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ISOLATION AND PARTIAL CHARACTERISATION OF A XYLOGLUCAN FROM THE CELL WALLS OF *Phaseolus coccineus*

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ABSTRACT

Cell-wall material from *Phaseolus coccineus* was fractionated by successive extraction with aqueous inorganic solvents. From the 4M KOH-soluble fraction, a polymer composed of L-arabinose (4.2%), L-fucose (6.0%), D-galactose (9.3%), D-xylose (34.1%), and D-glucose (46.4%) was isolated and purified by ion-exchange and cellulose column chromatography; the product was homogeneous by moving-boundary electrophoresis and ultracentrifugation. The $S^{o}_{20,w}$ and D_{obs} were 2.92 S and 1.7×10^{-7}, respectively, and the molecular weight was ~110,000. Methylation analysis suggested a (1→4)-linked glucan backbone with ~3 out of 4 glucosyl residues substituted through O-6 with xylose or oligosaccharides terminating in galactose, fucose, and arabinose. Limited acetolysis gave several di- and tri-saccharide derivatives of which four [D-Gal*p*-(1→2)-D-Xyl*p*, D-Glc*p*-(1→4)-D-Glc*p*, L-Fuc*p*-(1→2)-D-Gal*p*-(1→2)-D-Xyl*p*, and D-Glc*p*-(1→4)-D-Glc*p*-(1→4)-D-Glc*p*] were tentatively identified. Specific glycosidases were used to determine the configuration of the glycosidic linkages. The backbone was shown to be a (1→4)-β-D-glucan and the L-fucosyl groups were α. Neither α- nor β-D-galactosidase removed D-galactose. The structure of the xyloglucan is discussed in relation to other cell-wall polymers, especially cellulose.

INTRODUCTION

The cell walls of dicotyledons consist largely of polysaccharides which have been classified according to their solubility in aqueous inorganic solvents[1,2]. The groups are usually heterogeneous in composition, but it is possible to obtain relatively homogeneous preparations suitable for structural analysis.

The xyloglucans (amyloids) are major, structural, hemicellulosic polysaccharides that have been isolated from seeds[3–5], plant cell-walls[6–10], and the extracellular media of suspension-cultured plant cells[11,12]. These polymers contain (1→4)-β-D-glucan backbones with units substituted at O-6 by xylose and oligosaccharides terminating in galactose and, in some instances, fucose and/or arabinose[13]. Recently, xyloglucan-type polymers have been found as minor constituents of monocotyledon cell-walls[14].

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The function of the xyloglucan in the cell wall and its relationship to other cell-wall polymers is difficult to assess. Bauer *et al.*[6] showed that the xyloglucan of suspension-culture cell-walls of sycamore may be covalently linked to pectic polysaccharides and bound non-covalently to cellulose. Ring and Selvendran[10] found that the bulk of the xyloglucans of potato cell-wall were not covalently linked to pectic polysaccharides. Xyloglucans have been implicated in cell-wall extension[15], but the evidence is inconclusive[16,17].

We now report on a xyloglucan from the cell walls of *Phaseolus coccineus*.

EXPERIMENTAL

Materials. — Mature runner beans (*Phaseolus coccineus* var. Streamline, on average 30 × 2 cm) were collected from plants grown in experimental plots near the laboratory. Parenchyma was obtained by scraping the pods (split in half lengthwise) up to the parchment layer.

Methyl sulphoxide was purified by distillation from CaH_2. DEAE-Sephadex and DEAE-Sephacel were obtained from Pharmacia, cellulose powder CC31 from Whatman, and Dowex AG 50W-X8 resin from BDH Chemicals. α-D-Galactosidase (EC 3.2.1.22) from green coffee-beans, β-D-galactosidase (EC 3.2.1.23) from *Aspergillus niger*, and alpha-amylase (EC 3.2.1.1) from porcine pancreas (Type 1-A PMSF treated) were obtained from Sigma Chemical Co.; α-L-fucosidase (EC 3.2.1.51) from beef kidney was obtained from Boehringer (W. Germany); and cellulase (EC 3.2.1.4) from *Trichoderma viride* CS12 was prepared by the method of Stevens and Payne[18], and partially purified by ammonium sulphate precipitation (30–80% saturation).

Capillary glass tubing was prepared by using a Shimadzu Glass Drawing apparatus, Superox^{TM-4} was purchased from Fields Instrument Co., and OV-225 and OV-1 from JJ Chromatography Supplies.

Monosaccharide and amino acid analysis. — Neutral sugars released by Saemen or M H_2SO_4 hydrolysis were analysed[19] as their alditol acetates by g.l.c.[19]. Uronic acid was determined by a modified carbazole method[19]. Total sugar was determined by the phenol–sulphuric acid method[20]. Amino acids were analysed by g.l.c. as their propyl heptafluorobutyryl derivatives[21].

Degree of esterification of pectin. — This was determined[22] by using KBr discs and a Pye–Unicam SP200G i.r. spectrophotometer.

T.l.c. — Silica gel G was used, with two irrigations with aqueous 65% 2-propanol–ethyl acetate (1:1) and detection with aniline hydrogenphthalate[23].

Moving-boundary electrophoresis. — A Perkin–Elmer Model 38A Tiselius Electrophoresis apparatus was used with 2-mL cells (open assembly). Polysaccharide (~20 mg/mL) was dissolved in 50mM sodium tetraborate (pH 8.0). Photographs were taken at intervals of 20 min.

Ultracentrifugation. — An MSE centriscan was used. Samples were prepared in 100mM sodium phosphate buffer (pH 7.2) containing 150mM NaCl. Sedimentation analysis (at various concentrations) was performed at 45,000 r.p.m. and 20°, with

scans taken at intervals of 20 min using Schlieren optics. Sedimentation values were extrapolated to zero concentration and corrected for solvent density and viscosity. The partial specific volume of the polysaccharide was determined from the sum of the partial specific volumes of the monomers, *i.e.*, 0.613 mL/g for hexoses, 0.678 mL/g for 6-deoxyhexoses[24], and 0.622 mL/g for pentoses[25]. An observed diffusion coefficient (D_{obs}) was obtained from successive Schlieren scans (at 10 mg/mL) from a plot of the second moment about the mean of the boundary gradient curve (σ) against time (s^{-1}). The plot gave a straight line with a slope equal to twice the diffusion coefficient[26].

Cell-wall preparation. — Cell-wall material of parenchymatous tissue was prepared by sequential extraction of the wet, ball-milled tissue with aqueous 1% sodium deoxycholate, phenol–acetic acid–water (2:1:1), and aqueous 90% methyl sulphoxide[27,28].

Fractionation of cell-wall material. — Cell-wall material (1 g/100 mL) was depectinated by sequential extraction twice with water (pH 4, 80°, 1 h) and then twice with aqueous 1% ammonium oxalate (pH 5.5, 80°, 2 h). The insoluble material (1 g/100 mL) was further extracted with M KOH containing 10mM $NaBH_4$ under argon for 2 h at 20°. The crude xyloglucan was isolated by extraction of the insoluble material (1 g/100 mL) with 4M KOH containing 10mM $NaBH_4$ under argon for 2 h at 20°. After extraction, the insoluble material was collected, and washed with 4M KOH (100 mL) and distilled water (500 mL). The combined filtrate and washings were adjusted to pH 5.5 with glacial acetic acid, dialysed exhaustively against distilled water, and freeze-dried.

Fractionation of the crude xyloglucan. — The 4M KOH-soluble fraction (100 mg) was dissolved in water and filtered onto a column (30 × 1 cm) of DEAE-Sephadex (Cl^- form) and eluted at 25 mL/h in sequence with water (50 mL), a linear gradient of 0→600mM NaCl (total vol., 100 mL), and M NaCl (50 mL). Fractions (2 mL) were collected and portions (100 μL) were analysed for total sugar. Appropriate fractions were combined, dialysed, and freeze-dried. A solution of the neutral fraction (75 mg) containing the xyloglucan in water (2 mL) was placed on a column (25 × 2 cm) of cellulose CC31 and eluted at 30 mL/h with water (100 mL) and then 500mM KOH containing 10mM $NaBH_4$ (250 mL). The alkali fraction was adjusted to pH 5.5 with glacial acetic acid, dialysed, and freeze-dried. A solution of the residue (60 mg) in 50mM sodium borate (pH 8) was filtered onto a column (20 × 1 cm) of DEAE-Sephacel (borate form) and eluted at 20 mL/h in sequence with 50mM sodium borate (50 mL), a linear gradient of 50→600mM sodium borate (total volume, 100 mL), and 800mM sodium borate containing 500mM NaCl (50 mL). Fractions (2 mL) were collected and portions (100 μL) assayed for total sugar. Appropriate fractions were combined, adjusted to pH 4.0 with acetic acid, dialysed, and freeze-dried, to give the purified xyloglucan (51 mg).

Partial, acid hydrolysis. — The purified xyloglucan (7.5 mg) was treated with 12.5mM oxalic acid for 3 h at 100°. The solution was dialysed and freeze-dried (yield, 6.2 mg).

Methylation analysis. — (*a*) *Polymeric material.* Methylation analysis of the purified xyloglucan (complete or acid-treated) was carried out as previously described[28]. Partially methylated alditol acetates were separated by g.l.c. on a wall-coated, OV-225 glass-capillary column (25 m × 0.25 mm). G.l.c.–m.s. was performed on an AEI MS30 mass spectrometer in the e.i. mode[27], using a wide bore (25 m × 0.55 mm), wall-coated glass-capillary column. Data were acquired with continuous scanning (3 s/decade) on an AEI DS 50SM computer system.

(*b*) *Oligosaccharides.* These (~2 mg) were reduced with aqueous NaB^2H_4 at room temperature for 5 h, the mixture was deionised with Dowex AG50W-X8 (H^+) resin, and boric acid was removed conventionally as methyl borate. The products were dried *in vacuo* over P_2O_5 for 12 h at 50°. Methylation and extraction of the methylated products was performed as described by Jansson *et al.*[29]. The methylated oligosaccharide alditols were separated by g.l.c. on a column (45 cm × 4 mm) containing 1% of OV-1, with a temperature programme 150→310° at 2°/min. G.l.c.–m.s. (e.i. mode) was performed on an AEI MS30 mass spectrometer with continual scanning over the mass range *m/z* 28–847 at 10 s/decade. For g.l.c.–m.s. (c.i. mode), ammonia was the reagent gas[30].

Capillary g.l.c. — (*a*) *Narrow bore.* A soda-glass column (25 m × 0.25 mm) was leached and deactivated[31] with Superox™-4, and then dynamically coated with OV-225 (25% in dichloromethane) using the Schomberg mercury-plug method[32]. A Perkin–Elmer Sigma 1 instrument with Grob splitless injection was used with a column pressure of 16 p.s.i., the flame-ionisation detector at 250°, and an oven programme of 40° for 2 min, 40°/min to 150°, and 150°→210° at 1.5°/min.

(*b*) *Wide bore.* A Pyrex-glass column (25 m × 0.55 mm) was prepared as described above, except that the coating mixture was 30% OV-225 in dichloromethane.

Acetolysis. — Purified xyloglucan (15 mg) was dried *in vacuo* over P_2O_5 for 12 h at 50° and then treated with acetic anhydride–pyridine (1:1, 1 mL) for 12 h at room temperature followed by 8 h at 100°. The cooled mixture was concentrated, and a dispersion of the syrupy residue in acetic anhydride–glacial acetic acid–conc. sulphuric acid (1:1:0.1, 1.5 mL) was stored at 37° for 12 h, neutralised with pyridine (5 mL), and concentrated. The syrupy residue was dispersed in water (5 mL) and extracted with chloroform (4 × 5 mL). The combined extracts were dried (Na_2SO_4), filtered, concentrated to ~500 μL, added to methanol (1 mL) at 50°, and treated with ethanolic M sodium ethoxide (1 mL). After storage for 2 h at room temperature, Dowex AG50W-X8 (H^+) resin was added, the mixture was filtered and concentrated to dryness, and a solution of the residue in distilled water (1 mL) was clarified by centrifugation. The products were examined by t.l.c. and identified as the methylated oligosaccharide alditols by g.l.c.–m.s.[10,33].

Enzymic degradations. — (*a*) *With cellulase.* A solution of purified xyloglucan (2 mg) in 50mM sodium acetate buffer (2 mL, pH 5.2) was incubated with 100 μL [10,000 units; 1 unit produces 1 μg/h of soluble carbohydrate (glucose) from ball-milled filter paper at pH 5.2 and 37°] of *Trichoderma viride* CS12 cellulase at 37°

for 48 h in the presence of toluene. The mixture was deionised, concentrated to 100 μL, and analysed by t.l.c.

(*b*) *With alpha-amylase.* A solution of purified xyloglucan (2 mg) in 50mM sodium phosphate buffer (2 mL, pH 6.8) containing 10mM NaCl was incubated[34] with 100 μL (1000 units; 1 unit of enzyme will liberate 1 mg of maltose from starch in 3 min at pH 6.9 and 20°) of alpha-amylase at 37° for 48 h, in the presence of toluene. The mixture was deionised, concentrated, and analysed by t.l.c.

(*c*) *With α-L-fucosidase.* A solution of xyloglucan (1 mg) in 50mM sodium citrate buffer (1 mL, pH 6.5) was incubated with 100 μL (0.5 unit; 1 unit of enzyme hydrolysed 1 μmol/min of *p*-nitrophenyl α-L-fucopyranoside at pH 6.5 and 25°) of beef-kidney α-L-fucosidase at 37° for 3 days in the presence of toluene. The mixture was dialysed, freeze-dried, and analysed for sugars by g.l.c.

(*d*) *With α- and β-D-galactosidase.* Purified xyloglucan, completely or partially acid-hydrolysed, was treated with α- or β-D-galactosidase as described earlier[35].

RESULTS AND DISCUSSION

Isolation and fractionation of the cell-wall material. — The cell-wall material used in this study was free from contamination with cytoplasmic material and starch[28].

Cell walls were depectinated (~85%) with hot water and ammonium oxalate. These fractions were rich in uronic acid, but differed in neutral sugar composition and degree of esterification (Table I, columns 2 and 3). Extraction with M KOH solubilised a small amount of polymeric material (Table I, column 5) containing polysaccharide and hydroxyproline-poor protein[36]. The crude xyloglucan was solubilised on extraction with 4M KOH and contained small amounts of uronic acid and protein (Table I, column 6). Methylation analysis revealed traces of xylan and arabinan (unpublished data). Similar observations have been made on potato cell-wall xyloglucan[10]. The insoluble residue (α-cellulose), after alkali extraction, contained small amounts of pectic material and hydroxyproline-rich glycoprotein (Table I, column 7). Treatment of the α-cellulose with a cellulase preparation from *Trichoderma viride* solubilised a component (~12%) of high molecular weight that was rich in uronic acid (Rha 7.2, Ara 14.8, Xyl 2.6, Man 1.1, Gal 25.9, Glc 13.3, and uronic acid 35.1 mol%). The hydroxyproline-rich glycoprotein remained in the insoluble residue (Ara 16.3, Xyl 14.2, Man 1.6, Gal 3.8, Glc 46.2, uronic acid 5.4, protein 12.5 mol%). The protein contained 20% of hydroxyproline and 12% of serine. This finding adds further support to the suggestion that the hydroxyproline-rich glycoprotein is strongly associated with cellulose[35-37]. Similar observations on the association of some pectic material with the α-cellulose fraction have been made with cabbage cell-walls[38].

Fractionation of the crude xyloglucan. — Contaminating polymers were removed by successive ion-exchange, cellulose, and ion-exchange column chromatography. Fractionation of the 4M KOH extract on DEAE-Sephadex gave a neutral fraction

TABLE I

COMPOSITION OF FRACTIONATED CELL-WALL MATERIAL FROM RUNNER BEANS

Component	*Fraction*						
	(1) Unfractionated cell-wall	*(2) Hot water-soluble*	*(3) Hot oxalate-soluble*	*(4) Depectinated cell-wall*	*(5) M KOH-soluble*	*(6) 4M KOH-soluble*	*(7) α-Cellulose*
Monosaccharide[a]							
6-Deoxyhexose	1.9	2.9	2.4	1.7	2.5	5.8	1.5
Arabinose	6.7	21.5	6.2	6.9	18.2	13.7	4.7
Xylose	3.2	1.4	0.6	4.8	23.5	29.0	1.7
Mannose	1.7	0.9	—	2.7	0.5	0.7	3.0
Galactose	10.9	25.9	17.0	9.6	16.8	9.1	8.6
Glucose	36.0	4.7	0.6	59.0	6.8	37.1	63.7
Uronic acid	39.6	42.7	73.2	15.3	31.7	4.6	16.8
Total carbohydrate (%)	78.7	71.2	89.5	68.2	44.8	87.6	83.7
Protein (%)	4.0	<1.0	<1.0	6.0	16.0	3.0	6.0
Degree of esterification (%)	—	52.0	17.0	—	—	—	—
Fraction of cell wall (%)	—	10	30	60	5.0	10.0	45

[a]Expressed as mol/100 mol of monosaccharide.

TABLE II

MONOSACCHARIDE COMPOSITION OF XYLOGLUCAN FRACTIONS DURING PURIFICATION

Component	*Fraction*			
	(1) DEAE-Sephadex Neutral fraction	*(2) Cellulose Alkali eluate*	*(3) DEAE-Sephacel Borate eluate*	*(4) Partially hydrolysed xyloglucan*
Monosaccharide[a]				
Fucose	5.3	5.6	6.0	1.0
Arabinose	6.9	3.8	4.2	1.0
Xylose	27.6	31.0	34.1	37.3
Mannose	1.2	—	—	—
Galactose	9.2	9.4	9.3	9.7
Glucose	41.7	46.8	46.4	51.0
Uronic acid	8.1	3.4	<1.0	<1.0
Recovery from column (%)	78.2	85.8	86.4	—

[a]Expressed as mol/100 mol of monosaccharide.

(78.2%, eluted with water) and several minor components (eluted with the NaCl gradient) (Fig. 1*a*). The neutral fraction was rich in glucose and xylose (Table II, column 1), whereas the minor fractions differed in both neutral sugar and uronic acid content. Methylation analysis of the neutral fraction revealed small quantities of arabinan-type material. Studies with xyloglucans from *Nicotiana tabacum* showed that contaminating material may be removed by cellulose column chromatography[39]. Fractionation of the neutral component on cellulose gave a minor component (10%, eluted with water) composed mainly of arabinose. The major component (90%, eluted with 500mM KOH) was rich in glucose and xylose (Table II, column 2). The latter material was purified on DEAE-Sephacel (borate form). The purified xyloglucan was eluted as a single, symmetrical peak between 130 and 250mM sodium borate (Fig. 1*b*), and the composition shown in Table II (column 3) is similar to that of xyloglucans isolated from a variety of plants[13].

Moving-boundary electrophoreses. — In 50mM sodium borate (pH 8.0), the purified xyloglucan gave a single boundary in both ascending and descending limbs of the electrophoresis cell.

Ultracentrifugation. — A single, symmetrical peak was observed at 45,000 r.p.m., and the absence of "hyperfine" peaks suggested that the xyloglucan did not aggregate. Sedimentation coefficients were determined at 2.0, 5.0, 7.5, and 10.0 mg/mL, and S_{obs} was extrapolated to zero. The extrapolated S° value was corrected for solvent viscosity and density, to give $S^\circ_{20,w}$ of 2.92 S. An observed diffusion coefficient (D_{obs}) was calculated to be 1.7×10^{-7}. Using the $S^\circ_{20,w}$ and D_{obs} values, a molecular weight of 110,000 was estimated. The frictional ratio (f/fo) was calculated as 4.146 which, for a prolate elipsoid, is equivalent to an axial ratio of ~100, suggesting a highly asymmetrical molecule.

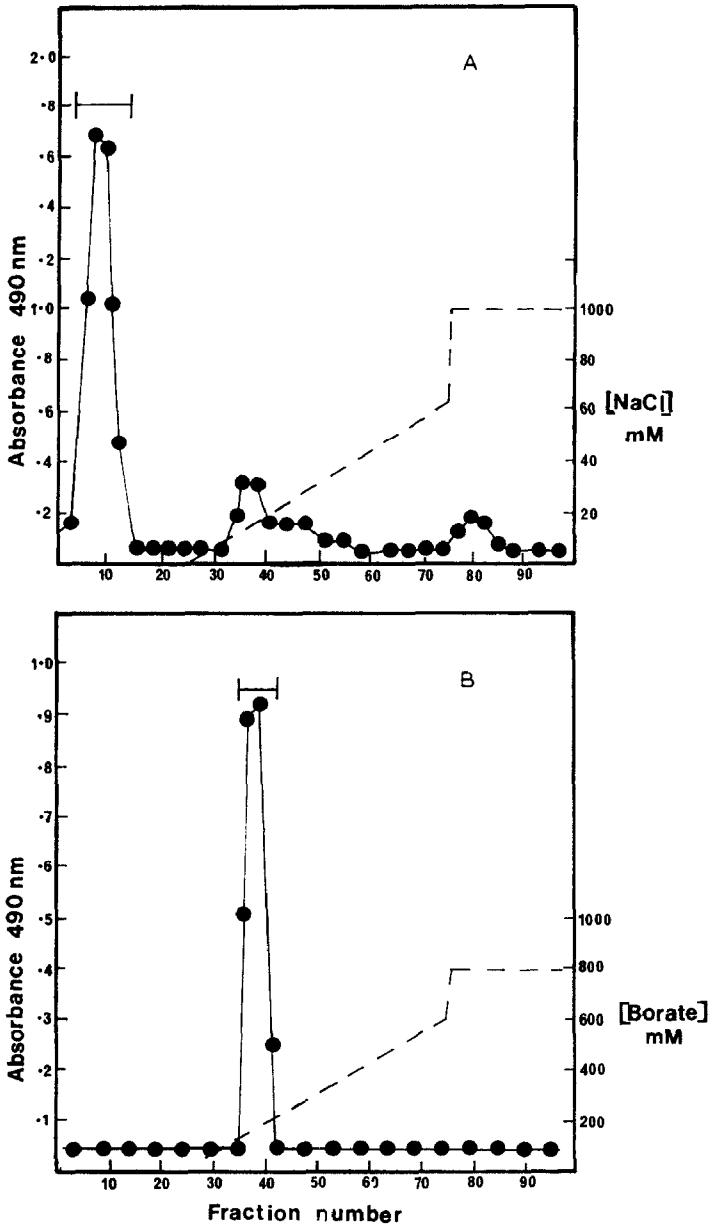

Fig. 1. Fractionation of the 4M KOH-soluble fraction from runner-bean cell-walls: A, on DEAE-Sephadex; B, alkali eluate from cellulose on DEAE-Sephacel (borate form); —●—, total carbohydrate; ---, solvent gradient. For details, see text.

TABLE III

PARTIALLY METHYLATED ALDITOL ACETATES OBTAINED FROM RUNNER-BEAN XYLOGLUCAN BEFORE AND AFTER PARTIAL HYDROLYSIS WITH ACID

Partially methylated alditol acetate[a]	*Native xyloglucan*	*Partially hydrolysed xyloglucan*	*Deduced linkage*
2,3,4-Me_3-fucitol[b]	6.4	0.5	T-Fuc*p*-(1→
2,3,5-Me_3-arabinitol	3.0	0.5	T-Ara*f*-(1→
2,3,4-Me_3-xylitol	18.0	23.5	T-Xyl*p*-(1→
3,4-Me_2-xylitol	12.2	11.3	→2)-Xyl*p*-(1→
2,3,4,6-Me_4-galactitol	5.2	13.1	T-Gal*p*-(1→
3,4,6-Me_3-galactitol	6.2	0.6	→2)-Gal*p*-(1→
2,3,6-Me_3-glucitol	13.3	14.6	→4)-Glc*p*-(1→
2,3-Me_2-glucitol	35.7	35.9	→4,6)-Glc*p*-(1→

[a]Expressed as area percentages of the total methylated alditol derivatives. [b]Connotes 2,3,4-tri-*O*-methylfucitol, *etc.*

Partial, acid hydrolysis. — Analysis after partial, acid hydrolysis of the purified xyloglucan indicated that only arabinose and fucose residues had been hydrolysed (Table II, column 4), suggesting that the arabinose existed in the furanoid form. Arabinose and fucose were the only sugars detected in the acid hydrolysate, indicating that each monosaccharide occurred as a terminal residue.

Methylation analysis. — The purified and partially hydrolysed xyloglucans were subjected to methylation analysis, and the results are shown in Table III. The overall recoveries of methylated alditol acetates were in good agreement with sugar values obtained by direct analysis.

The results suggested that the xyloglucan contained a (1→4)-glucan backbone, ~75% of the units of which were substituted at position 6. The high proportion (~35%) of terminal sugars indicated that side-chain oligosaccharides had a low d.p. which is consistent with the structure of xyloglucans from other plant sources[13]. Fucose, arabinose, xylose, and galactose in the ratios 1.0:0.5:3.0:1.0 occurred as terminal residues. The arabinose was furanoid and the other sugars were pyranoid. The total amount of terminal residues agreed with the amount of branch points, *i.e.*, (1→4,1→6)-linked glucose. Evidence that the terminal sugars were not all linked directly to the glucan backbone was obtained from the occurrence of interchain sugar derivatives corresponding to (1→2)-linked xylose and galactose residues. This finding is consistent with the structure of xyloglucans from other plant sources[13].

Methylation analysis of the partially hydrolysed xyloglucan revealed that the terminal fucose, terminal arabinose, and (1→2)-linked galactose residues had been substantially decreased with a lesser decrease of the (1→2)-linked xylose residues. There was a concomitant increase in the relative amounts of terminal xylose and galactose residues (Table III, column 2). These data were consistent with structures

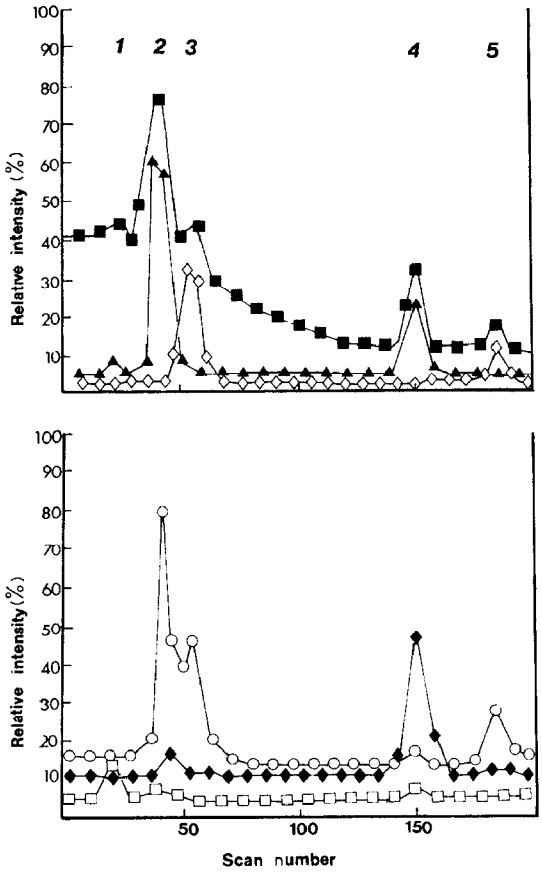

Fig. 2. G.l.c. of methylated oligosaccharide alditols with s.i.m. profile; using a column (45 × 0.44 cm) containing 1% OV-1 held at 150° for 5 min and then 2°/min to 320°; —■—, flame-ionisation detector response; —▲—, *m/z* 192; —◇—, *m/z* 236; —○—, *m/z* 219; —◆—, *m/z* 189; and —□—, *m/z* 175. See text and Table IV for details of the relative abundance and origins of the ions.

in which the acid-labile, terminal fucosyl and arabinosyl groups were attached to O-2 of xylose and/or galactose. The relative amounts of (1→4)- and (1→4,1→6)-linked glucose residues were not appreciably affected. However, the possibility that some fucose and/or arabinose residues were directly linked to the glucan backbone cannot be discounted.

Acetolysis. — Acetolysis, which preferentially cleaves (1→6) linkages[40,31], has been used for structural studies of yeast mannans[42,43] and rape-seed xyloglucan[7]. The purified xyloglucan, when subjected to limited acetolysis, gave oligosaccharide derivatives that were deacetylated, reduced with NaB^2H_4, and identified as the methylated oligosaccharide alditols by g.l.c.–m.s.

Five major peaks (1–5, 1.0:13.0:4.0:6.0:1.3 ratio) were detected by g.l.c. and selective ion-monitoring (s.i.m.) across the area of the peaks (Fig. 2). Although g.l.c. showed considerable overlap between peaks 2 and 3, s.i.m. gave satisfactory

peak resolution. The trailing of peak 2 was caused by co-chromatography of contaminating material (hydrocarbon), but this did not interfere with the interpretation of the mass-spectral data.

S.i.m. permitted the identification of non-reducing termini (pentose, *m/z* 175; deoxyhexose, *m/z* 189; and hexose, *m/z* 219) and the alditol moiety (pentitol, *m/z* 192; and hexitol, *m/z* 236), and is shown in Fig. 2.

The oligosaccharide derivatives were characterised by (*a*) retention time (*R*, relative to that of methylated cellobi-itol), (*b*) the diagnostic ions of the mass spectra, (*c*) the molecular weight of the derivatives deduced from c.i.–m.s. using the ions $(M + NH_4)^+$ and $(M + 1)^+$, and (*d*) methylation analysis. Sequence information was obtained by using established principles[44–47] as applied to methylated oligosaccharides from plant cell-wall polysaccharides[10,33]. The nomenclature for the degradation of methylated oligosaccharide alditols and the symbols employed correspond to those of Kochetkov and Chizhov[48].

Peak *1* (*R* 0.53) was eluted in the methylated disaccharide alditol region. C.i.-m.s. gave ions at *m/z* 401 and 384, corresponding to $(M + NH_4)^+$ and $(M + 1)^+$, respectively, and a parent disaccharide derivative containing two pentose units. E.i.-m.s. gave ions at *m/z* 175 and 192, indicating a pentosylpentitol derivative. Unlike the peaks *2–5*, peak *1* gave a weak mass spectrum; thus, the nature of the glycosidic linkage could not be determined unambiguously. However, it is probable that the component was Ara*f*-(1→2)-xylitol, as can be inferred from methylation analysis of the undegraded and partially hydrolysed xyloglucan (Table III). Xyloglucans carrying the substituent Ara*f*-(1→2)-Xyl*p* on the glucan backbone have been isolated from *Nicotiana tabacum*[39] and potato[10].

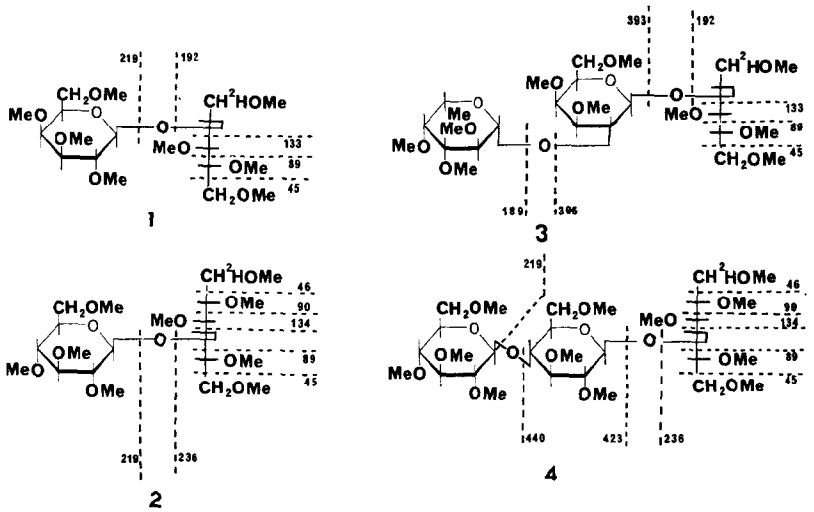

Fig. 3. Mass-spectral fragmentation patterns of the methylated oligosaccharide alditols; structures **1–4** correspond to components from peaks *2–5* of Fig. 2. See text and Table IV for details of the relative abundance of the ions.

TABLE IV

DIAGNOSTIC IONS OBTAINED FROM G.L.C.–M.S. OF PERMETHYLATED OLIGOSACCHARIDE ALDITOLS DERIVED FROM AN ACETOLYSATE OF RUNNER-BEAN XYOLOGLUCAN[a]

Ion (m/z)	*Relative abundance*				*Symbol*[b]
	Peak 2	*Peak 3*	*Peak 4*	*Peak 5*	
45	76.2	50.5	82.6	76.4	
46	14.9	10.5	16.5	16.1	
71	38.9	52.5	40.7	17.4	
75	30.2	43.1	38.4	36.4	
88	100.0	100.0	100.0	100.0	
89	25.8	36.1	37.3	33.4	
90	3.2	9.5	3.7	8.3	
101	77.9	84.6	68.6	66.0	
111	26.0	51.2	5.1	22.5	
133	7.1	3.8	5.4	—	
134	1.5	7.0	0.9	2.7	
155	4.2	15.1	2.6	12.7	
175	0.7	0.4	1.0	—	
177	—	1.2	—	—	
178	0.3	0.7	0.1	—	
187	15.4	58.0	4.1	33.6	aA_2
189	0.8	3.0	20.8	2.2	aA_1
192	56.4	5.7	81.8	—	Pentitol
219	2.9	9.0	0.8	4.6	aA_1
221	—	0.6	—	0.7	
222	—	0.6	—	0.6	
236	0.9	30.2	—	21.9	Hexitol
252	1.3	—	1.7	—	abJ_1*; bcJ_1†
296	0.1	3.0	—	1.0	abJ_1‡; bcJ_1§
329	—	0.5	0.5	—	baA_3
349	—	1.2	—	—	
361	—	—	1.6	—	baA_2
363	—	—	0.1	—	
393	—	—	0.9	—	baA_1
396	—	—	0.5	0.3	
423	—	—	—	0.4	baA_1
440	—	—	—	0.7	bcA_1
456	—	—	—	1.2	$abcJ_1$ −44

[a]See Fig. 3 and text for further details. [b]Symbols correspond to those used by Kochetkov and Chizhev[48]; superscripts *, †, ‡, and § correspond to peaks *2*, *4*, *3*, and *5*, respectively.

Peak *2* (*R* 0.83) was also eluted in the methylated disaccharide alditol region. C.i.-m.s. gave ions at *m/z* 445 and 428, corresponding to $(M + NH_4)^+$ and $(M + 1)^+$, and a parent disaccharide derivative containing one hexose and one pentose unit. E.i.-m.s. gave intense ions at *m/z* 192 and 219 consistent with a hexosylpentitol derivative, and this was confirmed by the ion *m/z* 252 − 192 + 60 (abJ_1) (Fig. 3, **1**). The nature of the linkage can be deduced from ions produced by the cleavage of carbon–carbon bonds of the methylated pentitol (Fig. 3, **1**). Characteristic fragments

are obtained[44] from (1→2), (1→3), and (1→4) linkages after incorporation of deuterium at C-1. The mass spectrum (Table IV) contained a relatively intense ion at *m/z* 133, which was diagnostic of a methylated pentitol substituted at position 2 and hence a (1→2) linkage. Methylation analysis showed that xylose was the only pentose linked through position 2 and galactose was the only non-reducing, terminal hexose. Thus, peak *2* most probably contained Gal*p*-(1→2)-Xyl*p*. This disaccharide has been isolated from rape-seed xyloglucan[7] and shown to contain a *β* linkage.

Peak *3* (*R* 1.00) was eluted in the methylated disaccharide-alditol region. C.i.-m.s. gave ions at *m/z* 489 $(M + NH_4)^+$ and 472 $(M + 1)^+$, indicating a parent disaccharide containing two hexose residues. E.i.-m.s. gave ions at *m/z* 219 and 236 consistent with a hexosylhexitol derivative and this was confirmed by the ion at *m/z* 296 − 236 + 60 (abJ_1) (Fig. 3, **2**). A relatively intense ion at *m/z* 134 indicated a (1→4) linkage (Table IV). The ion at *m/z* 133 probably arose from the component in peak *2*. Methylation analysis showed that glucose was the only (1→4)-linked hexose. Therefore, the disaccharide in peak *3* was inferred to be derived from cellobiitol. Cellobiose has been characterised in acetolysates of rape-seed[7] and mung-bean[49] xyloglucan.

Peak *4* (*R* 2.31) was eluted in the methylated trisaccharide-alditol region. C.i.-m.s. gave ions at *m/z* 619 $(M + NH_4)^+$ and 602 $(M + 1)^+$, corresponding to a trisaccharide derivative containing deoxyhexose, hexose, and pentose residues. E.i.-m.s. gave intense ions at *m/z* 189 and 192 (Table IV), showing the presence of terminal, non-reducing deoxyhexose and pentitol residues. Ions at *m/z* 252 − 192 + 60 (bcJ_1) and 456 − 396 + 60 ($abcJ_1$) supported the trisaccharide structure, and ions at *m/z* 393 (baA_1) and 396 (cbA_1) derived from deoxyhexose-hexose and hexose-pentitol moieties, respectively, showed that the internal sugar was hexose (Fig. 3, **3**). The ions at *m/z* 133 or 134, of which the former was considerably more intense, suggested that the pentitol was linked through position 2.

That the linkage between the deoxyhexose and hexose residues was (1→2) was indicated (*a*) by methylation analysis of the undegraded and partially hydrolysed xyloglucan (Table III), which showed that removal of the terminal fucose resulted in a decrease in (1→2)-linked galactose and a corresponding increase in terminal galactose, and (*b*) from the relative abundance of the baA series of ions. This series (*m/z* 393, 361, and 329) for the trisaccharide derivative (Table IV) showed that $baA_2 > baA_1 > baA_3$, suggesting a (1→2) linkage[10,33,45]. Thus, the parent trisaccharide was Fuc*p*-(1→2)-Gal*p*-(1→2)-Xyl*p*. A similar trisaccharide has been isolated from mung-bean xyloglucan[49,50].

Peak *5* (*R* 2.85) was eluted in the methylated trisaccharide-alditol region. C.i.-m.s. gave ions at *m/z* 693 $(M + NH_4)^+$ and 676 $(M + 1)^+$, suggesting a trisaccharide derivative containing three hexose residues. E.i.-m.s. gave ions at *m/z* 219 and 236, showing the presence of terminal, non-reducing hexose and hexitol residues. Ions at *m/z* 296 − 236 + 60 (bcJ_1) and 456 ($abcJ_1$ − 44) confirmed the trisaccharide nature. The occurrence of ions at *m/z* 423 (baA_1) and 440 (bcA_1) indicated hexose-hexose and hexose-hexitol linkages, respectively (Fig. 3, **4**). The occurrence of an

ion at *m/z* 134 (Table IV) suggested that the hexitol was linked through position 4, but mass spectrometry cannot distinguish between (1→4)- and (1→6)-linked hexose-hexose units[45]; since acetolysis preferentially cleaves (1→6) linkages, the trisaccharide alditol derivative is probably derived from Glc*p*-(1→4)-Glc*p*-(1→4)-Glc*p*, which could have arisen from the glucan backbone.

A minor component that was eluted in the methylated tetrasaccharide-alditol region gave a very weak mass spectrum. The occurrence of ions at *m/z* 187 (219 − 32) and 236 suggests the presence of terminal, non-reducing hexose and hexitol residues.

The oligosaccharides in peaks *1–5* were major components (>95%) of the respective peaks. However, certain ions in the mass spectra (notably in peaks *2* and *3*) suggested the presence of other disaccharides. The presence of weak ions at *m/z* 175 and 189 indicated derivatives having terminal, non-reducing pentose and deoxyhexose residues. Therefore, the presence of small quantities of pentosyl-hexitol or deoxyhexosyl-hexitol derivatives cannot be discounted. Aspinall *et al.*[7] have isolated Xyl*p*-(1→6)-Glc*p* from acetolysates of rape-seed hull xyloglucan.

Treatments with cellulase and alpha-amylase. — When the purified xyloglucan was treated for 48 h with cellulase or alpha-amylase, only the former released oligosaccharides tentatively identified as di-, tri-, and tetra-saccharides by comparison of R_F values with that of cellobiose. This evidence combined with data from methylation analysis shows that the xyloglucan contained a (1→4)-linked β-D-glucan backbone, as has been demonstrated for other xyloglucans[13].

Studies with glycosidases. — Treatment of the purified xyloglucan with α-L-fucosidase released only fucose residues (~85%). This finding, in combination with methylation analysis, showed fucose to be present solely as the terminal, α-linked residue.

Purified or partially hydrolysed xyloglucans were resistant to α- and β-D-galactosidases, which may be due to steric hindrance caused by the oligosaccharide side-chains wrapping around the glucan backbone[6]. Also, the enzymes may not have been specific for the linkages present. Studies with xyloglucans from other plant sources suggest galactose to exist as the β anomer[3,7].

GENERAL DISCUSSION

By using ion-exchange and cellulose column chromatography, a xyloglucan has been isolated from the cell wall of *Phaseolus coccineus* and purified. The polymer was homogeneous in moving-boundary electrophoresis and ultracentrifugation, and had a molecular weight of 110,000. The molecular weights of xyloglucans vary considerably: *Annona muricata*[4], 10,000; *Simmondsia chinensis*[5], 174,000; and *Glycine max*[12], 60,000 and 180,000. However, these values are probably dependent on the plant source and the method of isolation.

Methylation analysis of the intact and partially hydrolysed xyloglucan, limited acetolysis, and degradation studies with cellulase revealed the xyloglucan to be based on a (1→4)-linked D-glucan backbone with a high proportion of the glucosyl residues

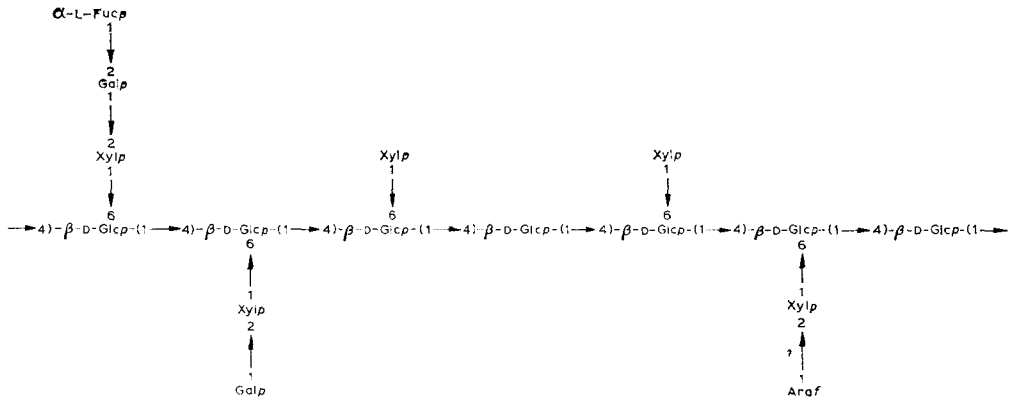

Fig. 4. Tentative structure for a portion of the xyloglucan from runner-bean cell-walls; the order of the side-chain substituents is arbitrary.

substituted at position 6. The combined data are consistent with the structure shown in Fig. 4. Whereas the terminal fucose could be removed with α-L-fucosidase, the terminal galactosyl groups were resistant to α- and β-D-galactosidase.

The function of the side-chains is not clear, but may involve regulation of cell wall–xyloglucan interactions[6]. The degree of side-chain substitution may regulate xyloglucan–xyloglucan interactions. In xyloglucans isolated from *A. muricata*, the ratio of (1→4)- and (1→4,1→6)-linked glucose residues was[4] 1.0:0.3 and it was soluble only in 0.1M alkali. In the xyloglucan from *P. coccineus* and other plants[13], the ratio of (1→4)- and (1→4,1→6)-linked glucose residues was 1.0:3.0, and these polymers were water-soluble.

Sequential extraction and fractionation studies showed that the xyloglucan was probably hydrogen-bonded to cellulose *in vivo*, but the bulk of it is not covalently linked to pectic polysaccharides. The latter finding accords with the work on potato cell-walls[10], but contrasts with the work on cultured sycamore cells[6] where it was shown that an appreciable proportion of the xyloglucan polymers may have been covalently linked to rhamnogalacturonan backbones *via* (arabino) galactan chains. This concept was used in the formulation of a model for the primary cell-wall complex[6,51]. This view and variations thereof[52,53] have become widely accepted and cited in text books[54,55]. However, a recent review[13] stated that, in later experiments, large amounts of xyloglucan covalently linked with pectic polysaccharides could not be isolated. Our data and that from potato cell-walls[10] have clarified this point in connection with the proposed model for primary cell-walls. However, it is possible that some xyloglucan polymers may be linked to pectic polysaccharides by alkali-labile (phenolic or glycosidic) cross-links.

The xyloglucan from *P. coccineus* closely resembles those isolated from a variety of plants. This conservation of structure suggests a fundamental role in cell-wall biochemistry. However, any non-structural role has yet to be demonstrated.

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