5} M) of UDP-D-xylose, compared with 0.2 – 0.3×10^{-4} μmole in the presence of the usual 1.3×10^{-4} μmole (0.25×10^{-4} M) of the UDP-D-xylose- C^{14} (Fig. 2).

Sucrose at 0.4 M concentration in the incubation mixture markedly improved the rate of xylan synthesis, as shown in Table 1. The effect on xylan synthesis of the addition of 0.1 μmole of various nucleotides to standard enzyme mixtures is summarized in Table 2. UTP, UDP, and UMP depressed xylan synthesis by 33–55, 68, and 85–90 per cent, respectively, whereas uridine, GTP, and ATP did not markedly depress the rate of synthesis, and AMP and GMP only depressed it by 45–50 per cent. Neither cysteine nor magnesium ion in the presence or absence of albumin stimulated xylan synthesis.

always showed on chromatograms developed with solvent (a) components identical with xylose- C^{14} and arabinose- C^{14} . The latter sugar with an R_{xylose} of 0.91 in this solvent moved clearly behind xylose. In xylan synthesized from UDP-D-xylose- C^{14} , the L-arabinose- C^{14} peak contained 10 per cent of the radioactivity present, and in xylan synthesized from UDP-pentose- C^{14} , 20 per cent.

Identification of oligosaccharides from the partial acid hydrolysate of synthetic xylan: The pattern of oligosaccharides obtained when a partial fuming HCl hydrolysate of synthesized xylan was analyzed by paper chromatography in solvent (a) is shown in Figure 3. The series of compounds which moved at the same rates as the members of the homologous series of β -1,4-xylohextrins from plant xylan were always obtained together with one other possible trisaccharide (trisaccharide-B-1) trailing just behind the position of 1,4-xylotriose. Individual oligosaccharides from appropriate regions of several such hydrolysates were eluted for further examination. The chromatographic mobilities of the various compounds are summarized in Table 3. These results show that disaccharide-A, trisaccharide-B, tetrasaccharide-C, and pentasaccharide-D are chromatographically identical with the β -1,4-xylohextrins of D.P. 2, 3, 4, and 5, respectively. All four compounds yielded xylose- C^{14} but no arabinose- C^{14} on total acid hydrolysis, while partial acid hydrolyses of the labeled tetra- and pentasaccharides yielded components chromatographically identical in solvent (a) with xylobiose, xylotriose, and xylohextraose.

Disaccharide-A was oxidized with lead tetraacetate⁴ and the oxidized sugar reduced with sodium borohydride and then hydrolyzed. A chromatogram of the hydrolysate developed with solvent (c) showed the presence of two radioactive components identical with D-xylose (204 cpm) and glycerol (97 cpm). In this solvent, glycerol has an R_{xylose} of 1.35. The ratio of D-xylose- C^{14} to glycerol- C^{14} was 2.1:1.0 (theoretical 1.66:1.0). The preceding series of reactions has been shown¹⁹ to yield glycerol from the original reducing sugar unit of a 1,4-linked xylobiose, but gives erythritol from a 1,3-linked pentobiose and pentitol from a 1,2-linked pentobiose, respectively. The only other xylobiose so far obtained from a natural xylan is rhodymenabiose, β -1,3-xylobiose, from seaweed rhodymenan, and this compound moves on chromatograms at different rates from the 1,4-xylobiose; for example, in solvent (b) it has R_{xylose} 1.05.¹⁶ Disaccharide-A is identified, therefore, as 1,4-xylobiose and trisaccharide-B, tetrasaccharide-C, and pentasaccharide-D as 1,4-xylohextrins of degree of polymerization 3, 4, and 5, respectively. The possible trisaccharide-B-1 on total hydrolysis gave on a chromatogram developed with solvent (a) compounds corresponding to both xylose- C^{14} and arabinose- C^{14} in the ratio of xylose:arabinose of 1.9:1.0. The compound was not examined further.

Labeling pattern of oligosaccharide alcohols: Treatment of individual oligosaccharides isolated from the fuming acid hydrolysate (Fig. 3) with sodium borohydride followed by acid hydrolysis and electrophoresis in ammonium molybdate buffer yielded xylose- C^{14} and xylitol- C^{14} in the ratios listed in Table 4. All of the xylohextrins up to D.P. = 5 produced labeled xylitol ($M_{picrate}$ 1.8) which clearly separates from xylose ($M_{picrate}$ 0.15) under these conditions.

Stepwise partial acid hydrolysis of synthetic xylan: Partial acid hydrolysis of the xylan synthesized from UDP-pentose- C^{14} under conditions which cleave furanoside but not pyranoside links liberated labeled arabinose but not labeled xylose from the

TABLE 4

D-XYLOSE-C¹⁴ AND D-XYLITOL-C¹⁴ CONTENT OF RADIOACTIVE XYLODEXTRIN ALCOHOLS

Oligosaccharide	Cpm Obtained		Xylose:Xylitol	
	D-Xylose-C ¹⁴	D-Xylitol-C ¹⁴	Found	Theoretical for uniformly labeled oligosaccharide
A. 1,4-xylobiose	204	217	0.94:1	1:1
B. 1,4-xylotriose	140	80	1.75:1	2:1
C. 1,4-xylotetraose	709	214	3.20:1	3:1
D. 1,4-xylopentaose	251	75	3.60:1	4:1

Borohydride-reduced xyloextrins were hydrolyzed and the hydrolysates electrophoresed in molybdate buffer.

xylan (Table 5), showing that at least a large part of the incorporated arabinose is present as terminal arabinofuranoside residues.

Action of hemicellulase on synthetic xylan: The synthetic xylan and its oligosaccharides were readily hydrolyzed by incubation at 30° overnight with hemicellulase in 0.1 potassium phosphate buffer (pH 7.0). Labeled arabinose, xylose, and xylobiose were detected on chromatograms of the hydrolysates. The hemicellulase was shown to hydrolyze wheat flour xylan, water-insoluble xylan, lichenin, laminarin, yeast glucan, starch, and isolichenin but not cellulose, dextran, or nigeran.

Discussion.—Corncob xylans are typical plant xylans^{13, 20, 21} in that they consist essentially of linear chains of β -1,4-linked D-xylopyranosyl residues with L-arabinosyl and D-glucuronosyl residues attached. The L-arabinose is present as 1,3-linked, acid-labile L-arabinofuranosyl residues mainly as single-unit side chains or as the terminal units of longer side chains, but with some as nonterminal side-chain units.

We consider that the structure of the labeled polymer synthesized by the corncob particulate preparations from UDP-D-xylose-C¹⁴ and UDP-pentose-C¹⁴ is essentially that of corncob xylan for the following reasons. The xylobiose liberated from the synthesized polymer is 1,4-linked and the xyloextrin series obtained by partial acid hydrolysis is chromatographically identical to the β -1,4-xyloextrin series. Much of the arabinose is liberated by the very mild acid conditions which cleave furanoside, but not pyranoside glycosidic links. The detection of a possible trisaccharide (B-1) containing arabinose, in the fuming hydrochloric acid hydrolysate, suggests the presence of nonterminal or pyranosyl arabinose residues.

The presence of L-arabinose-C¹⁴ in the polysaccharide synthesized from pure UDP-D-xylose-C¹⁴ is not surprising, as

UDP-D-xylose epimerase has been found in many plant tissues including corn shoots.¹¹ However, the mechanism for the formation of L-arabinofuranose units from UDP-D-xylopyranose cannot be explained at present.

Although effective xylan synthetase activity could be obtained by corncob particles prepared and assayed in the absence of albumin and sucrose, the presence of these compounds markedly improved the rate of xylan synthesis. This appears to be a similar effect to that already reported for the synthesis

TABLE 5
STEPWISE PARTIAL ACID HYDROLYSIS OF
SYNTHETIC XYLAN

Consecutive steps of xylan hydrolysis	D-Xylose-C ¹⁴ (cpm)	L-Arabinose-C ¹⁴ (cpm)
(a) Xylan containing 5000 cpm		
0.01 N acid	26	400
0.01 N acid	51	172
0.01 N acid	22	39
(b) Xylan containing 3000 cpm		
2 $\times$ 0.01 N acid	70	365
1 N acid	1799	250

Radioactive xylan was hydrolyzed and unhydrolyzed polymer recovered by precipitation with 3 vol of ethanol after each treatment. Liberated radioactive sugars in the hydrolysates were separated on paper chromatograms developed with solvent (a) and counted.

by mung bean shoot particles of polygalacturonic acid (pectin) from UDP-D-galacturonic acid³ and a possible cellulose from UDP-D-glucose.²² In general, the results from the *in vitro* synthesis of pectin, cellulose, and xylan point to similar enzymes, or enzymes associated with the same plant particles or fragments, being involved in all of the syntheses. This is probably not surprising in view of the intimate relationship of these polysaccharides in the plant cell walls.

Summary.—Particulate preparations from immature corn cobs contain enzymes which catalyze the epimerization of UDP-D-xylose-C¹⁴ to UDP-L-arabinose-C¹⁴ and the transfer of the sugars from both of these sugar nucleotides to polysaccharide. The synthetic polysaccharide is shown to be similar to natural corn xylan as it is soluble in alkali and contains acid-labile L-arabinofuranosyl units and chains of 1,4-linked xylosyl units which appear to have a β -configuration. The xylan-synthesizing activity of the particulate preparation is considerably enhanced by preparation and assay in the presence of 1 per cent bovine serum albumin and 0.4 M sucrose and is depressed by UTP, UDP, UMP, GMP, and AMP, but not by uridine, ATP, or GTP.

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