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The Structure of Plant Cell Walls

II. THE HEMICELLULOSE OF THE WALLS OF SUSPENSION-CULTURED SYCAMORE CELLS[1]

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ABSTRACT

The molecular structure, chemical properties, and biological function of the xyloglucan polysaccharide isolated from cell walls of suspension-cultured sycamore (*Acer pseudoplatanus*) cells are described. The sycamore wall xyloglucan is compared to the extracellular xyloglucan secreted by suspension-cultured sycamore cells into their culture medium and is also compared to the seed "amyloid" xyloglucans.

Xyloglucan—or fragments of xyloglucan—and acidic fragments of the pectic polysaccharides are released from endopolygalacturonase-pretreated sycamore walls by treatment of these walls with 8 M urea, endoglucanase, or 0.5 N NaOH. Some of the xyloglucan thus released is found to cochromatograph with the acidic pectic fragments on diethylaminoethyl Sephadex. The chemical or enzymic treatments required for the release of xyloglucan from the walls and the cochromatography of xyloglucan with the acidic pectic fragments indicate that xyloglucan is covalently linked to the pectic polysaccharides and is noncovalently bound to the cellulose fibrils of the sycamore cell wall.

The molecular structure of sycamore xyloglucan was characterized by methylation analysis of the oligosaccharides obtained by endoglucanase treatment of the polymer. The structure of the polymer is based on a repeating heptasaccharide unit which consists of 4 residues of β-1-4-linked glucose and 3 residues of terminal xylose. A single xylose residue is glycosidically linked to carbon 6 of 3 of the glucosyl residues.

In the first paper of this series (24), recently developed techniques for the methylation analysis of polysaccharides were used in order to study the structure of the primary cell wall of suspension-cultured sycamore cells and in order to characterize structurally the defined fragments released from these walls by a highly purified endopolygalacturonase. This study indicated that the sycamore cell wall contains a pectic rhamnogalacturonan polymer, and that arabinan and galactan side chains are attached to the rhamnogalacturonan polymer.

We report here the isolation and characterization of the hemicellulosic xyloglucan from endopolygalacturonase-pretreated sycamore cell walls. Evidence is presented which indicates that the xylogucan is covalently linked to the pectic polysaccharides and is noncovalently bound to the cellulose fibrils of these walls.

METHODS

Cell walls from suspension-cultured sycamore (*Acer pseudoplatanus*) cells were obtained as previously described (24). Neutral sugar compositions were quantitatively determined by gas chromatography by the method of Albersheim *et al.* (1). Some samples were hydrolyzed for 1.5 hr rather than 1.0 hr in order to hydrolyze fully the β-(1 → 4)-glucosyl linkage present in the backbone of xyloglucan. Uronic acid compositions were, in some cases, determined by the gas chromatographic procedure of Jones and Albersheim (12). Neutral sugars and uronic acids were assayed colorimetrically by the anthrone and carbazole methods as previously described (24). The methods used to determine sugar linkage compositions have been described in the preceding paper (24).

Isolation of SEPS.[6] A modified M-6 medium was used for the growth of the sycamore cells in suspension culture when the cultures filtrates were used for the isolation of SEPS. This modified medium was used in order to avoid contamination of the SEPS with the ethanol-precipitable mannan present in the yeast extract component of M-6 medium. The modified medium was prepared in the following manner. The yeast extract was dissolved in water (30 g/liter), and ethanol was added to a concentration of 70% (v/v). After storage at 2 C for several hours, the insoluble material was removed by centrifugation, and the supernatant solution was concentrated under reduced pressure at 40 C. After the ethanol had been completely evaporated, the remaining solution was diluted with water to give 10 ml of solution for every gram of original yeast extract. The sycamore culture medium was then prepared as described above except that 10 ml of the above solution were used in place of 1 g of yeast extract.

SEPS was isolated from culture medium in which sycamore cells had been grown to late log phase (10–12 days) before removal on a coarse sintered glass funnel. Ethanol was added to the cell-free culture medium to a concentration of 70% (v/v), and the resulting suspension was stored for several hours at 2 C. The insoluble material was removed by centrifugation at 10,000*g* for 10 min. This pellet was resuspended in water (50

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[6] Abbreviation: SEPS: sycamore extracellular polysaccharides.

ml of water for each liter of original medium), and any material remaining insoluble was removed by centrifugation. The soluble portion was reprecipitated with 70% ethanol, and the precipitate was re-extracted with water.

The methyl galacturonate residues of the SEPS polymers (4) were then de-esterified at 2 C by titrating the solution to pH 12.0. The pH of the magnetically stirred solution was periodically readjusted to 12.0. After 3 to 4 hr the solution was titrated to pH 6.5. The resulting gelatinous solution was clarified by the addition of EDTA to a final concentration of 10 mM. This solution was exhaustively dialyzed at 2 C against 10 mM potassium phosphate, pH 6.5, containing 1 mM EDTA.

Isolation of [^{14}C]SEPS. Radiolabeled SEPS were obtained by growing suspension-cultured sycamore cells in 1 liter of medium containing 500 μc of [^{14}C]sucrose (Schwarz BioResearch). The cells were harvested after 5 days, and the radiolabeled SEPS were isolated from the culture filtrate and fractionated as described for unlabeled SEPS.

Enzymes. Endopolygalacturonase was obtained from culture filtrates of *Colletotrichum lindemuthianum* and was assayed and purified as previously described (7).

A partially purified endoglucanase (EC 3.2.1.4, β-1,4-glucan 4-glucanohydrolase) from *Trichoderma viride* was the gift of Dr. Joseph B. Jurale of this laboratory. This enzyme was obtained from culture filtrates of *T. viride* strain 92027, which was the gift of K. Selby and C. C. Maitland, Shirley Institute, Didsbury, Manchester. The fungus has been maintained on potato dextrose slants, and it was cultured using the medium and procedure of Selby and Maitland (21) except that the peptone, yeast extract, and malt extract were obtained from Difco, the distiller's solubles were omitted, and 2% Whatman standard grade cellulose powder was used as the carbon source instead of cotton. The cultures were harvested when they turned yellowish green to green (18–28 days). The culture filtrates were centrifuged and concentrated on an Amicon UM-10 membrane. The concentrate, in 10 mM sodium acetate, pH 5.2, was passed through a Bio-Gel P-6 column to remove low molecular weight material. The void peak protein was eluted with buffer containing 100 mM sodium chloride, concentrated on the Amicon, and then dialyzed in an animal membrane against 20 mM potassium phosphate, pH 7.0. The endoglucanase was purified from this solution by the procedure of Mandels and Reese (19) using a DEAE-Sephadex column equilibrated with the phosphate buffer and eluted with a linear gradient of sodium chloride from 0 to 500 mM. β-Glucosidase passed rapidly through the column, while the C_x endoglucanase eluted in two major peaks, one just after the β-glucosidase (C_x-A) and the other at about 100 mM sodium chloride (C_x-B). The fractions containing both peaks of the C_x endoglucanase were combined for use. A second endoglucanase preparation from *T. viride*, described as electrophoretically homogeneous cellulase, was purchased from Miles Laboratories.

A partially purified preparation of endoglucanase from *C. lindemuthianum* was also provided by Dr. Joseph Jurale. The fungus was cultured as previously described (7) except that 2% Whatman CF-11 cellulose was used as the carbon source. The culture filtrate was centrifuged and then concentrated by rotary evaporation at 35 to 40 C. The concentrate was dialyzed in an animal membrane against 10 mM sodium acetate, pH 5.2, and then fractionated on a DEAE-Sephadex column pre-equilibrated with this buffer. The endoglucanase was eluted from the column with 50 mM sodium acetate, pH 5.2, and dialyzed against 10 mM potassium phosphate, pH 7.0, containing 100 mM KCl. The endoglucanase was then chromatographed on Sephadex G-75 in this buffer. The enzyme voided this column.

The endoglucanase was assayed in 50 mM sodium acetate, pH 5.2, at 30 C, using 0.1% carboxymethylcellulose as the substrate. As with the endopolygalacturonase, 1 unit of endoglucanase is defined as that amount which releases 1 μmole of reducing sugar per hour under the assay conditions.

Endoglucanase Treatment of Endopolygalacturonase-pretreated Walls. Untreated sycamore cell walls (1.82 g dry weight) were exhaustively treated with endopolygalacturonase as described previously (24). These treated and washed walls were suspended in 200 ml of 10 mM sodium acetate, pH 5.2. *T. viride* endoglucanase (55 units) was added to the wall suspension, and the stirred suspension was incubated for 5 hr at 40 C. The wall material was removed by centrifugation at 15,000*g* for 10 min, and the supernatant solution was saved. The wall residue was again allowed to react with endoglucanase in the same manner, but for 11 hr. The suspension was centrifuged, and this supernatant solution was combined with the first. The combined supernatant solutions (sycamore wall fraction C) were filtered through Whatman GF/C glass fiber paper to remove small wall particles, and then titrated to pH 6.5.

Urea Extraction of Walls. Lyophilized walls, or pellets of wall material obtained after washing by centrifugation, were suspended to give a concentration of 5 mg of dry wall material per milliliter of solution containing 8 M urea (J. T. Baker, reagent grade) in 50 mM sodium acetate, pH 5.5. The suspension was magnetically stirred for 1 hr, then centrifuged. The wall pellet obtained was washed once with 50 ml of the 8 M urea solution and then twice with 200 ml of water. If the material extracted from the walls by this procedure was to be analyzed, the extracts were filtered through Whatman GF/C glass fiber paper and then dialyzed extensively against water at 2 C and lyophilized.

Base Extraction of Endopolygalacturonase-pretreated Walls. Endopolygalacturonase-pretreated cell walls (50 mg) were suspended in 10 ml of 0.5 N NaOH containing 100 mM sodium borohydride and allowed to stand at 2 C for 24 hr. The insoluble material was removed by centrifugation and the supernatant solution was neutralized with 1 N HCl and then desalted by gel filtration in 50 mM sodium acetate, pH 5.2, on Bio-Gel P-2. The column fractions were assayed for carbohydrate by the anthrone and carbazole tests. All of the carbohydrate voided the Bio-Gel P-2 column. Those fractions containing carbohydrate were pooled (sycamore wall fraction B), and the sodium ions were removed by passing the solution through a small column (1.3 × 3.0 cm) of Dowex 50W X-8 (H^+ form).

Endoglucanase Treatment of Xyloglucan. Samples of SEPS xyloglucan or sycamore wall xyloglucan (sycamore wall fraction C-1) were exhaustively treated with 0.7 unit of *T. viride* endoglucanase per mg of polysaccharide. The samples were incubated for 48 hr at room temperature in 4 ml of 50 mM sodium acetate, pH 5.2, containing 0.01% Thimerosal (Sigma Chemical Co.) to prevent bacterial growth. The hydrolyzed samples were lyophilized and dissolved in 2 ml of water prior to fractionation on Bio-Gel P-2.

Scintillation Counting. Aliquots (50–1000 μl) of samples containing ^{14}C-radiolabeled material were mixed with 13 ml of solution containing 0.5% 2,5-diphenyloxazole and 0.03% 1,4-bis-(2-(5)-phenyloxazole)benzene in toluene and with 2 ml of Beckman Biosolve 3. Samples were counted with 93% efficiency in a Beckman LS-250 liquid scintillation counter.

Protein Assay. Protein content was assayed as described by Lowry *et al.* (18), with bovine serum albumin as a standard.

Cellulose Binding Assay. A suspension containing 100 mg of thoroughly washed Whatman CC-41 cellulose per ml of water was prepared, and 2.0-ml aliquots of the stirred suspension were pipetted into conical centrifuge tubes. Aliquots of the

samples to be assayed and sufficient water to give a final volume of 2.5 ml were then added to the tubes, and the suspensions were mixed thoroughly. The suspensions were incubated for 30 min with frequent stirring and then centrifuged in a clinical centrifuge. If the samples contained radiolabeled material for assay by scintillation counting, aliquots for counting were taken directly from the supernatant solutions. If the samples were to be assayed by the anthrone and carbazole methods, the supernatant solutions were first filtered through Whatman GF/C glass fiber paper to remove residual particles of cellulose.

Ion Exchange and Gel Filtration Chromatography. DEAE-Sephadex A-25 was obtained from Sigma Chemical Co. and was equilibrated for use with 10 mM potassium phosphate, pH 6.5. Agarose 1.5m and Bio-Gel P-2 and P-30 polyacrylamide gels were obtained from BioRad Laboratories. The columns of Agarose 1.5m (60 × 1.3 cm) and Bio-Gel P-30 (90 × 1.3 cm) were equilibrated and eluted with 50 mM sodium acetate, pH 5.2. Pooled fractions from these columns were passed through Dowex 50W X-8 (H^+ form, obtained from J. T. Baker Chemical Co.) to convert the sodium acetate to acetic acid prior to lyophilization and gas chromatographic analysis. The Bio-Gel P-2 column (90 × 1.3 cm) was equilibrated and eluted with water. The jacketed column was maintained at 50 C with a circulating water bath.

Determination of Molecular Weight of Xyloglucan. The molecular weight of SEPS xyloglucan was estimated by the hypoiodite method described by Croon and Manley (6).

RESULTS

Isolation and Characterization of Xyloglucan from Sycamore Extracellular Polysaccharides. Crude, de-esterified SEPS were obtained by the procedure described in "Methods" and fractionated on DEAE A-25 Sephadex in order to separate the neutral polysaccharides from those which contain significant amounts of uronic acids. Xyloglucan is found in the neutral polysaccharide fraction of SEPS (SEPS fraction 1), the fraction which washes through the DEAE-Sephadex column in 10 mM phosphate buffer. The xyloglucan thus obtained can be further purified by column chromatography on cellulose as described by Aspinall *et al.* (2).

Table I. *Mole Per Cent Sugar Compositions of Total SEPS and Various Purified Xyloglucan Fractions*

The neutral sugar compositions of the following samples were determined as described in "Methods": crude SEPS (before fractionation on DEAE-Sephadex), SEPS xyloglucan (SEPS fraction 1 from DEAE-Sephadex), cellulose-purified SEPS xyloglucan, and xyloglucan from the three indicated regions of the peak obtained by chromatography of cellulose-purified SEPS xyloglucan on Agarose 1.5 m. The preparation of these samples is described in the text and in "Methods." Uronic acids were determined by the corrected bazole test, and the sugar compositions were computed as mole per cent of the total carbohydrate (24).

Sugar	Total SEPS	SEPS Xyloglucan	Cellulose-purified Xyloglucan	Agarose 1.5 m Fraction		
				1	2	3
Rhamnose	1.7	0.1	0.2	0.2	0	0
Fucose	3.1	5.2	6.0	6.7	6.9	5.6
Arabinose	14.7	1.8	2.0	1.8	1.4	1.2
Xylose	17.5	34.0	37.0	37.4	38.4	39.4
Mannose	1.0	1.3	0.3	1.5	0	0
Galactose	14.8	7.9	7.1	7.2	6.7	7.0
Glucose	21.6	48.4	46.5	44.5	45.9	46.0
Uronic acids[1]	25.6	<1.5	0	0	0	0

[1] The sample of total SEPS was found by methylation analysis of the reduced polysaccharides to contain both galacturonic and glucuronic acids.

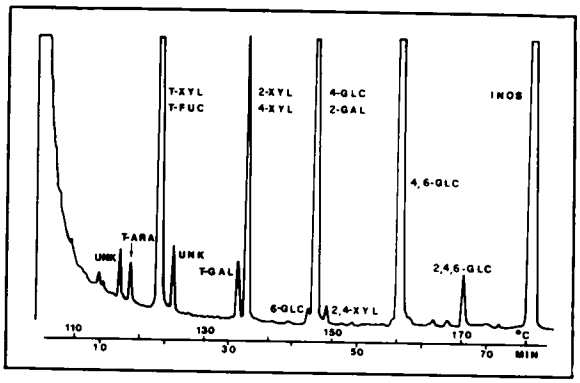

FIG. 1. Gas chromatogram of the methylated alditol acetates obtained from cellulose-purified SEPS xyloglucan. The methylation analysis of cellulose-purified SEPS xyloglucan was performed as described (24). The chromatogram was obtained on a Hewlett Packard model 7620A gas chromatograph using 120- × 0.3-cm columns packed with a liquid phase of 0.2% poly(ethylene glycol succinate), 0.2% poly(ethylene glycol adipate), and 0.4% silicone XF-1150 on a solid support of Gas Chrom P. The 1-μl sample was injected at a column temperature of 110 C, and after 6 min the temperature was raised at 1 C/min to 180 C. The initial peak in the chromatogram is due to the acetic anhydride used as the solvent. The glycosidic linkages to each sugar derivative are indicated by numerical prefixes: thus, 4,6-Glc indicates that sugars are glycosidically linked in the polysaccharide to the 4- and the 6-carbons of glucosyl residues. Terminal residues are indicated by T- (*e.g.*, T-Xyl). The inositol hexa-acetate used as a standard is indicated by INOS.

The sugar compositions of total SEPS, of SEPS xyloglucan (SEPS fraction 1), and of cellulose-purified SEPS xyloglucan are given in Table I.

Cellulose-purified SEPS xyloglucan was examined by gel filtration on Agarose 1.5m in order to separate from the xyloglucan any arabinose- and galactose-containing polysaccharides which might be present, and in order to estimate the size of the xyloglucan polymers. Cellulose-purified SEPS xyloglucan eluted as a single peak with an elution volume of approximately 1.9 V_0. The sugar compositions of material from the leading edge, the middle, and the trailing edge of the xyloglucan peak were determined. These compositions are included in Table I.

Samples of cellulose-purified SEPS xyloglucan were methylated. The gas chromatogram of the alditol acetate derivatives obtained from the methylated polymer is given in Figure 1, and the sugar linkage composition of the polymer appears in Table II.

Isolation of Xyloglucan from Sycamore Cell Walls. Evidence that xyloglucan is present in the sycamore cell wall, as well as in SEPS, was obtained by the isolation of this polymer, and characteristic fragments of this polymer, from the wall. A flow diagram of the procedures used to isolate xyloglucan from the wall is given in Figure 2.

Urea Extraction of Sycamore Walls. Aspinall *et al.* (2) have found that SEPS xyloglucan binds strongly, but noncovalently, to commercial cellulose, and that approximately one-third of the cellulose-bound xyloglucan could be recovered by elution with 8 M urea. Samples of untreated sycamore walls were therefore extracted with 8 M urea, and the sugar and sugar linkage compositions of the urea-extractable material were determined as described (24). It was found that 1 to 2% of the untreated wall is solubilized by 8 M urea and that over 90% of the

Table II. *Mole Per Cent Sugar Linkage Compositions of SEPS, Xyloglucan, the Chloroform-methanol-soluble Fraction of Methylated Sycamore Walls, and Sycamore Wall Fractions U and C-2*

The sugar linkage compositions are expressed as mole per cent of the total carbohydrate detected by gas chromatography of the partially methylated alditol acetates (24). The glycosidic linkages to each sugar derivative are indicated in the table by numerical prefixes: thus, 2,4-Rha indicates that sugars are glycosidically linked to the 2- and the 4-carbons of these rhamnosyl residues. Terminal residues are indicated by T- (*e.g.*, T-Xyl). Superscript letters in the table designate those sugar derivatives which elute in a single, unresolved gas chromatographic peak from the column described in the legend of Figure 1. Where all the derivatives with the same superscript are given values, these derivatives were separated on the S.C.O.T. capillary gas chromatographic column described in the legend of Figure 1 of Reference 24 (*e.g.*, superscript "b," T-Fuc and T-Xyl). In those instances where derivatives with the same superscript have not been separated, the total amount of material in the peak is given under the principle derivative (*e.g.*, superscript "a," T-Fuc and T-Xyl). The residues of 2-Xyl and 4-Xyl present in SEPS xyloglucan and in the chloroform-methanol-soluble fraction of methylated sycamore walls were quantitatively estimated by mass spectrometry (24). These residues occur in the xyloglucan polymer in the ratio of approximately 3.5:1, respectively, and occur in the chloroform-methanol-soluble fraction in the ratio of approximately 1:1. Uronic acids do not appear in the gas chromatograms of the partially methylated alditol acetates. Small peaks in the gas chromatograms due to unidentified derivatives are not included in the table, nor are peaks containing less than 0.5% of the recovered carbohydrate. The total of the unidentified derivatives represents 1 to 4% of the recovered carbohydrate. The type of polysaccharide in which each sugar derivative is normally found in sycamore walls is shown in the table by A = arabinan, AG = 3,6-linked arabinogalactan, G = 4-linked galactan, R = rhamnogalacturonan, and X = xyloglucan. Total SEPS is crude, de-esterified SEPS which has not been fractionated on DEAE-Sephadex. $CHCl_3$-CH_3OH-soluble walls is that fraction of methylated sycamore walls which is soluble in 1:1 chloroform-methanol (v/v). This fraction represents approximately 55% of the total wall and contains about 18 mole per cent of 4-linked glucose which arises from a starch contaminant of the wall preparations (24). The 4-Glc arising from starch is not included in this table. Purified xyloglucan is SEPS xyloglucan which has been purified by cellulose column chromatography as described by Aspinall *et al.* (2). The sample designated as pH 2.0 xyloglucan was obtained by hydrolysis of SEPS [^{14}C]xyloglucan with trifluoroacetic acid at pH 2.0 as described in the text. Sycamore wall fraction U was obtained by extraction of endopolygalacturonase-pretreated walls with 8 M urea as described in the text. Sycamore wall fraction C-2 is the first fraction of acidic polysaccharides obtained by DEAE-Sephadex chromatography (Fig. 5) of the products released from endopolygalacturonase-pretreated walls by endoglucanase (sycamore wall fraction C).

Sugar and Linkage	Polymer	Total SEPS	$CHCl_3$-CH_3OH-soluble Walls	Purified Xyloglucan	pH 2.0 Xyloglucan	Wall Fraction U	Wall Fraction C-2	Sugar and Linkage	Polymer	Total SEPS	$CHCl_3$-CH_3OH-soluble Walls	Purified Xyloglucan	pH 2.0 Xyloglucan	Wall Fraction U	Wall Fraction C-2
Rhamnose								Xylose (cont'd)							
T-Rha	AG	2.3	0	0	0	0	0	2- and 4-Xyl[e]	X	5.5	5.8	8.3	6.9	8.3	2.0
2-Rha	R	0	2.1	0	0	0	1.2	2,4-Xyl	X	1.9	0	0.6	1.9	1.8	0
2,4-Rha	R	0	2.1	0	0	1.3	3.6	Galactose							
Fucose								T-Gal	X;G	2.9	5.0	1.8	5.3	4.0	7.2
T-Fuc	X	[a]	3.1[b]	5.3[b]	0[b]	3.3[b]	[a]	2-Gal	X	[d]	[d]	5.6[e]	0.5[e]	[f]	[g]
Arabinose								3-Gal	AG	3.2	2.6	0	0	1.0	2.0
T-Ara	X;A	9.2	11.5	1.0	0	8.3	17.9	4-Gal	G	0	9.2	0	0	8.9	32.7[g]
2-Ara	A	0.7	1.1	0	0	0	0	6-Gal	G;AG	3.0	1.3	0	0	2.0	4.6
3-Ara	A	0	2.0	0	0	0	1.8	3,6-Gal	AG	11.5	1.5	0	0	0	0.8
5-Ara	A	1.1	5.9	0	0	4.0	7.7	Glucose							
2,5-Ara	A	0	1.3	0	0	0	0	T-Glc	X	0	0	0	0.6	1.1	0
3,5-Ara	A	0	4.2	0	0	4.5	9.4	4-Glc	X	17.6[d]	9.4[d]	13.4[e]	20.7[e]	19.8[f]	[g]
2,3,5-Ara	A	0	1.3	0	0	0	0	6-Glc	X	0	0	0.5	1.4	[f]	[g]
Xylose								4,6-Glc	X	23.6	14.7	31.6	30.5	15.5	0.6
T-Xyl	X	18.5[a]	13.0[b]	27.8[b]	28.5[b]	15.0[b]	3.1[a]	2,4,6-Glc	X	0	0	1.3	0.8	1.2	0

carbohydrate in this material is 4-linked glucose. When treated with amylase, this material yields negligible reducing groups as measured by the method of Nelson (20) as modified by Somogyi (23). The polysaccharide material extracted from *untreated* walls by 8 M urea is therefore probably cellulose.

Further samples of sycamore walls were treated with 8 M urea to remove the extractable glucan and then treated with highly purified endopolygalacturonase as described (24). These walls were then carefully washed with water and re-extracted with 8 M urea. About 2% of the wall is solubilized by this second urea extraction (wall fraction U). The sugar composition of fraction U is given in Table III, and the sugar linkage composition is included in Table II. Material from fraction U forms dark brown to violet complexes when mixed with 0.15 M iodine in aqueous potassium iodide. This is a characteristic reaction of xyloglucans from a wide variety of sources (15–17, 22).

Base Extraction of Sycamore Walls. Dilute base was reported by Aspinall *et al.* (2) to partially extract SEPS xyloglucan bound noncovalently to commercial cellulose. Fifty milligrams of endopolygalacturonase-pretreated sycamore walls were therefore extracted with base as described in "Methods," and the extracted material was lyophilized to yield 8 mg of sycamore wall fraction B. Wall fraction B represents about 16% of the endopolygalacturonase-pretreated walls and about 14% of the untreated walls. Fraction B polysaccharides form dark brown to violet complexes with iodine.

Treatment of Sycamore Walls with Endoglucanases. Samples of *untreated* sycamore walls were allowed to react extensively with the endoglucanase from *T. viride* as described in "Methods" for endoglucanase treatment of endopolygalacturonase-pretreated walls. Only about 1% of the wall material is solubilized by the endoglucanase. When endopolygalacturonase-pretreated walls were used, however, the *T. viride* endoglucanase released approximately 10 to 15% of these walls as soluble products (sycamore wall fraction C). These products

were fractionated on DEAE-Sephadex, and the results are shown in Figure 3. The sugar compositions of the endoglucanase-derived acidic carbohydrate fractions (sycamore wall fractions C-2, C-3, and C-4) were determined and are presented in Table III. The methylation analysis of fraction C-2 was performed, and the results from this analysis are included in Table II. Fraction C-1 was further fractionated on Bio-Gel P-2, as described below, in order to obtain discrete oligosaccharide fragments for a more detailed analysis of xyloglucan structure.

Bio-Gel P-2 Fractionation of Xyloglucan Fragments. Samples of SEPS xyloglucan and of the sycamore wall xyloglucan fragments released by endoglucanase (sycamore wall fraction C-1) were treated exhaustively with *T. viride* endoglucanase as described in "Methods." The reaction products were fractionated on a Bio-Gel P-2 column operated at 50 C. The results of these fractionations are shown in Figure 4. A mixture of untreated xyloglucan, stachyose, raffinose, cellobiose, and glucose was fractionated on the same column, and aliquots of each fraction were assayed for carbohydrate by the anthrone method. The elution volume of each of these standards is indicated in Figure 4. Fractions from each of the major xyloglucan peaks shown in Figure 4 were pooled, and aliquots

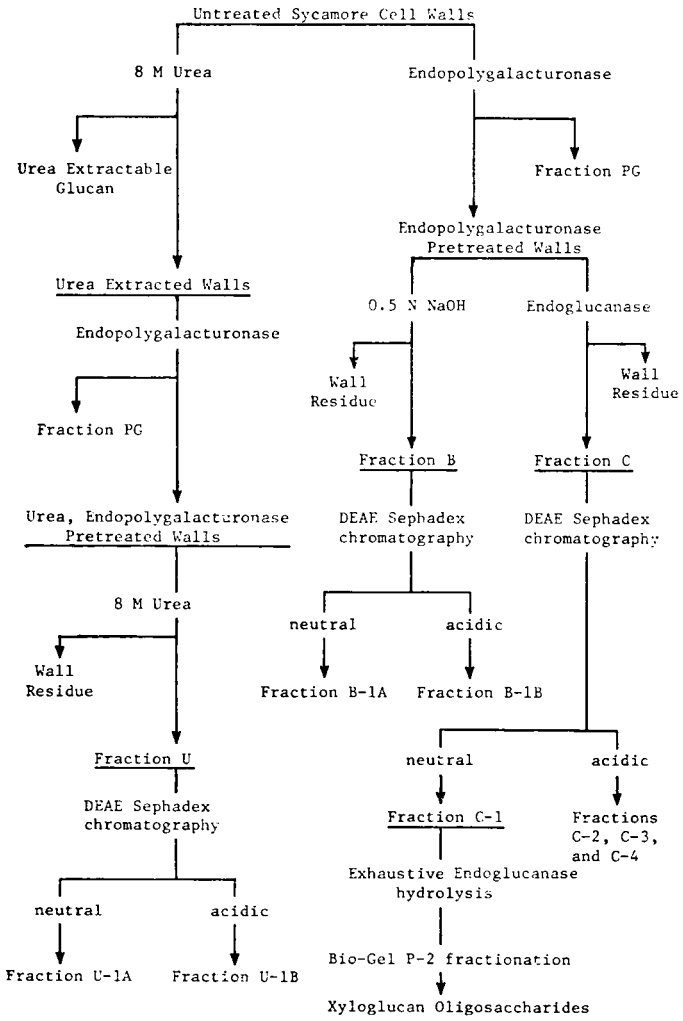

FIG. 2. Outline of the fractionation of xyloglucan-containing sycamore cell wall fractions. Xyloglucan-containing sycamore wall fractions were obtained by treatment of sycamore walls with urea, base, or endoglucanase. Details are given in the text.

Table III. *Mole Per Cent Sugar Compositions of Xyloglucan Fractions fromSycamore Cell Walls*

The neutral sugar compositions of the sycamore wall fractions were determined as described (24). These fractions were isolated from endopolygalacturonase-pretreated sycamore walls with 8 M urea, endoglucanase, or 0.5 N NaOH. Details of the isolation of each fraction are given in the text. Uronic acids were determined by the corrected carbazole test, and the sugar compositions were computed as mole per cent of the total carbohydrate (24).

Sugar	Sycamore Wall Fraction					
	U	C-1	C-2	C-3	C-4	B
Rhamnose	3.5	1.4	4.6	6.2	6.5	5.6
Fucose	3.0	6.6	1.0	0.9	1.3	2.1
Arabinose	16.1	9.1	37.4	44.3	46.0	20.1
Xylose	21.7	35.8	2.9	2.7	2.9	18.0
Mannose	1.6	0.9	0.4	0.2	0.1	0
Galactose	18.3	14.4	42.4	30.0	23.8	21.0
Glucose	28.3	30.4	4.0	4.2	4.4	19.5
GalUA	7.6	2.4	7.5	11.5	15.9	13.5

were taken for the determination of their sugar and sugar linkage compositions. The sugar and sugar linkage compositions of Bio-Gel P-2 peaks 1–5 are presented in Tables IV and V. The percentage of the recovered xyloglucan present in each of the Bio-Gel P-2 peaks was determined by the anthrone method. These percentages are indicated in Table IV.

When the partially purified endoglucanase from culture filtrates of *C. lindemuthianum* was used to treat endopolygalacturonase-pretreated walls, products corresponding to *T. viride* endoglucanase wall fraction C were obtained. These products were fractionated on DEAE-Sephadex as described above, and DEAE-Sephadex fractions corresponding to *T. viride* endoglucanase fraction C-1 and combined *T. viride* endoglucanase fractions C-2, C-3, and C-4 were obtained. The neutral sugar compositions of these *C. lindemuthianum* endoglucanase-derived DEAE-Sephadex fractions were determined and were found to be quite similar to the compositions of the corresponding *T. viride* endoglucanase fractions.

The endoglucanase purchased from Miles Laboratories was used to treat an additional sample of endopolygalacturonase-pretreated sycamore walls and to hydrolyze exhaustively the sycamore wall fraction C-1 material obtained after DEAE-Sephadex fractionation. This material was fractionated on Bio-Gel P-2 as described above. The results obtained with the Miles endoglucanase were significantly different from those obtained with the *T. viride* endoglucanase prepared in this laboratory. The Miles endoglucanase did not hydrolyze the xyloglucan as extensively as did the *T. viride* endoglucanse. The Miles endoglucanase produced a substantial Bio-Gel P-2 peak corresponding in elution volume and sugar composition to the peak 4 oligosaccharide obtained with the *T. viride* endoglucanase, but the Miles preparation did not produce peaks corresponding to either the peak 3 oligosaccharide or the peak 5 oligosaccharide. The Miles endoglucanase produced a very large peak corresponding in elution volume and in sugar composition to cellobiose and a small peak corresponding to glucose. The Miles endoglucanase preparation has not been further characterized, but these results demonstrate that the results reported in this section were critically dependent on the enzyme preparation used.

Identification of Sugar Residues Located at Reducing Ends of Endoglucanase-derived Xyloglucan Oligosaccharide Fragments. Samples of SEPS xyloglucan material from Bio-Gel P-2

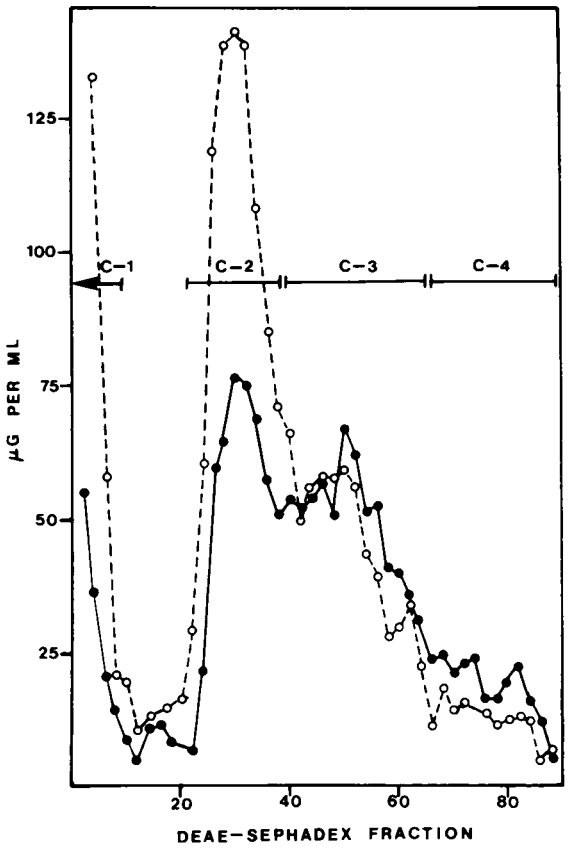

FIG. 3. Chromatography of fraction C on DEAE-Sephadex A-25. The combined reaction and wash solutions from the endoglucanase treatment of endopolygalacturonse-pretreated sycamore walls (wall fraction C) were titrated to pH 6.5 and then passed through a DEAE-Sephadex A-25 column (12.5 × 2.2 cm) equilibrated in 10 mM potassium phosphate, pH 6.5. The effluent was collected as one large fraction (680 ml). The column was then washed with 100 ml of the phosphate buffer, collecting 6.6-ml fractions. This was followed from fractions 15 to 90 by elution with a linear gradient of sodium chloride (0–800 mM) in buffer. Each fraction was assayed for neutral sugars by the anthrone test (○) and for uronic acids by the carbazole test (●). The sample eluate and buffer wash solution containing neutral carbohydrate was combined (wall fraction C-1). Column fractions 21 to 38 were combined as wall fraction C-2, 39 to 65 were combined as wall fraction C-3, and 66 to 90 were combined as wall fraction C-4.

peaks 2, 3, 4, and 5 (Fig. 4) were allowed to react for 1 hr with sodium borohydride (5 mg/ml) in 1 N ammonium hydroxide *prior* to methylation so that the sugar residues at the reducing ends of the oligosaccharide fragments would be reduced to the corresponding alditols. Methylation analyses of these samples were then carried out as described (24). The gas chromatograms of the partially methylated alditol acetates obtained from these samples were compared with the gas chromatograms obtained from samples of the same SEPS xyloglucan Bio-Gel P-2 peaks which had not been reduced prior to methylation. It was found that prior reduction of the samples resulted in the appearance of a single new peak which was the same in each of the gas chromatograms. The material in this peak was identified by mass spectrometry and found to be 4-*O*-acetyl-1,2,3,5,6-penta-*O*-methyl sorbitol. The reaction conditions given above result in only partial reduction (about 10–20%) of the reducing ends of these oligosaccharide fragments.

Hydrolysis of Xyloglucan at pH 2.0. The effect of dilute acid hydrolysis on both the structure and cellulose-binding properties of xyloglucan was studied. SEPS [^{14}C]xyloglucan, prepared as described in "Methods," was titrated to pH 2.0 with trifluoroacetic acid and hydrolyzed in sealed tubes for 1 hr at 110 C. After filtration of the hydrolyzed sample through Whatman GF/C glass fiber paper to remove traces of insoluble material, an aliquot of the solution was taken for methylation analysis. The results of this analysis appear in Table II. The remainder of the sample was fractionated on a Bio-Gel P-30 column. Each fraction from the column was assayed for carbohydrate by the anthrone method, for the presence of radiolabel by scintillation counting, and for binding of the radiolabeled material to cellulose, as described in "Methods." The results of this experiment are shown in Figure 5. Aliquots of fractions from several regions of the P-30 peak were pooled as indicated in the legend of Figure 5, and their sugar compositions were determined. It was found that virtually all of the fucose and arabinose and most of the rhamnose in these fractions appeared in regions 5 and 6. Fragments in regions 1 through 4 consisted almost exclusively of galactose, xylose, and glucose in the ratio of approximately 1:5:7, respectively.

Binding of Endoglucanase-derived Xyloglucan Oligosaccharides to Cellulose. A sample of SEPS [^{14}C]xyloglucan was treated exhaustively with endoglucanase and fractionated on the same Bio-Gel P-2 column that was described previously.

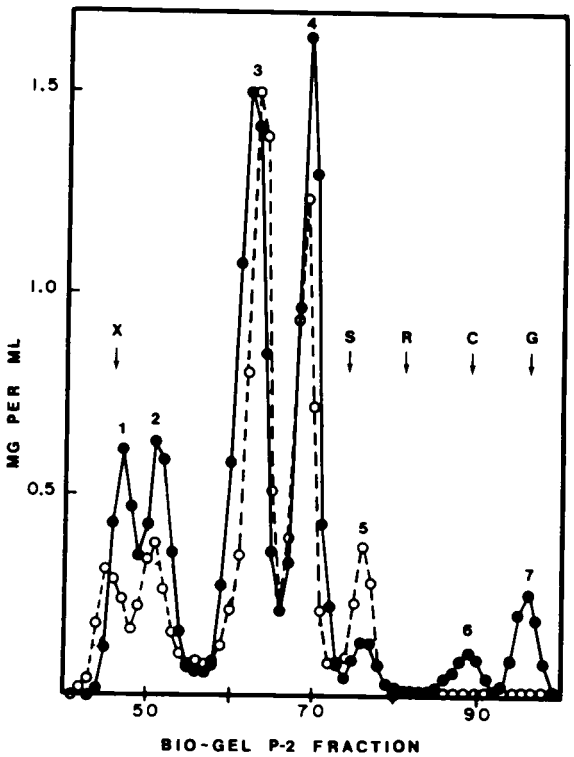

FIG. 4. Bio-Gel P-2 fractionation of endoglucanase-treated xyloglucan from SEPS and sycamore cell walls. A 30-mg sample of SEPS (○) and a separate 40-mg sample of sycamore wall xyloglucan (fraction C-1) (●) were treated exhaustively with endoglucanase as described in the text. These samples were then chromatographed on a Bio-Gel P-2 column operated at 50 C as described in "Methods." Fractions of 1.5 ml were collected at a flow rate of 0.1 ml/min, and a 50-μl aliquot of each fraction was assayed for carbohydrate by the anthrone test (24). Each of the major peaks is numbered in the order of its elution from the column. A mixture of carbohydrate standards was fractionated on the same column. Untreated SEPS xyloglucan voids the column. The elution volumes of untreated SEPS xlyoglucan (X), stachyose (S), raffinose (R), cellobiose (C), and glucose (G) are indicated in the figure by arrows.

Table IV. *Sugar Compositions and Cellulose Binding of Endoglucanase-derived Xyloglucan Oligosaccharides*

The sugar compositions of the xyloglucan oligosaccharides obtained by Bio-Gel P-2 chromatography after treatment of xyloglucan with endoglucanase were determined as described in "Methods." Each oligosaccharide is referred to by the number assigned to it in Figure 4. The source of the xyloglucan oligosaccharides is indicated by SEPS = sycamore extracellular polysaccharides and SCW = sycamore cell walls. The sugar compositions are expressed as mole per cent of the total carbohydrate detected by gas chromatography of the alditol acetate derivatives. The amount of carbohydrate material in each peak, expressed as per cent of the total recovered xyloglucan, was determined by the anthrone assay as described (24). The sugar compositions of the two small peaks (Bio-Gel P-2 peaks 6 and 7) seen in the elution pattern (Fig. 4) of sycamore wall fraction C-1 were determined but are not shown. The mole per cent sugar composition of peak 6 was found to be galactose = 15, glucose = 85. The mole per cent sugar composition of peak 7 was found to be xylose = 15, glucose = 85. A sample of SEPS [^{14}C]xyloglucan was treated with endoglucanase and fractionated on the same Bio-Gel P-2 column as described in the text. Aliquots of fractions across each of the major SEPS [^{14}C]xyloglucan peaks were assayed for binding to Whatman Cellulose as described in "Methods." The percentage of radiolabeled material from each peak which bound to cellulose is indicated in the table.

Sugar	Bio-Gel P-2 Peak									
	1		2		3		4		5	
	SEPS	SCW	SEPS	SCW	SEPS	SCW	SEPS	SCW	SEPS	SCW
Fucose	6.1	6.2	6.5	5.5	10.5	10.0	0	0	0	0
Arabinose	7.1	6.3	3.9	4.9	0	0	0	0	0	7.1
Xylose	30.5	36.3	34.3	36.7	33.5	32.4	45.4	42.9	40.4	33.5
Mannose	4.1	0	0	0	0	0	0	0	0	0
Galactose	11.8	9.1	8.8	9.1	11.5	12.7	0	0	0	6.6
Glucose	39.9	42.0	45.9	43.8	45.7	44.8	54.6	57.2	59.6	53.7
Per cent of xyloglucan in peak	9.0	10.4	11.2	13.3	37.8	35.2	28.6	28.4	9.0	2.8
Per cent binding to cellulose	84		36		1		3		2	

The ability of the radiolabeled oligosaccharides from each of the Bio-Gel P-2 peaks to bind to Whatman cellulose was determined as described in "Methods." The results of this experiment are included in Table IV.

Binding of Xyloglucan to Cellulose. The rate of binding of SEPS [^{14}C]xyloglucan to Whatman cellulose was examined by the assay described in "Methods." It was found that all of the xyloglucan binds to Whatman cellulose in less than 5 min. Approximately 15 to 20% of the cellulose-bound xyloglucan is released from the cellulose by overnight incubation with *T. viride* endoglucanase.

Iodine Staining Properties of Xyloglucan. Samples of SEPS xyloglucan were mixed with 0.15 M I_2 in aqueous KI. The resulting solutions turned dark brown to violet, depending on the final concentrations of iodine and xyloglucan. Color formation increased slowly over a period of several hours. The colored complex did not sediment when the solutions were centrifuged. Samples of xyloglucan which had been extensively pretreated with endoglucanase did not form colored complexes with the iodine reagent. Further 10-mg samples of xyloglucan were incubated in 2 ml of water with 100-mg samples of Whatman CC-41 cellulose. The cellulose was then washed twice with water by centrifugation to remove any xyloglucan which did not bind to the cellulose. Iodine reagent was then added to the washed pellet and to a similar cellulose pellet which had not been incubated with xyloglucan. The control cellulose pellet was not visibly stained, whereas the cellulose pellet which had been preincubated with xyloglucan stained dark grey to violet.

Molecular Weight of Xyloglucan. A sample containing 150 mg of SEPS xyloglucan was fractionated on Agarose 1.5m. Fractions from the center one-third of the xyloglucan peak were pooled and dialyzed against water. After lyophilization, 49.2 mg of average-sized SEPS xyloglucan were obtained. The molecular weight of this material was determined by the hypoiodite procedure as described in "Methods" and found to be approximately 7600.

DISCUSSION

SEPS Xyloglucan. De-esterified sycamore extracellular polysaccharides were fractionated on DEAE-Sephadex in order to separate the neutral polysaccharides from those which contain uronic acids. The neutral fraction of SEPS (SEPS fraction 1) passes directly through the column in 10 mM potassium phosphate, pH 7.0, and consists almost exclusively of xyloglucan (Table I).

The xyloglucan of SEPS fraction 1 was further purified by binding to cellulose and by eluting the noncovalently bound xyloglucan with either 8 M urea or 1 N NaOH as described by Aspinall *et al.* (2). The cellulose-purified SEPS xyloglucan thus obtained contains significant and reproducible amounts of arabinose and galactose (Table I). Attempts to separate the arabinose and galactose from the xyloglucan by gel filtration on Agarose 1.5m were not successful. Only one peak, eluting at approximately 1.9 V_0, was obtained. The sugar compositions of polymers from the leading edge, the center, and the trailing edge of the xyloglucan peak are quite similar (Table I), suggesting that the polymers from these three regions are structurally homogeneous but differ in size or degree of aggregation. Small amounts of rhamnose and of mannose are present exclusively in the highest molecular weight region of the peak and are therefore probably not integral parts of the xyloglucan polymer.

The methylation analysis of SEPS xyloglucan (Fig. 1 and Table II) confirms the major linkages reported by Aspinall *et al.* (2). In addition, this analysis shows the presence of resi-

Table V. *Sugar Linkage Compositions of Endoglucanase-derived Xyloglucan Oligosaccharides*

The sugar linkage compositions are expressed as mole per cent of the total carbohydrate detected by gas chromatography of the partially methylated alditol acetates (24). The source of the xyloglucan oligosaccharides is indicated by SEPS = sycamore extracellular polysaccharides and SCW = sycamore cell walls. Each oligosaccharide is referred to by the number assigned to it in Figure 4. The type of polysaccharide in which each sugar derivative is normally found in sycamore walls is shown in the table by A = arabinan, AG = 3,6-linked arabinogalactan, G = 4-linked galactan, R = rhamnogalactan, and X = xyloglucan. Refer to the legend of Table II for further details.

Sugar and Linkage	Polymer	Bio-Gel P-2 Peak								
		1		2		3		4		5
		SEPS	SCW	SEPS	SCW	SEPS	SCW	SEPS	SCW	SEPS
Rhamnose										
2,4-Rha	R	0	1.4	0	0	0	0	0	0	0
Fucose										
T-Fuc[a]	X	6.4	3.8	5.9	5.4	10.9	11.9	0	0	0
Arabinose										
T-Ara	X;A	3.4	8.1	2.1	3.2	0	0	0	0	0
5-Ara	A	0	3.5	0	0	0	0	0	0	0
3,5-Ara	A	0	3.5	0	0	0	0	0	0	0
Xylose										
T-Xyl[a]	X	19.9	18.7	25.1	27.1	20.2	20.5	38.7	43.9	39.3
2- and 4-Xyl[b]	X	7.1	4.7	6.8	6.7	11.2	11.3	1.4	0	0.9
2,4-Xyl	X	3.2	0.5	0	0.6	0	0	0	0	0
Galactose										
T-Gal	X;G	5.2	4.5	2.5	2.8	2.0	2.6	0.9	0	1.7
2-Gal[c]	X	4.9	0.6	6.8	5.2	7.8	10.9	0	0	0
3-Gal	AG	1.6	0	0	0	0	0	0	0	0
4-Gal	G	0	12.1[d]	0	0	0	0	0	0	0
6-Gal	AG,G	1.6	1.5	0	0	0	0	0	0	0
Glucose										
T-Glc	X	1.4	0.9	0.5	0	0	0	0	0	1.8
4-Glc[c]	X	9.7	11.2	12.9	14.2	13.0	12.5	14.4	11.1	18.8
6-Glc	X	3.7	[d]	4.8	5.4	10.4	10.1	12.5	14.2	19.0
4,6-Glc	X	18.8	13.8	23.7	22.3	21.6	20.5	27.8	26.8	18.9
2,4,6-Glc	X	7.3	10.0	4.5	5.0	0	0	0	0	0

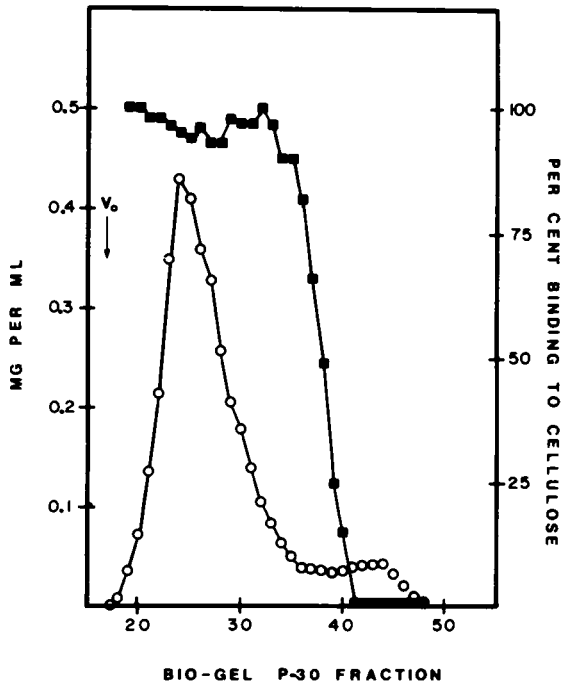

FIG. 5. Bio-Gel P-30 fractionation of pH 2.0 hydrolyzed SEPS [^{14}C]xyloglucan. A 15-mg sample of SEPS [^{14}C]xyloglucan was brought to pH 2.0 with trifluoroacetic acid and hydrolyzed at 110 C for 1 hr as described in the text. A 10-mg portion of the hydrolyzed sample was fractionated on Bio-Gel P-30 as described in "Methods." Fractions of 3 ml were collected at a flow rate of 0.2 ml/min, and 100-μl aliquots of each fraction were assayed for carbohydrate (○) by the anthrone test, for radioactivity (not shown), and for the binding of radiolabeled material to Whatman cellulose (■) as described in "Methods." The amount of radioactivity was found to correspond closely with the amount of carbohydrate in all fractions. The specific activity of the xlyoglucan was found to be 8.3 nc/mg of glucose-equivalent carbohydrate. The xyloglucan peak was divided into six regions for the determination of sugar compositions. The fractions pooled for this purpose were: region 1 = fractions 18 to 23, region 2 = fractions 24 to 25, region 3 = fractions 27 to 29, region 4 = fractions 30 to 33, region 5 = fractions 34 to 40, and region 6 = fractions 41–49. The void volume (V_o) of the Bio-Gel column is shown in the figure by an arrow.

dues of terminal arabinose, terminal and 2-linked galactose, and 2,4,6-linked glucose. Two unidentified sugar derivatives are also present as minor constituents.

Composition of Sycamore Walls. The sugar and sugar linkage compositions of isolated sycamore cell walls are presented in Tables II and III in the first paper of this series (24). An examination of these results shows that the sycamore cell wall contains all of the sugars, in the appropriate linkages, which are found in SEPS xyloglucan. It can be calculated from the amount of xylose *or* from the amount of 4,6-linked glucose

present in the cell wall and in SEPS xyloglucan that a *maximum* of about 21% of the dry weight of the cell wall could be xyloglucan of the type isolated from SEPS. It should be noted that the noncellulosic glucose value reported in Table II of Reference 24 is probably too low, since the acid hydrolysis procedure used to obtain this value converts some of the glucan backbone chains of xyloglucan into cellulose-like oligosaccharides. Cellulose is not appreciably hydrolyzed to glucose under the hydrolysis conditions used.

Isolation of Sycamore Wall Xyloglucan. There is considerable evidence that sycamore walls contain a xyloglucan polysaccharide and that this sycamore wall xyloglucan is the same as the xyloglucan isolated from SEPS. The fractions released from endopolygalacturonase-pretreated walls by urea, endoglucanase, or dilute base clearly contain the sugars and sugar linkages which are characteristic of SEPS xyloglucan (Tables I, II, and III). Moreover, the polysaccharides of fractions U and B show the same ability as SEPS xyloglucan to bind noncovalently to cellulose and to form colored complexes with iodine. In addition, endoglucanase releases fragments of xyloglucan from endopolygalacturonase-pretreated walls which give the same basic elution pattern when fractionated on Bio-Gel P-2 as the elution pattern obtained by fractionation of endoglucanase-pretreated SEPS xyloglucan (Fig. 4). This correspondence between the elution patterns of xyloglucan fragments from the two sources is extremely unlikely unless the wall-derived xyloglucan and SEPS xyloglucan have the same basic structure. A comparison of the sugar and sugar linkage compositions of these fragments (Tables IV and V) shows that the xyloglucans from the two sources are virtually identical. The demonstration that an extracellular polysaccharide is closely related to one of the major polysaccharides of the primary cell wall will hopefully lead to a more intensive study of the metabolic relationships between extracellular polysaccharides and the cell wall.

The fact that both xyloglucan and cellulose are present in the sycamore wall makes it very likely that the xyloglucan is noncovalently bound to the cellulose fibrils of the wall in a manner similar to the noncovalent binding of SEPS xyloglucan to commercial cellulose reported by Aspinall *et al.* (2). This possibility is supported by the observation that both 8 M urea and dilute base, which are able to extract partially xylogucan noncovalently bound to commercial cellulose, are also able to extract partially the xyloglucan from endopolygalacturonase-pretreated sycamore walls (wall fractions U and B).

The hypothesis that xyloglucan is bound noncovalently to cellulose fibrils in the walls leads to the prediction that such noncovalent binding should be chemically reversible. A more detailed examination of the results of the extraction with 8 M urea indicates that this prediction is satisfied.

The extraction of *untreated* sycamore walls with 8 M urea solubilizes *small* amounts of a 4-linked glucan which is probably cellulose but does not solubilize any other polysaccharide or sugar in appreciable amounts. Since 8 M urea is widely used for the disruption of hydrogen bonds, and since the cellulose fibrils of the cell wall are believed to be held together by interchain hydrogen bonds (8), the solubilization of some cellulose by 8 M urea might be expected. From the fact that other sugars and polysaccharides are not released from untreated walls by 8 M urea, it appears that urea does not cause the cleavage of covalent bonds in the wall matrix.

The extraction of endopolygalacturonase-pretreated walls with 8 M urea releases a wall fraction which contains xyloglucan (fraction U, Tables II and III). Recalling that the extraction of untreated walls with urea releases only the 4-linked glucan discussed above, it should be noted that the subsequent treatment of these urea-extracted walls with endopolygalacturonase results in the release of fraction PG material (see Reference 24), but does *not* result in the release of the xyloglucan-containing polysaccharides of fraction U. Thus, the effect of urea appears to be chemically reversible when the urea is removed. The release of the fraction U polysaccharides requires an additional urea extraction after the endopolygalacturonase treatment.

The extraction of endopolygalacturonase-pretreated sycamore walls with 0.5 N NaOH releases a polysaccharide fraction (sycamore wall fraction B) which contains xyloglucan, and which is very similar in composition to the fractions obtained by urea or endoglucanase treatments (Table III). The partial and nonspecific cleavage of chemical bonds in the cell wall by aqueous alkali makes it impossible to say with certainty how the polysaccharides of fraction B were attached to the endopolygalacturonase-pretreated walls. However, further evidence for the reversibility of xyloglucan binding in the cell wall is provided by the results of an experiment in which sycamore walls were pretreated with 0.01 N NaOH at 2 C for 4 hr in order to de-esterify the galacturonosyl residues, neutralized, and then treated with endopolygalacturonase. The endopolygalacturonase treatment of these base-pretreated walls released a normal PG fraction, but did *not* release the xyloglucan polysaccharides characteristic of fraction B. Base extraction with 0.01 N NaOH has been shown to partially release xyloglucan bound noncovalently to commercial cellulose or to isolated Red Kidney bean walls (unpublished results).

The proposal that the xyloglucan of sycamore walls is noncovalently bound to the cellulose fibrils of these walls is quite consistent with the results of the endoglucanase treatment of endopolygalacturonase-pretreated walls. Thus, xyloglucan polymers in the wall which are noncovalently bound to cellulose fibrils would be hydrolyzed by the endoglucanase to give oligosaccharide fragments (Fig. 4) which do not bind appreciably to cellulose (Table IV).

The existence of a *covalent* connection between xyloglucan and the pectic polysaccharides of the wall is also indicated by the results of the isolation and fractionation of xyloglucan from the walls. Sycamore wall fractions U, B, and C, obtained by urea, base, and endoglucanase treatment of endopolygalacturonase-pretreated walls, all contain substantial amounts of rhamnose, arabinose, galactose, and galacturonic acid (Table III). An examination of the sugar linkage compositions of these fractions (see fractions U and C-2, Table II) shows that these sugars appear in the linkages and proportions that are characteristic of wall fraction PG-1 (Table V in Reference 24). Fraction PG-1 is the higher molecular weight, neutral sugar-rich fraction released by endopolygalacturonase treatment of sycamore walls and contains highly branched arabinan and 4-linked galactan attached as side chains to rhamnosyl-rich acidic fragments of the rhamnogalacturonan. The appearance of these characteristically pectic polysaccharides in the xyloglucan wall fractions U, B, and C, rather than in the wall fractions released by endopolygalacturonase, is best explained by the hypothesis that some of the neutral-rich pectic polysaccharides are covalently attached to xyloglucan polymers and are released from the wall matrix only when the noncovalent bonding between xyloglucan and cellulose fibrils is disrupted or weakened by agents such as 8 M urea, base, or endoglucanase. This hypothesis is supported by the observation that some of the xyloglucan oligosaccharide fragments produced by endoglucanase treatment of endopolygalacturonase-pretreated walls are found to cochromatograph with the neutral-rich pectic polysaccharide fragments of wall fraction C on DEAE-Sephadex (Fig. 3, Table IV, and fraction C-2 of Table II). The neutral-rich pectic polysaccharide fragments chromatograph as negatively charged species in a manner consistent with their

proposed structure (24), but the xyloglucan oligosaccharide fragments, by themselves, are neutral species which do not bind to DEAE-Sephadex (fraction C-1, Fig. 3) and should not cochromatograph with the pectic polysaccharide fragments unless they are covalently attached. Additional evidence for such a covalent linkage is presented in the other papers of this series (13, 24).

The failure of 8 M urea to release xyloglucan from *untreated* sycamore walls can be interpreted as additional evidence that the xyloglucan is covalently linked to the pectic polysaccharides of the wall. However, the failure of endoglucanase to release xyloglucan from untreated walls cannot readily be explained by the presence of covalent linkages between xyloglucan polymers and the pectic polysaccharides, since the products of endoglucanase treatment of xyloglucan are oligosaccharide fragments (Fig. 4). Thus, endoglucanase treatment of untreated walls should release all of the wall xyloglucan as oligosaccharide fragments except those fragments which are covalently attached to the pectic polysaccharides. The fact that endoglucanase releases negligible amounts of xyloglucan oligosaccharides from untreated walls, but releases very substantial amounts of xyloglucan oligosaccharides from endopolygalacturonase-pretreated walls, suggests that the presence of the pectic polysaccharides in the untreated walls blocks the access of endoglucanase to xyloglucan and that removal of the pectic polysaccharides with endopolygalacturonase renders substantial amounts of the xyloglucan in the walls accessible to attack by the endoglucanase.

The inability of sequential treatments with endopolygalacturonase and endoglucanase to remove *all* of the sugars characteristic of the pectic polysaccharides and the xyloglucan from the cell walls may indicate that these sugars are present in polysaccharides which have different structures, but the same sugars in the same linkages and the same proportions, as those released by the two enzymes. Alternatively, the incomplete release of these sugars may indicate that they are present in polysaccharides or polysaccharide fragments which are still attached to other parts of the wall matrix, or may simply indicate that parts of the wall matrix are physically inaccessible to the enzymes. Although we favor the latter alternative, this is a matter which requires further investigation.

Structure and Cellulose Binding of Xyloglucan. The binding of pH 2 hydrolyzed SEPS xyloglucan to cellulose was examined. The elution volume of the polymer on Agarose 1.5m (1.9 V_0) and the elution volume of the hydrolysis fragments on Bio-Gel P-30 (Fig. 5) indicate that the average size of the xyloglucan fragments produced by partial acid hydrolysis of the polymer at pH 2.0 is considerably smaller than the average size of the original polymers. Despite their smaller average size, virtually all of the xyloglucan fragments produced by partial acid hydrolysis of the polymer at pH 2.0 retain their ability to bind to cellulose (Fig. 5). This finding suggests, and the results of the Bio-Gel P-2 fractionation (Fig. 4) confirm, that the cellulose-binding property of xyloglucan is *not* due to the presence of discrete, cellulose-like regions in the xyloglucan polymer which contain only residues of unbranched glucose. The results of the hydrolysis of xyloglucan at pH 2.0 also show that hydrolytic cleavage of most of the rhamnosyl, fucosyl, and arabinosyl residues from the larger xyloglucan fragments does not affect the cellulose binding of these fragments, and also that the xylosyl and galactosyl residues still attached to these fragments do not interfere with the binding to cellulose.

Considerable information about the molecular structure of xyloglucan was obtained by analysis of the fragments produced by endoglucanase hydrolysis. The xyloglucan fragments produced by *T. viride* endoglucanase hydrolysis appear as discrete peaks when fractionated on BioIGel P-2 (Fig. 4). Two small peaks (peaks 6 and 7, Fig. 4) appear in the elution pattern of fragments from sycamore wall fraction C-1 but do not appear in the elution pattern of fragments from SEPS xyloglucan. Bio-Gel P-2 peaks 6 and 7 have elution volumes corresponding to a disaccharide and a monosaccharide, respectively (Fig. 4), and appear from their sugar compositions to be mostly cellobiose and glucose, respectively (see legend of Table IV). We have no explanation for the presence of small amounts of galactose in peak 6 and of xylose in peak 7. The absence of cellobiose and of glucose from the endoglucanase hydrolysis products of SEPS xyloglucan shows that the SEPS polymer does not contain significant regions of unbranched glucose. These two peaks in the elution pattern of sycamore wall fraction C-1 probably arise from the enzymic hydrolysis of a small amount of cellulose from the wall.

Proposed structures for the endoglucanase-derived xyloglucan oligosaccharides present in Bio-Gel P-2 peaks 2, 3, 4, and 5 (Fig. 4) are presented in Figure 6. A comparison of the experimentally determined sugar linkage compositions of the xyloglucan oligosaccharides and the sugar linkage compositions calculated for the oligosaccharide structures presented in Figure 6 is given in Table VI.

The structure proposed (Fig. 6) for the oligosaccharide of peak 5 is based on the following observations.

1. The sugar composition of this oligosaccharide shows that it consists almost exclusively of glucose and xylose (Table IV). These two sugars appear in a molar ratio of approximately 3:2, respectively. (Small amounts of arabinose and galactose are present in peak 5 obtained from sycamore wall fraction C-1. The presence of these two sugars in peak 5 is not understood. Peak 5, however, represents only about 2.8% of the

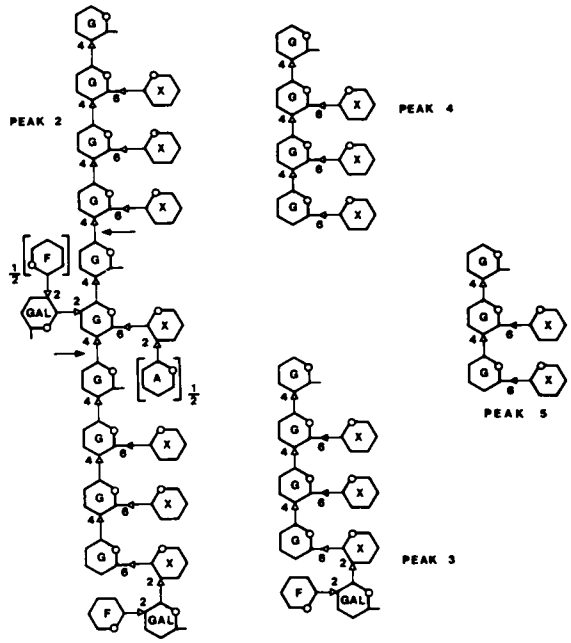

FIG. 6. The proposed structures of endoglucanase-derived xyloglucan oligosaccharides. The structures shown are based on the data presented in Tables IV and V and on the observations discussed in the text. Each of the xyloglucan oligosaccharides is referred to by the number assigned to it in Figure 4. For simplicity, the anomeric configurations of the glycosidic linkages are not indicated. The resistance to endoglucanase hydrolysis of the glycosidic linkages marked by arrows, in the structure proposed for the oligosaccharide of peak 2, is discussed in the text. The particular xylose residue to which the fucosyl-galatose is attached in the structure presented in peaks 2 and 3 is unknown. The structure of the oligosaccharide presented for peak 2 has not been fully established.

Table VI. *Actual and Calculated Sugar Linkage Compositions of Endoglucanase-derived Xyloglucan Oligosaccharides*

The experimentally determined sugar linkage compositions (from Table V) of the endoglucanase-derived xyloglucan oligosaccharides obtained by chromatography on Bio-Gel P-2 (Fig. 4) are compared with the sugar linkage compositions calculated for the proposed structures of these oligosaccharides shown in Fig. 6. Each oligosaccharide is referred to in the table by the peak number assigned to it in Figure 4. The sugar linkage compositions are presented in this table as mole per cent of the total carbohydrate. The values given in Table V for the sugar linkage compositions of the wall-derived xyloglucan oligosaccharides of peaks 2, 3, and 4 have been averaged with the values of the corresponding SEPS xyloglucan oligosaccharides. These average values are used in this table.

Sugar and Linkage	Bio-Gel P-2 Peak							
	2		3		4		5	
	Exptl	Calc	Exptl	Calc	Exptl	Calc	Exptl	Calc
Fucose								
T-Fuc	5.7	7.1	11.4	11.1	0	0	0	0
Arabinose								
T-Ara	2.7	2.4	0	0	0	0	0	0
Xylose								
T-Xyl	26.1	26.2	20.4	22.2	41.3	42.9	39.3	40.0
2-Xyl	6.8	7.1	11.3	11.1	0.7	0	0.9	0
Galactose								
T-Gal	2.7	2.4	2.3	0	0.5	0	1.7	0
2-Gal	6.0	7.1	9.4	11.1	0	0	0	0
Glucose								
4-Glc	13.8	14.3	12.8	11.1	12.7	14.3	18.8	20.0
6-Glc	5.1	4.8	10.3	11.1	13.4	14.3	19.0	20.0
4,6-Glc	23.0	23.9	21.1	22.2	27.3	28.6	18.9	20.0
2,4,6-Glc	4.8	4.8	0	0	0	0	0	0

total carbohydrate in fraction C-1, and the arabinose and galactose in this peak may be due to small amounts of a contaminant.)

2. The oligosaccharide of peak 5 (Fig. 4) has an elution volume between that of an unbranched tetrasaccharide (stachyose) and that of an unbranched trisaccharide (raffinose). Since the oligosaccharide of peak 5 is branched (Table V), its molecular asymmetry should be less than that of an unbranched oligosaccharide containing the same number of residues, and its gel filtration elution volume should be corresponding larger. The identification of the peak 5 oligosaccharide as a branched pentasaccharide is thus consistent with both the elution volume and the molar ratio of glucose to xylose of this oligosaccharide.

3. Both xylose residues of the pentasaccharide of peak 5 are terminal (Table V).

4. Partial reduction of the peak 5 pentasaccharide with sodium borohydride *prior* to methylation resulted in the appearance of a peak in the gas chromatogram of the subsequently synthesized partially methylated alditol acetate derivatives which has been identified as 4-*O*-acetyl-1,2,3,5,6-penta-*O*-methyl sorbitol ("Results"). Thus, in agreement with the known specificity of endoglucanase (3, 14), 4-linked glucose occurs at the reducing end of the pentasaccharide.

5. The methylation analysis of untreated xyloglucan shows that 6-linked glucose is not present in significant amounts in the polymer (Table II). After treatment of xyloglucan with endoglucanase, however, 6-linked glucose appears as a major component in the sugar linkage compositions of the oligosaccharide fragments (Table V). The appearance of 6-linked glucose is accompanied by a corresponding decrease in the percentage of 4,6-linked glucose. *T. viride* endoglucanase, therefore, generates xyloglucan oligosaccharides with 4-linked glucose at the reducing end of the glucan chain and 6-linked glucose at the nonreducing end. The enzyme specifically hydrolyzes the glycosidic bonds between unbranched glucosyl residues and carbon 4 of 4,6-linked glucosyl residues.

Similar considerations lead to the structures proposed (Fig. 6) for the oligosaccharides of Bio-Gel P-2 peaks 3 and 4. Both oligosaccharides contain glucose and xylose in a molar ratio of approximately 4:3 (Table IV), and, as the result of endoglucanase hydrolysis, both oligosaccharides have 4-linked glucose at the reducing end of the glucan chain and 6-linked glucose at the nonreducing end. The elution volumes of peaks 3 and 4 are consistent with those of a branched nonasaccharide and a branched heptasaccharide, respectively.

All of the xylosyl residues of the heptasaccharide of peak 4 are terminal, whereas one of the 3 xylosyl residues of the nonasaccharide of peak 3 is 2-linked rather than terminal (Table V). The nonasaccharide contains 1 residue of 2-linked galactose and 1 residue of terminal fucose in addition to the sugars of the heptasaccharide unit. It seems reasonable to assume that the structure of the heptasaccharide would be conserved in the biosynthesis of the nonasaccharide, so that the nonasaccharide would be synthesized by the addition of galactosyl and fucosyl residues to the heptasaccharide unit rather than by a synthesis involving rearrangements of this unit. Thus, the structure proposed for the nonasaccharide of peak 3 retains the basic structure given for the heptasaccharide and shows galactose linked to the 2 position of one of the xylosyl residues. It is not known to which of the xylosyl residues the galactose is attached. The partial acid hydrolysis of xyloglucan at pH 2.0 (Table II) resulted in a polymer which contained substantially greater amounts of terminal galactose and correspondingly lower amounts of 2-linked galactose and terminal fucose. The proposed structure for the nonasaccharide of peak 3 therefore shows fucose linked to the 2 position of the galactosyl residue. It may be noted that the experimentally determined sugar linkage composition of this oligosaccharide (Table V) indicates that approximately 20% of the galactosyl residues are terminal, and thus this fraction of the galactosyl residues cannot have fucose linked to the 2 position.

The structure proposed for the oligosaccharide of peak 2, Figure 4, is more complex and difficult to establish. The distinguishing feature of this oligosaccharide is the presence of the doubly branched, 2,4,6-linked glucose. There is approximately 1 residue of this component for every 20 residues in the oligosaccharide (Tables V and VI). The oligosaccharide cannot be much larger than 20 residues or it would have voided the Bio-Gel P-2 column despite its highly branched structure. If the structures of the heptasaccharide and nonasaccharide units of the polymer are used in the biosynthesis of the oligosaccharide of peak 2, then a structure for this oligosaccharide which is consistent with its approximate size and sugar linkage composition may be generated by the combination of one heptasaccharide unit, one nonasaccharide unit, 1 residue of 4-linked glucose, and 1 residue of 2,4,6-linked glucose with arabinosylxylose and fucosylgalactose side chains.

Any structure proposed for the oligosaccharide of peak 2 should account for the fact that the glycosidic bonds of the 2 internal residues of 4-linked glucose (which are indicated by arrows in the proposed structure shown in Fig. 6) are *not* hydrolyzed by the *T. viride* endoglucanase even though this enzyme produces the oligosaccharides of peaks 3, 4, and 5 by hydrolyzing the glycosidic bonds between residues of 4-linked

glucose and 4,6-linked glucose. It is suggested that the *T. viride* endoglucanase cannot hydrolyze the two indicated glycosidic bonds in the peak 2 oligosaccharide shown in Figure 6 because the side chain at the 2 position of the doubly branched, 2,4,6-linked glucosyl residue sterically hinders the enzyme.

No structure has been presented for the void peak material (peak 1, Fig. 4) since SEPS xyloglucan and sycamore wall xyloglucan show significant differences in this fraction (Table V). The void peak fraction obtained from sycamore walls contains substantial amounts of sugars in linkages which are characteristic of the pectic arabinan and galactan (5- and 3,5-linked arabinose and 4-linked galactose) and of the pectic rhamnogalactoronan (2,4-linked rhamnose). It has not been determined whether this peak contains separate or covalently linked pectic and xyloglucan oligosaccharides.

A structure which is generally consistent with the sugar linkage composition of peak 1 from SEPS xyloglucan can be generated by adding to the oligosaccharide of peak 2 1 residue of 2,4,6-linked glucose with the disaccharide side chains shown for this residue in Figure 6. It seems unlikely, however, that the resulting oligosaccharide would be sufficiently larger than the peak 2 oligosaccharide to account for the observed difference in the elution volumes of peaks 1 and 2. Thus, peak 1 may contain a dimer of the oligosaccharide suggested above. The presence of 4 residues of the doubly branched glucose could account for the resistance of the dimer to endoglucanase. However, it is clear from the low percentage of 2,4,6-linked glucose in cellulose-purified SEPS xyloglucan (Table II) that dimers of this sort are not frequently synthesized.

Comparison of Sycamore Xyloglucan and "Amyloids." As previously noted by Aspinall *et al.* (2), the xyloglucan from SEPS is structurally similar to the so-called seed "amyloids." These amyloids, which are also xyloglucans, are found in the seeds of a wide variety of plants and derive their name from the fact that they form colored complexes with iodine—as does starch [amylose] (15–17, 22). Sycamore xyloglucan also forms colored complexes with iodine. It has been suggested (9) that the failure of Aspinall *et al.* (2) to detect formation of an iodine complex with SEPS xyloglucan may have been due to the presence of protein in their preparation. The chemical basis of xyloglucan-iodine complex formation is not known, although Gould *et al.* (9) have recently suggested that formation of the complex between iodine and the amyloid from white mustard seeds involves the interaction of iodine molecules or iodide ions within the interstices between *aggregated* xyloglucan chains. This suggestion is consistent with our observation that the oligosaccharides produced by endoglucanase hydrolysis of SEPS xyloglucan do not form colored iodine complexes. Since the endoglucanase-derived xyloglucan oligosaccharides have little or no ability to bind to cellulose (Table IV), it seems unlikely that they would be able to associate strongly enough with each other to provide interchain "holes" for complex formation with iodine. We have also observed that SEPS xyloglucan bound to cellulose is able to form colored iodine complexes, which, in the model of Gould *et al.* (9), would indicate that suitable "holes" for iodine complexing are formed when chains of xyloglucan associate with the glucan chains of cellulose.

There is a striking similarity between the structure proposed here for sycamore xyloglucan and that proposed in the elegant study by Kooiman for the amyloid obtained from the seeds of *Tamarindus indicus* (16). Kooiman has isolated and characterized the oligosaccharides produced by endoglucanase hydrolysis of the tamarind amyloid. Three such oligosaccharides were obtained in significant amounts. One of these oligosaccharides was shown to have a structure identical to that proposed here for the heptasaccharide unit of sycamore xyloglucan. The other two oligosaccharides were found by Kooiman to contain either 1 or 2 residues of terminal galactose linked to the 2 position of xylosyl residues in the heptasaccharide unit. Using an enzyme preparation called "Luizym" (Luitpoldwerke, München, West Germany), Kooiman (16) was also able to hydrolyze the tamarind xyloglucan so that almost all of the xylosyl residues of the polymer were recovered in the disaccharide 6-*O*-α-D-xylopyranosyl-D-glucopyranose. The essentially quantitative isolation of this disaccharide clearly demonstrates that all of the glucosyl resides in the polymer are present in a cellulose-like glucan backbone, and that all of the xylosyl residues occur as mono-xylosyl side chains linked to carbon 6 of the glucosyl residues in the glucan backbone.

The sugar linkage compositions of the sycamore xyloglucan oligosaccharides (TableV) do not prove that all of the glucosyl residues of sycamore xyloglucan are present in the cellulose-like, β-(1 → 4)-linked glucan backbone. The sugar linkage compositions are equally consistent with oligosaccharide structures in which 1 of the 4,6-linked glucosyl residues occurs as a side chain linked 1 → 6 to either a 6- or a 4,6-linked glucosyl residue of the cellulose-like glucan chain. We have based the structures of the sycamore xyloglucan oligosaccharides presented in Figure 6 on the clear demonstration by Kooiman (16) (see above) that all of the glucosyl residues of the closely related tamarind xyloglucan are present in the cellulose-like glucan backbone of this polymer.

Preliminary investigations in this laboratory of the anomeric configuration of the glycosidic linkages in sycamore xyloglucan, using the chromium trioxide-acetic acid oxidation method of Hoffman *et al.* (11), suggest that the fucosyl residues of this polymer are linked in the α configuration, and that all of the other glycosidic linkages exist in the β configuration. Kooiman (16) has reported that the galactosyl and glucosyl residues of the tamarind xyloglucan are glycosidically linked in the β configuration but has presented strong evidence that the xylosyl residues are linked α-(1 → 6), rather than β-(1 → 6), to the glucosyl residues. Our evidence for the β-(1 → 6) linkage of xylosyl residues in sycamore xyloglucan is not conclusive. Therefore, we cannot, at this point, conclude that the xyloglucans from the two plants are different in this respect. Studies with CPK models of xyloglucan indicate that both α- and β-linked xylosyl residues stick out from the sides of the β-(1 → 4)-linked glucan chain, and that both configurations lead to very similar structures for the polymer even when the fucosylgalactose side chains are attached to some of the xylosyl residues.

The molecular weight of SEPS xyloglucan is estimated from the results of the hypoiodite oxidation experiment to be approximately 7600, which corresponds to about 56 sugar residues in the polymer. About 25 to 30 of these residues would be glucosyl residues in the (1 → 4)-linked backbone of the polymer. The molecular weights of the amyloid xyloglucans from *Tamarindus indicus* and *Annona muricata* L. were estimated by Kooiman (17), using a similar hypoiodite method, and found to be approximately 10,750 and 11,500, respectively. Using a periodate oxidation method, however, Kooiman (17) found the molecular weight of the *A. muricata* xyloglucan to be only 8,800. Since the methods used for molecular weight determinations yield only rough estimates of the average size of polymers this large, these three xyloglucans can be considered to have comparable molecular weights.

In view of the structural similarities between sycamore xyloglucan and the seed amyloid xyloglucans (16, 17, 22), it will be of interest to ascertain whether or not the amyloid

xyloglucans are incorporated into the structure of the primary cell walls of the germinating seedlings. The fate of the amyloid xyloglucan from white mustard seeds has recently been studied by Gould *et al.* (9). These investigators have found that, before germination, two xyloglucan fractions could be obtained from the cotyledons: a soluble xyloglucan which could be extracted with hot EDTA solutions, and an insoluble xyloglucan which required further extraction with aqueous alkali or lithium thiocyanate. After germination, the soluble xyloglucan fraction was not detected, but the insoluble xyloglucan was still present. The disappearance of the soluble xyloglucan after germination led Gould *et al.* (9) to the conclusion that the soluble xyloglucan is a storage polysaccharide which is metabolized upon germination. It seems likely to us that the difference between the soluble and insoluble xyloglucans is the binding of the latter to cellulose, and that quite possibly the disappearance of the soluble xyloglucan after germination involves the binding of this fraction to cellulose or its incorporation into newly synthesized cell walls.

Conclusions. The structure of xyloglucan consists basically of a cellulose-like, β-(1 → 4)-linked glucan backbone with frequent xylosyl side chains attached to carbon 6 of the glucosyl residues in the backbone. On the basis of x-ray data and model studies, it is believed that cellulose fibrils are held together by hydrogen bonds between oxygens of alternating glycosidic bonds in one glucan chain and the primary hydroxyl groups at position 6 of glucosyl residues in another chain (8). Thus, when xyloglucan binds to cellulose, every second glycosidic oxygen of the glucan chain of xyloglucan is available to act as an acceptor for hydrogen bond formation with the hydrogen of a primary hydroxyl group at position 6 of a glucosyl residue of a given cellulose chain. However, only about one-fourth of the glucosyl residues in the xyloglucan polymer have primary hydroxyl groups available at position 6 to act as donors for interchain hydrogen bonding. This feature of xyloglucan structure may reduce further lateral associations, giving a monolayer of xyloglucan on the surface of the cellulose fibril. The fucosyl-(1 → 2)-galactosyl-(1 → 2)-xylose side chains of sycamore xyloglucan may play a more important role in preventing further lateral associations. Xyloglucan structures built with CPK models show that the 1 → 2 linkages of the fucosylgalactosylxylose side chains cause them to curl over either the top or the bottom face of the β-(1 → 4)-linked glucan backbone. If the bottom face of the glucan backbone of xyloglucan is hydrogen-bonded to a cellulose fibril, then the trisaccharide side chains would hinder access of other β-(1 → 4)-linked glucan chains to the top face of the xyloglucan backbone.

It can be calculated that there is approximately enough xyloglucan in sycamore cell walls to encapsulate all of the cellulose fibrils in these walls with a monolayer of xyloglucan. This rough calculation is based on the relative amounts of cellulose and xyloglucan in the sycamore wall, on the approximate proportion of cellulose chains which are exposed at the surface of an elementary fibril (estimated to be about 50%), and on the assumption that xyloglucan can bind to all of the cellulose chains which are at the surface of a cellulose fibril.

Xyloglucan is not the only plant cell wall polysaccharide with a structure suited to the formation of interchain hydrogen bonds with cellulose. Polysaccharides appearing in the classical hemicellulose fraction of plant cell walls (xylans, arabinoxylans, mannans, glucomannans, and galactoglucomannans) also have structures which are well suited for hydrogen bonding to cellulose chains. Several such polysaccharides have, in fact, been reported to bind to cellulose *in vitro* (5, 10). These polysaccharides and the xyloglucans may belong to a single class of functionally related polymers which have in common the ability to bind noncovalently to cellulose. We suggest that the structural function of the hemicellulosic polysaccharides is to interconnect the cellulose fibrils and the pectic polysaccharides of the wall, and that this function is based on the ability of the hemicellulosic polysaccharides to bind noncovalently to cellulose and to bind covalently, through glycosidic bonds at their reducing ends, to the pectic polysaccharides.

The partial and nonspecific cleavage of chemical bonds in the plant cell wall by the aqueous alkali used to obtain the classical hemicellulose fraction of the wall has made it difficult to recognize the polysaccharides appearing in this classical fraction as discrete, but interconnected, polymers, and has also made it difficult to correlate the appearance of these polysaccharides in the classical hemicellulosic fraction with their place and structural function in the native cell wall. We tentatively suggest, therefore, that the term "hemicellulose" be redefined to include only those plant cell wall polysaccharides which are found to bind noncovalently to cellulose. This operational definition is based on a chemical property of the polysaccharides which is relatively easy to measure and which is clearly related to the proposed biological function of these polymers.

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LITERATURE CITED

1. Albersheim, P., D. J. Nevins, P. D. English, and A. Karr. 1967. A method for the analysis of sugars in plant cell-wall polysaccharides by gas-liquid chromatography. Carbohyd. Res. 5: 340–345.
2. Aspinall, G. O., J. A. Molloy, and J. W. T. Craig. 1969. Extracellular polysaccharides from suspension-cultured sycamore cells. Can. J. Biochem. 47: 1063–1070.
3. Barras, D. R., A. E. Moore, and B. A. Stone. 1969. *In:* Cellulases and Their Applications. American Chemical Society, Washington, D.C. pp. 105–138.
4. Becker, G. E., P. A. Hui, and P. Albersheim. 1964. Synthesis of extracellular polysaccharides by suspensions of *Acer pseudoplatanus* cells. Plant Physiol. 39: 913–920.
5. Blake, J. D. and G. N. Richards. 1971. An examination of some methods for fractionation of plant hemicelluloses. Carbohyd. Res. 17: 253–268.
6. Croon, I. and R. St. J. Manley. 1963. *In:* R. L. Whistler and M. L. Wolfrom, eds., Methods in Carbohydrate Chemistry, Vol. III. Academic Press, New York. pp. 286–287.
7. English, P. D., A. Maglothin, K. Keegstra, and P. Albersheim. 1972. A cell wall-degrading endopolygalacturonase secreted by *Colletotrichum lindemuthianum*. Plant Physiol. 49: 293–297.
8. Frey-Wyssling, A. 1969. The ultrastructure and biogenesis of native cellulose. Fortschr. Chem. Org. Naturst. 27: 1–30.
9. Gould, S. E. B., D. A. Rees, and N. J. Wight. 1971. Polysaccharides in germination. Xyloglucans ('amyloids') from the cotyledons of white mustard. Biochem. J. 124: 47–53.
10. Grant, G. T., C. McNab, D. A. Rees, and R. J. Skerrett. 1969. Seed mucilages as examples of polysaccharide denaturation. Chem. Commun. pp. 805–806.
11. Hoffman, J., B. Lindberg, and S. Svensson. 1972. Determination of the anomeric configuration of sugar residues in acetylated oligo- and polysaccharides by oxidation with chromium trioxide in acetic acid. Acta Chem. Scand. 26: 644.
12. Jones, T. M. and P. Albersheim. 1972. A gas chromatographic method for the determination of aldose and uronic acid constituents of plant cell wall polysaccharides. Plant Physiol. 49: 926–936.
13. Keegstra, K., K. T. Talmadge, W. D. Bauer, and P. Albersheim. 1973. The structure of plant cell walls. III. A model of the walls of suspension-cultured sycamore cells based on the interconnections of the macromolecular components. Plant Physiol. 51: 188–196.
14. Klop, W. and P. Kooiman. 1965. The action of cellulolytic enzymes on substituted celluloses. Biochim. Biophys. Acta 99: 102–120.
15. Kooiman, P. 1960. On the occurrence of amyloids in plant seeds. Acta Bot. Neer. 9: 208–219.
16. Kooiman, P. 1961. The constitution of *Tamarindus*-amyloid. Rec. Trav. Chim. Pays-Bas 80: 849–865.
17. Kooiman, P. 1967. The constitution of the amyloid from seeds of *Annona muricata* L. Phytochemistry 6: 1665–1673.

18. LOWRY, O. H., N. J. ROSEBROUGH, L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.
19. MANDELS, M. AND E. T. REESE. 1964. Fungal cellulases and the microbial decomposition of cellulosic fabric. Develop. Ind. Microbiol. 5: 5–20.
20. NELSON, N. J. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. 153: 375–380.
21. SELBY, K. AND C. C. MAITLAND. 1967. The cellulase of *Trichoderma viride*. Separation of the components involved in the solubilization of cotton. Biochem. J. 104: 716–724.
22. SIDDIQUI, I. R. AND P. J. WOOD. 1971. Structural investigation of water-soluble, rape-seed (*Brassica campestris*) polysaccharides. Carbohyd. Res. 17: 97–108.
23. SOMOGYI, M. 1952. Notes on sugar determination. J. Biol. Chem. 195: 19–23.
24. TALMADGE, K., K. KEEGSTRA, W. D. BAUER, AND P. ALBERSHEIM. 1973. The structure of plant cell walls. I. The macromolecular components of the walls of suspension-cultured sycamore cells with a detailed analysis of the pectic polysaccharides. Plant Physiol. 51: 158–173.