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STRUCTURAL ANALYSIS OF THE XYLOGLUCAN FROM *Phaseolus coccineus* CELL-WALLS USING CELLULASE-DERIVED OLIGOSACCHARIDES

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ABSTRACT

Oligosaccharides, obtained by digestion of a xyloglucan from the cell walls of *Phaseolus coccineus* with cellulase, have been isolated by gel filtration. Oligosaccharides containing 2–6 residues accounted for ~57% of the hydrolysate, with larger oligosaccharides (d.p. ~10) and partially degraded xyloglucan accounting for ~32% of the polymer. The major glycosidic linkages were determined by methylation analysis. Methylated penta- and hexa-saccharide alditols were isolated by reverse-phase h.p.l.c. and characterised by e.i.-m.s. and f.a.b.-m.s. Methylated derivatives of the di-, tri-, and tetra-saccharide alditols were examined by g.l.c.–m.s. in the e.i. and c.i. modes. A structure based on these results is proposed.

INTRODUCTION

The isolation and partial characterisation of a xyloglucan from the cell walls of *Phaseolus coccineus* has been reported[1]. The polymer contained a (1→4)-linked β-D-glucan backbone substituted through positions 6 with D-Xyl*p*, D-Gal*p*-(1→2)-D-Xyl*p*, and α-L-Fuc*p*-(1→2)-D-Gal*p*-(1→2)-D-Xyl*p*. Using these data, a tentative structure was proposed[1].

We now report the relative amounts and structures of a range of oligosaccharides isolated from a digest of the partially purified xyloglucan with *Trichoderma viride* cellulase. The results have confirmed the earlier work[1] and allowed a more detailed structure of the xyloglucan to be proposed.

EXPERIMENTAL

Plant tissue. — Cell-wall material from the parenchyma of *P. coccineus* was prepared as previously described[2].

Monosaccharide analysis. — Neutral sugars, released by hydrolysis with M H_2SO_4 (100°, 2.5 h), were determined[3] by g.l.c. of their alditol acetates.

Isolation of the xyloglucan. — Depectinated cell-walls were extracted (1 g/100

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mL) with M and 4M KOH, and the xyloglucan was isolated from the latter extract, after neutralisation and dialysis, by chromatography on DEAE-Sephadex[1].

Degradation with cellulase. — A solution of the partially purified xyloglucan (420 mg) in 50mM sodium acetate buffer (pH 5.2, 40 mL) was incubated for 48 h at 37° in the presence of toluene with a cellulase preparation[4] (2 mL, 2 × 10^5 units) from *T. viride*, which had been partially purified by ammonium sulphate precipitation. One unit of enzyme liberated 1 μg of soluble carbohydrate, as glucose, from ball-milled filter paper per h at 37° and pH 5.2.

Gel filtration of the oligosaccharides. — The cellulase-degraded material was desalted by passage through a column (15 × 1 cm) of Dowex AG50W-X8 (H^+) resin. The eluate was concentrated to ~5 mL and filtered, and the clear supernatant was eluted from a column (150 × 1 cm) of Bio-Gel P2 (−400 mesh) at 55° with water. Fractions (1 mL) were collected and portions (25 μL) were assayed for total carbohydrate with phenol–sulphuric acid[5]. Appropriate fractions were combined, reduced in volume to ~1 mL, and re-chromatographed as described above except that 0.5-mL fractions were collected.

Material eluted in the void volume of the Bio-Gel P2 column was refractionated on a column (90 × 1 cm) of Sephadex G-25 (fine) by elution with water. Fractions (1 mL) were collected and portions (50 μL) were assayed for total carbohydrate[5]. Appropriate fractions were combined and freeze-dried.

Methylation analysis. — The isolated oligosaccharides (1.0–2.0 mg) were reduced with NaB^2H_4 and desalted[1]. The oligosaccharide-alditols were methylated, extracted with dichloromethane[6], and analysed[1] by g.l.c. Partially methylated alditol acetates were prepared and analysed[2] by g.l.c.–m.s.

Reverse-phase h.p.l.c. — The penta- and hexa-saccharide alditols were methylated, and each reaction mixture was purged with argon to remove excess methyl iodide and diluted with water to give aqueous ~20% methyl sulphoxide. The methylated derivatives were isolated by using Sep-Pak C_{18} cartridges (Waters Associates) essentially as described by Waeghe *et al.*[7] except that 10 mL of aqueous 60% acetonitrile (HPLC grade) was used to elute the derivatives. Each eluate was concentrated to dryness, and a solution of the residue in aqueous 60% acetonitrile (250 μL) was filtered through a 0.45-μm fluoropore membrane using an MF-1 centrifugal microfilter (Alltech Associates). H.p.l.c. was performed with a Perkin–Elmer Series 2 chromatograph, using a Zorbax ODS column (25 cm × 4.5 mm) and a Brownlee C_{18} guard column. Samples (50 μL) were introduced through a Rheodyne 7125 injector (100-μL loop) and eluted[8] with aqueous 60% acetonitrile (pre-filtered through 0.45-μm fluoropore filters) at 0.5 mL/min. The eluate was monitored with a Waters 401 differential refractometer and fractions were collected manually. Combined fractions were concentrated to dryness and a solution of each residue in acetone (50 μL) was used for direct-insertion m.s.

Mass spectrometry. — Methylated oligosaccharide-alditols were examined by direct-insertion[9] m.s. or g.l.c.–m.s. using e.i. and c.i. modes (NH_3 as the reagent gas). F.a.b.-m.s. was performed on an AEI MS9 mass spectrometer with xenon as the primary bombarding-gas beam[10].

Polarimetry. — Optical rotations were determined with a Perkin–Elmer 141 polarimeter and a 10-cm cell at 20°.

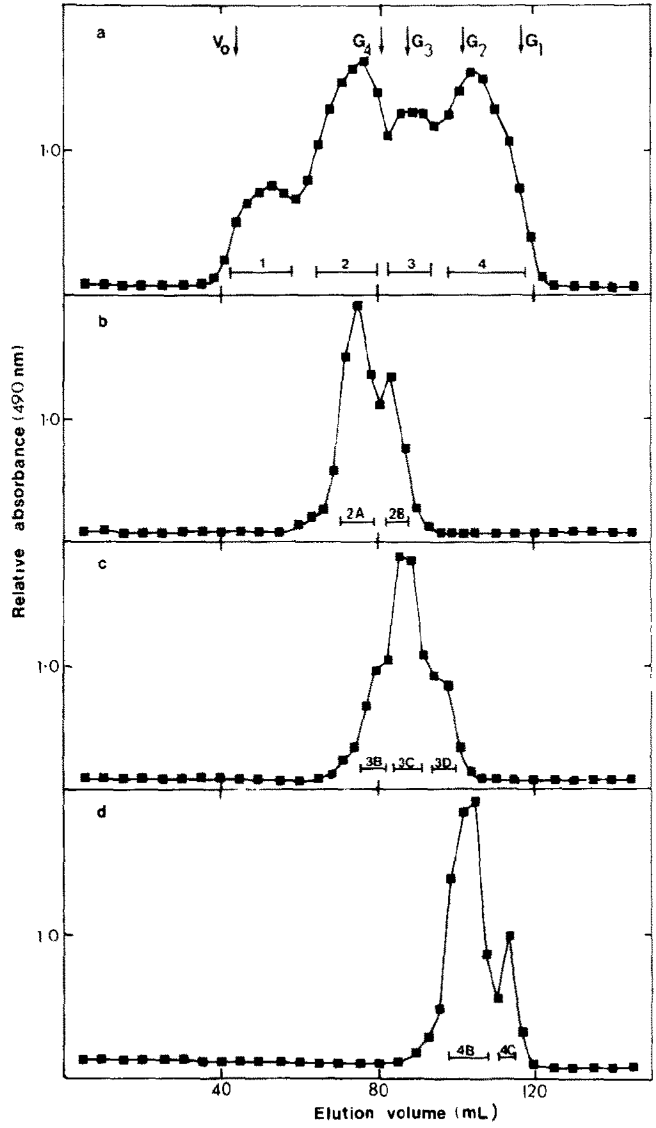


Fig. 1. Fractionation of the oligosaccharides derived from the cellulase digest of *P. coccineus* xyloglucan on Bio-Gel P2: (a) fractionation of the total digest; (b)–(d) refractionation of fractions 2–4, respectively. In (c) and (d), 3A and 4A (not shown) would correspond to the leading edge of the respective peaks. Void volume (V_0) was determined with Blue Dextran; G_1–G_4: D-glucose, cellobiose, raffinose, and stachyose, respectively.

RESULTS

Fractionation of the oligosaccharides. — Gel filtration of the cellulase-degraded xyloglucan on Bio-Gel P2 gave four major fractions (Fig. 1a) of which three (2–4) could be further resolved by re-chromatography on the same column (Fig. 1b–d). Re-chromatography of fraction 2A on Sephadex G-25 gave a single symmetrical peak with d.p. ~10. Fraction 1, when chromatographed on Sephadex G-25, gave three components which probably corresponded to cellulase and undegraded xyloglucan (1A and 1B) and partially degraded xyloglucan (1C).

The isolated oligosaccharides were analysed for neutral sugars (Table I); each, except 4C, was rich in glucose and xylose with various amounts of galactose, fucose, and arabinose. Fraction 4C was composed mainly of monosaccharides.

Methylation analysis. — Of the oligosaccharide fractions obtained by gel filtration, six (4B, 3D, 3C, 3B, 2B, and 2A) were reduced with NaB^2H_4 and subjected to methylation analysis (Table II). Each oligosaccharide gave a glucitol derivative, derived from the reducing terminus, a large proportion of which had been linked through positions 4 or 6. However, significant amounts of 4,6-disubstituted reducing end-groups were detected in some of the fractions. Various amounts of derivatives from Fuc*p*, Ara*f*, Xyl*p*, (1→2)-Gal*p*, (1→6)-, (1→4)-, and (1→4,6)-Glc*p* accounted for the bulk of the remaining components (Table II).

Oligosaccharide sequencing. — The principles of sequencing oligosaccharides have been described[11,12] and applied to plant cell-wall polysaccharides[1,13,14]. The nomenclature used is that of Kotchetkov and Chizhov[15] except that the alditol fragment will be referred to as "*ald*"[16] to distinguish it from the terminal non-reducing sugar residue. The ion containing the alditol moiety (*ald*J_2) arises[8] by loss of 60 mass units from *ald*J_1.

The methylated di-, tri-, and tetra-saccharide alditols could be separated and characterised by g.l.c.–m.s. The penta- and hexa-saccharide derivatives could be

TABLE I

NEUTRAL-SUGAR COMPOSITION OF CELLULASE-DERIVED XYLOGLUCAN OLIGOSACCHARIDES

Main fraction obtained by gel filtration	*Monosaccharide (molar ratio relative to Glc)*					*Percent of xyloglucan*
	Glc	*Xyl*	*Gal*	*Fuc*	*Ara*	
1C	1.00	0.70	0.44	0.25	0.45	1.1
2A	1.00	0.75	0.49	0.26	0.16	22.0
2B	1.00	0.57	0.36	0.27	0.24	8.8
3B	1.00	0.41	0.36	0.13	0.11	4.9
3C	1.00	0.62	0.29	0.15	0.23	8.8
3D	1.00	0.75	0.12	0.06	0.20	5.3
4B	1.00	0.78	0	0.06	0.10	29.3
4C	1.00	0.12	0.03	0.13	0.09	5.9

TABLE II

METHYLATED ALDITOL ACETATES OBTAINED FROM CELLULASE-DERIVED XYLOGLUCAN OLIGOSACCHARIDES

Methylated alditol acetate[a]	*Oligosaccharide fraction*						*Deduced linkage*
	4B	*3D*	*3C*	*3B*	*2B*	*2A*	
2,3,4-Me_3-Fuc[b]	—	0.7	4.5	9.5	9.5	8.7	Fuc*p*
2,3,5-Me_3Ara	—	7.8	4.9	2.8	3.3	4.8	Ara*f*
3,5-Me_2-Ara	—	—	0.9	—	—	—	(1→2)-Ara*f*
2,5-Me_2-Ara	—	—	1.4	—	—	—	(1→3)-Ara*f*
2,3,4-Me_3-Xyl	37.0	23.8	8.5	6.2	6.9	4.2	Xyl*p*
3,4-Me_2-Xyl	—	11.5	14.1	12.0	15.9	18.9	(1→2)-Xyl*p*
2,3-Me_2-Xyl	—	3.3	3.2	—	—	—	(1→3)-Xyl*p*
2,3,4,6-Me_4-Gal	—	2.2	7.5	3.0	4.0	8.5	Gal*p*
3,4,6-Me_3-Gal	—	1.1	3.1	12.5	11.7	8.6	(1→2)-Gal*p*
1,2,3,4,5-Me_5-Glc	63.0	17.2	4.3	—	—	—	→6)-Glucitol
1,2,3,5,6-Me_5-Glc	—	5.9	12.4	16.5	6.5	3.8	→4)-Glucitol
1,2,3,5-Me_4-Glc	—	8.0	4.3	2.8	9.4	4.5	→4,6)-Glucitol
2,3,4,6-Me_4-Glc	—	8.0	4.3	2.7	—	1.8	Glc*p*
2,3,4-Me_3-Glc	—	10.3	18.3	18.9	17.8	8.7	(1→6)-Glc*p*
2,3,6-Me_3-Glc	—	—	6.1	8.1	6.9	8.7	(1→4)-Glc*p*
2,3-Me_2-Glc	—	—	2.2	6.0	8.1	18.9	(1→4,6)-Glc*p*

[a] Expressed as peak area %, as determined by g.l.c. of the methylated alditol acetates. [b] 2,3,4-Me_3-Fuc = 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylfucitol, *etc.*

resolved only by reverse-phase h.p.l.c., and the structures deduced from direct-insertion m.s. data in conjunction with the results of methylation analysis.

H.p.l.c. of fraction 2B. — Reverse-phase h.p.l.c. of reduced and methylated 2B gave 2 major and 4 minor components (Fig. 2). The pentasaccharide derivatives accounted for ~65% of the material applied to the column. A similar elution profile was obtained for fraction 3B except that the pentasaccharide derivatives accounted for ~80% of the material.

F.a.b.-m.s. of the compound in peak 1 (Fig. 2) gave an ion at *m/z* 1010, corresponding to $(M + 1)^+$ from a methylated oligosaccharide-alditol containing one deoxyhexosyl, one pentosyl, two hexosyl, and one hexitol residue. E.i.-m.s. gave ions of the aA [*m/z* 189 (33.7%), 157 (25.7%), 125 (7.0%)], baA [393 (2.4%), 361 (10.6%), 329 (0.8%)], cbaA [553 (0.1%), 521 (2.6%), 489 (0.6%)], and dcbaA [757 (0.1%)] series (see **1**). The ions at *m/z* 236 (*ald*J_2, 26.6%), 440 (d*ald*J_2, 0.2%), 600 (cd*ald*J_2, 0.1%), 804 (bcd*ald*J_2, 0.1%), and the corresponding ions of the J series, 296 (d*ald*J_1, 1.3%), 500 (cd*ald*J_1, 0.2%), 660 (bcd*ald*J_1, 0.9%), and 864 (abcd*ald*J_1, 0.1%) were consistent with the empirical structure 6-deoxyhexosyl-(1→?)-hexosyl-(1→?)-pentosyl-(1→?)-hexosyl-(1→?)-hexitol for the parent oligosaccharide-alditol (**1**). The relative abundance of the ions at *m/z* 134 and 133 (4.2:1.0) showed that the hexitol residue was substituted through position 4. The ratios of the baA and cbaA series of ions (1.0:4.4:0.3 and 1.0:26.0:6.0, respectively) and the data from methylation analysis (Table II) were consistent with the

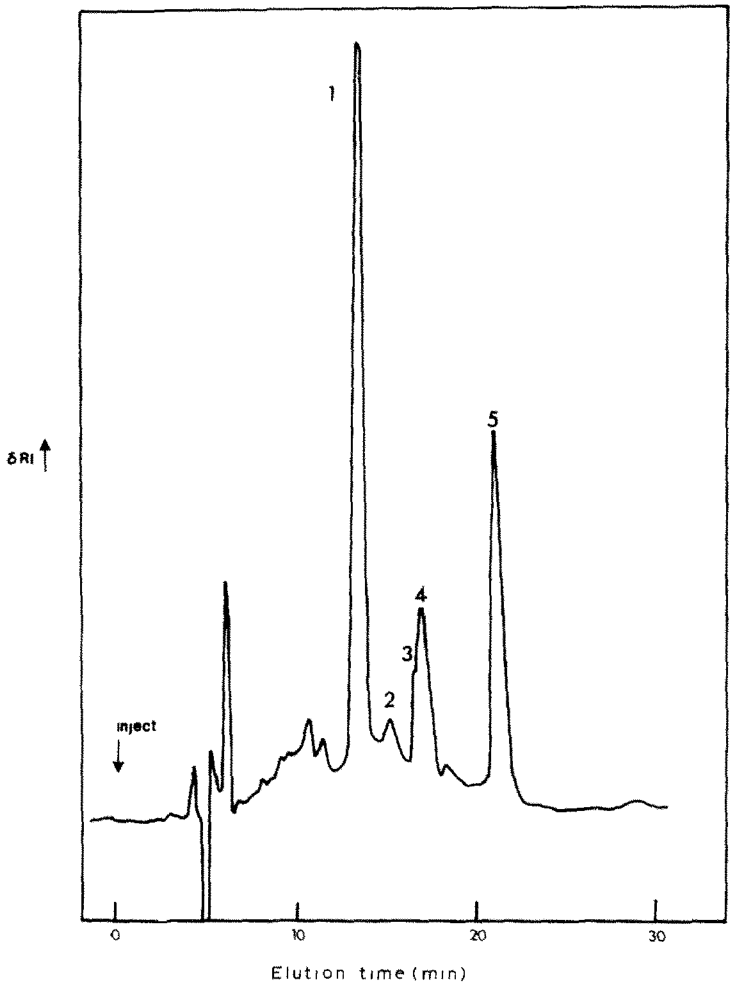
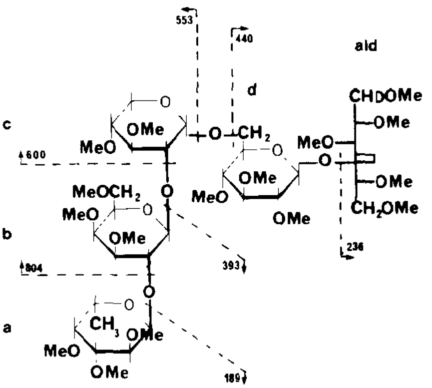


Fig. 2. Reverse-phase h.p.l.c of the methylated oligosaccharide-alditols derived from Bio-Gel P2 fraction 2B in Fig. 1.

sequence Fuc*p*-(1→2)-Gal*p*-(1→2)-Xyl*p*-(1→6)-Glc*p*-(1→4)-Glc*p*, for the parent oligosaccharide (see Fig. 4 inset II).

F.a.b.-m.s. of the compound in peak 5 gave an ion at *m/z* 1200, corresponding to (M + 1)$^+$ from a methylated oligosaccharide-alditol containing two pentosyl residues, three hexosyl residues, and one hexitol residue. Ions of the aA [*m/z* 219 (9.9%), 187 (66.6%), 155 (39.8%)], baA [379 (0.8%), 347 (12.3%), 315 (1.3%)], and cbaA [583 (0.1%)] series identified the sequence hexosyl-(1→?)-pentosyl-(1→?)-hexosyl-(1→?). Intense ions at *m/z* 236 (*ald*J$_2$, 29.3%) and 134 (10.3%) showed that the hexitol residue was substituted through position 4 (**2**). The origin of the ions at *m/z* 175 (eA$_1$, 14.0%), 296 (d*ald*J$_1$, 1.8%), 600 (de*ald*J$_2$, 0.3%), and 660 (cde*ald*J$_1$, 0.2%) can be inferred from **2**. The abundance ratios of



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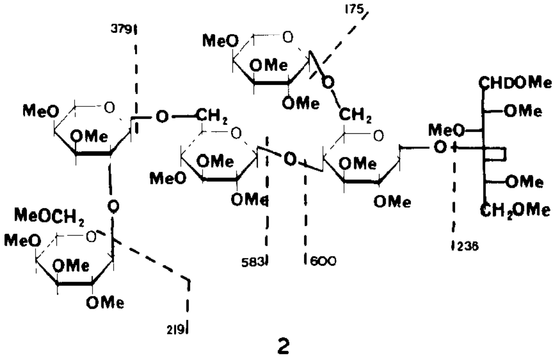

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the baA series of ions (baA$_2$>baA$_3$>baA$_1$) was consistent with the non-reducing, terminal hexosyl residue being linked to position 2 of the pentosyl moiety. The above data, in combination with the results of methylation analysis, suggested that the native oligosaccharide contained a cellotriosyl backbone substituted through position 6 of the terminal residue with Gal*p*-(1→2)-Xyl*p*-(1→) and through position 6 of the internal residue with a single Xyl*p* residue (see Fig. 4, inset IV).

F.a.b.-m.s. of the minor components (peaks 2–4, Fig. 2) obtained from h.p.l.c. gave (M + 1)$^+$ ions at *m/z* 996 (peak 2), 1214 and 1170 (peak 3), and 1156 (peak 4). The combined data provided evidence for the occurrence of methylated oligosaccharide-alditols derived from (*a*) cellobiose substituted with Gal*p*-(1→2)-Xyl*p* and Xyl*p* (peak 2), (*b*) cellotriose substituted with Fuc*p*-(1→2)-Gal*p*-(1→2)-Xyl*p* at the non-reducing terminus and cellobiose substituted with both Fuc*p*-(1→2)-Gal*p*-(1→2)-Xyl*p* and Xyl*p* (peak 3), and (*c*) cellotriose substituted with three Xyl*p* residues (peak 4).

Characterisation of di-, tri-, and tetra-saccharide alditols. — G.l.c. of the methylated fractions 3C, 3D, and 4B (Fig. 3a–c) showed them to contain mainly tetra-, tri-, and di-saccharide derivatives, respectively.

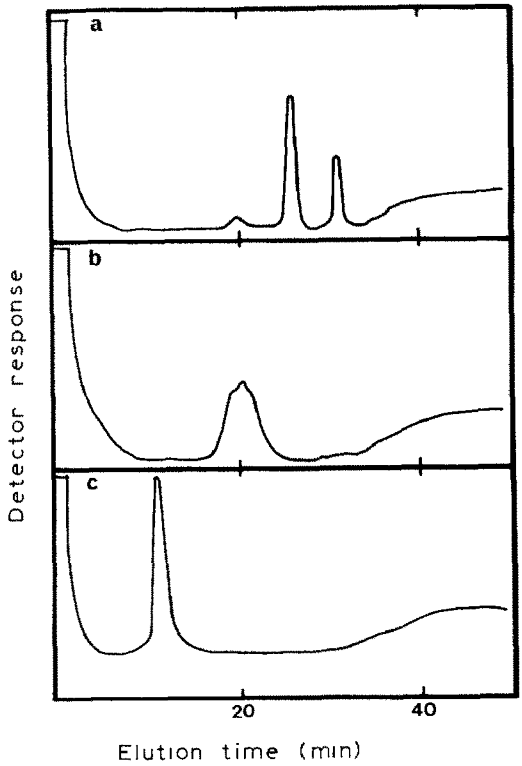


Fig 3. G.l c of the methylated oligosaccharide-alditols derived from Bio-Gel P2 fractions: a, 3C, b, 3D; and c, 4B on 1% OV-1. See text for details

G.l.c. resolved reduced and methylated 3C into 3 fractions (Fig. 3a) which were eluted in the region corresponding to tri- (5.6%), tetra- (75.8%), and pentasaccharide (18.6%) alditol derivatives. Only the tetrasaccharide-alditol fraction will be discussed.

G.l.c.–c.i.-m.s. of the compounds in the tetrasaccharide-alditol region gave significant amounts of ions at m/z 853 [$(M + 18)^+$, 69.2%], 836 [$(M + 1)^+$, 3.8%], 809 [$(M + 18)^+$, 22.0%], and 792 [$(M + 1)^+$, 6.1%] derived from derivatives containing one pentosyl, two hexosyl, and one hexitol residue; and two pentosyl, one hexosyl, and one hexitol residue, respectively. Selected ion monitoring with g.l.c.–e.i.-m.s. for the ions at m/z 175, 219, and 236 suggested the presence of five components of which three were present in significant amounts. Evidence for the structural features of these components is given below.

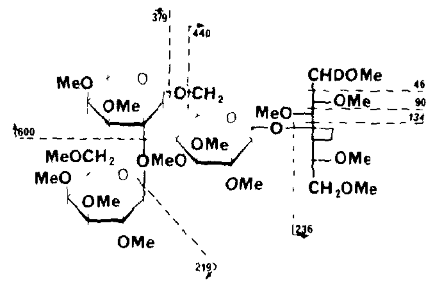
The first component eluted gave ions of the aA [m/z 175 (32.3%), 143 (55.3%), 111 (39.8%)] and baA [335 (1.3%), 303 (2.5%), 271 (0.5%)] series which identified the sequence pentosyl-(1→?)-pentosyl-(1→?). An intense ion at m/z 236 (*ald*J$_2$, 40.2%) demonstrated the presence of a substituted hexitol residue. Ions at m/z 296 (*ald*J$_1$, 3.8%), 440 (c*ald*J$_2$, 0.5%), 500 (bc*ald*J$_1$, 0.4%), 600 (bc*ald*J$_2$, 2.5%), and 660 (abc*ald*J$_1$, 0.8%), in conjunction with the aA and baA series of ions, were consistent with the empirical structure pentosyl-(1→?)-pentosyl-(1→?)-hexosyl-(1→?)-hexitol. The relative abundance of the baA series of ions (baA$_2$>baA$_1$>baA$_3$) provided evidence that the internal pentosyl residue was substituted at position 2. Previous work[1] and the methylation analysis data (Table II) are consistent with the structure Ara*f*-(1→2)-Xyl*p*-(1→6)-Glc*p*-(1→4)-Glc*p* for the parent oligosaccharide.

The major tetrasaccharide derivative was found to contain one pentosyl, two hexosyl, and one hexitol residue. G.l.c.-e.i.-m.s. gave ions of the aA [*m/z* 219 (11.2%), 187 (36.4%), 155 (9.7%)] series and an intense ion at *m/z* 236 (*ald*J$_2$, 51.6%) showing non-reducing terminal hexosyl and hexitol residues respectively. Ions of the baA series [*m/z* 379 (1.4%), 347 (33.4%), 315 (3.0%)] in conjunction with the ions at *m/z* 296 (c*ald*J$_1$, 4.9%), 440 (c*ald*J$_2$, 0.8%), 600 (bc*ald*J$_2$, 0.3%), and 660 (abc*ald*J$_1$, 4.2%) were consistent with the structure hexosyl-(1→?)-pentosyl-(1→?)-hexosyl-(1→?)-hexitol. The relative abundance of the baA series (baA$_2$>baA$_3$>baA$_1$) indicated that the internal pentosyl residue was substituted at position 2 (**3**). The above data, in conjunction with the results of methylation analysis, were consistent with the structure Gal*p*-(1→2)-Xyl*p*-(1→6)-Glc*p*-(1→4)-Glc*p* (see Fig. 4, inset I) which has been found in cellulase digests of xyloglucans from potato[14] and *Simmondsia*[17].

A component which was eluted at the trailing edge of the tetrasaccharide-alditol peak gave, in g.l.c.-e.i.-m.s., ions of the aA [*m/z* 175 (35.8%), 143 (62.2%), 111 (41.0%)] and baA [379 (6.2%), 347 (21.8%), 315 (2.6%)] series which identified the sequence pentosyl-(1→?)-hexosyl-(1→?). The presence of ions at *m/z* 396 (*ald*cJ$_2$, 3.3%), 456 (b*ald*cJ$_1$, 2.3%), 600 (b*ald*cJ$_2$, 0.4%), and 660 (ab*ald*cJ$_1$, 2.4%) suggested that the alditol moiety was substituted with pentosyl and pentosyl-(1→?)-hexosyl-(1→?) residues. These data in conjunction with those of methylation analysis (Table II) provided evidence for the presence of a tetrasaccharide corresponding to cellobiose substituted through both 6 positions with single Xyl*p* residues.

G.l.c. of the reduced and methylated fraction 3D gave a diffuse peak which was eluted in the region for methylated trisaccharide-alditols (Fig. 3b). G.l.c.-c.i.-m.s. gave ions at *m/z* 605 (21.6%) and 649 (12.8%), corresponding to $(M + 18)^+$ from derivatives containing two pentosyl and one hexitol, and one pentosyl, one hexosyl, and one hexitol residue, respectively. Selected ion monitoring for the ions at *m/z* 175, 219, and 236 indicated the presence of several overlapping trisaccharide-alditols, of which three were present in significant amounts.

The first component eluted gave ions of the aA [*m/z* 175 (49.0%) 143 (80.2%), 111 (21.8%)] and baA [335 (0.9%), 303 (3.3%), 271 (0.1%)] series which identified the sequence pentosyl-(1→?)-pentosyl-(1→?). Ions at *m/z* 236 (*ald*J$_2$, 23.4%), 296 (b*ald*J$_1$, 2.1%), 134 (4.8%), and 178 (1.6%) demonstrated a hexitol residue substituted through position 6 (**4**). The relative abundance of the baA series (baA$_2$>baA$_1$>baA$_3$) and the data from methylation analysis would be consistent



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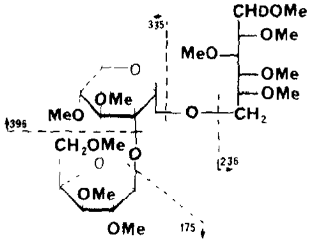

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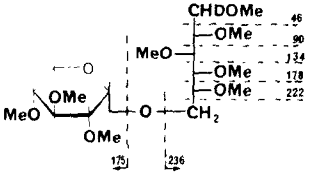

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with the structure Ara*f*-(1→2)-Xyl*p*-(1→6)-Glc*p* for the parent oligosaccharide (see Fig. 4, inset III).

Selected ion monitoring for the ion at *m/z* 649 (g.l.c.–c.i.-m.s.) showed it to be partially resolved into two major components. G.l.c.–e.i.-m.s. showed both derivatives to contain non-reducing pentosyl, hexosyl, and hexitol residues. At least two trisaccharides, Xyl*p*-(1→6)-Glc*p*-(1→4)-Glc*p* and Gal*p*-(1→2)-Xyl*p*-(1→6)-Glc*p*, are probably present. Furthermore, the presence of an ion at *m/z* 396 (6.7%) in the second of the two components suggests that the trisaccharide Glc*p*-(1→4)-Glc*p*-(6←1)-Xyl*p* may also be present.

Examination of reduced and methylated fraction 3D by direct-insertion c.i.-m.s. revealed an ion at *m/z* 619 (1.8%) corresponding to (M + 18)$^+$ for a trisaccharide alditol containing deoxyhexosyl, pentosyl, and hexitol residues. G.l.c.–e.i.-m.s. gave weak ions of the aA series [*m/z* 189 (4.5%), 157 (5.0%), 125 (0.8)] demonstrating non-reducing, terminal 6-deoxyhexose. Although insufficient data were available to define the remaining sequence, two structures are possible, namely, Fuc*p*-(1→2)-Gal*p*-(1→2)-Xyl*p*, which could be derived from cleavage of

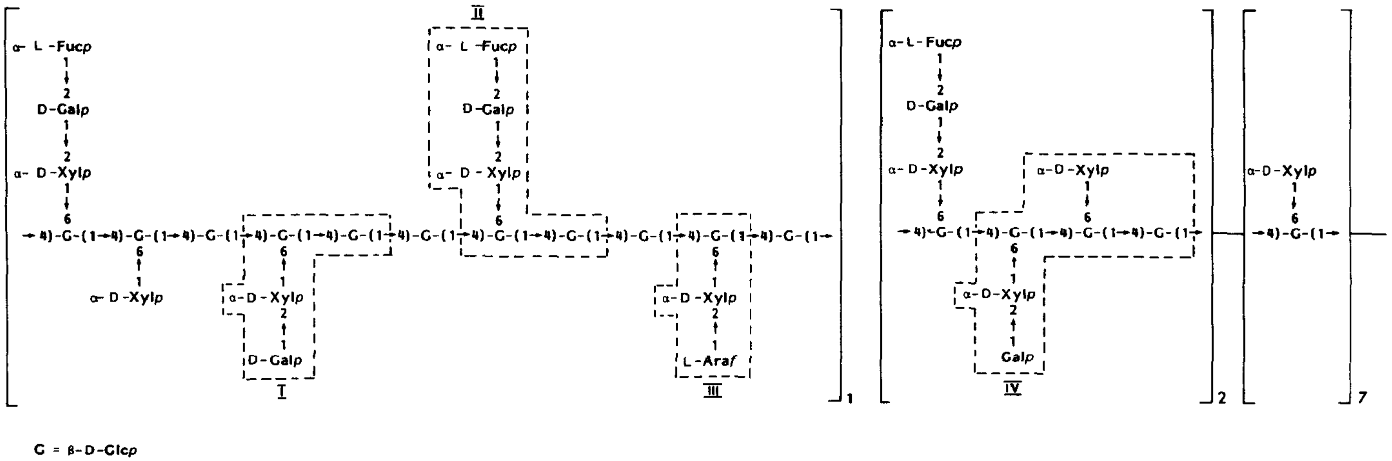

Fig. 4. Tentative structure for the xyloglucan from *P. coccineus* cell walls. The regions I–IV correspond to oligosaccharides characterised from Bio-Gel P2 fractions 3C, 2B, 3D, and 2B, respectively.

side-chain oligosaccharides, or Fuc*p*-(1→2)-Xyl*p*-(1→6)-Glc*p*, which has not been found in other cell-wall xyloglucans.

G.l.c. of reduced and methylated fraction 4B gave a single peak which was eluted in the region for a disaccharide alditol (Fig. 3c). G.l.c.–c.i.-m.s. gave intense ions at *m/z* 428 (30.7%) and 445 (100%), corresponding to $(M + 1)^+$ and $(M + 18)^+$, respectively, from a disaccharide derivative containing pentosyl and hexitol residues. G.l.c.–e.i.-m.s. gave intense ions of the aA series [*m/z* 175 (87.7%), 143 (86.1%), 111 (13.8%)] which showed the presence of non-reducing, terminal pentosyl groups. Ions at *m/z* 134 (27.2%), 178 (41.4%), 222 (5.0%), 236 (*ald*J$_2$, 51.0%), and 296 (a*ald*J$_1$, 42.4%) demonstrated a hexitol derivative substituted through position 6 (**5**). Methylation analysis (Table II) showed xylose to be the sole non-reducing terminal sugar with 6-linked glucose at the reducing terminus. Methylation analysis of reduced and unreduced fraction 4B showed the respective xylose and glucose derivatives to be present in the ratio 1:0:1.7. The reasons for this discrepancy are unclear, but may be due to loss of the xylose derivative during work-up procedures. The above data, in combination with optical rotation measurements of the native disaccharide, $[\alpha]_{580}^{20}$ +88.9° (*c* 0.2, water), identify the disaccharide as α-D-Xyl*p*-(1→6)-D-Glc*p* (isoprimeverose), which has been found in cellulase digests of various xyloglucans[14,17,18].

Attempts to obtain structural information by direct probe insertion e.i.- and c.i.-m.s. of f.a.b.-m.s. of reduced and methylated fraction 2A were unsuccessful, suggesting the presence of relatively large oligosaccharides. Gel filtration of fraction 2A on Sephadex G-25 gave a major component (>95%) which was eluted in the region d.p. 10–12. Neutral-sugar analysis (Table I) gave fucose, galactose, xylose, and glucose in the ratios 1.0:1.9:2.9:3.9 which are, with the exception of glucose, in broad agreement with those obtained by methylation analysis (Table II). These data indicated the presence of oligosaccharides having d.p. ~10, possibly corresponding to substituted cellotetraose (Fig. 4, middle-region structure). Similar oligosaccharides have been isolated from mung bean[19] and pea[20] xyloglucans. A nonasaccharide has also been isolated from a cellulase digest of sycamore xyloglucan[21] and its structure rigorously characterised[22].

Fraction 1 was eluted in the void volume of the BioGel P2 column (Fig. 1a) but could be partially fractionated on Sephadex G-25 into three components. The major fraction, 1A (52.0%), was eluted at the column V_0 and contained both protein and carbohydrate (cellulase and undegraded xyloglucan). Fraction 1B (23.0%) contained mainly carbohydrate with a high content of xylose and arabinose, and fraction 1C (25.0%) was rich in carbohydrate with a neutral sugar composition (Table I) similar to that of the native xyloglucan[1].

DISCUSSION

The structural features of a xyloglucan that can be obtained by such chemical methods as methylation analysis and acetolysis are limited in that they give little information on the distribution of oligosaccharide side-chains along the β-D-glucan backbone. Enzymic methods are diagnostic, as the relative susceptibility of the (1→4)-linked-β-D-glucosyl residues to cellulase is dependent on both the degree and type of side-chain substitution and the duration of enzymic hydrolysis.

Gel filtration of the enzymic digest showed that 57.2% of the xyloglucan could be degraded to oligosaccharides with d.p. $\leqslant 6$. Oligosaccharides with d.p. ~10 and undegraded material accounted for 22.0 and ~10.0%, respectively. Studies of cellulase digests of xyloglucans from *Simmondsia*[15], mung bean[19], and pea[21] have shown that ~75% of the oligosaccharides have d.p. ~10. Thus, differences in xyloglucan and enzyme preparations may be reflected in the types of oligosaccharides obtained.

The major component of oligosaccharides with d.p. $\leqslant 6$ obtained from *P. coccineus* xyloglucan was characterised as α-D-Xyl*p*-(1→6)-D-Glc*p* with lesser amounts of glucose, cellobiose, and cellotriose fragments variously substituted through position 6 with Xyl*p*, Ara*f*-(1→2)-Xyl*p*, Gal*p*-(1→2)-Xyl*p*, and Fuc*p*-(1→2)-Gal*p*-(1→2)-Xyl*p*. The absence of detectable amounts of cellobiose and the presence of relatively low levels of glucose in the enzyme digest suggest that "long" regions of unsubstituted glucosyl residues do not occur. However, small amounts of oligosaccharides containing cellotriose substituted through position 6 of a single glucosyl residue were found. Therefore, it is possible that side-chain substitution restricts enzymic hydrolysis of neighbouring glucosyl residues.

From the range of oligosaccharides obtained from the cellulase digest of the xyloglucan, it appears that it does not possess a "simple" repeating-unit such as those found in many microbial polysaccharides[23], although the possibility of large repeating units cannot be discounted. A plausible interpretation of the data presented here is that the xyloglucan contains a "block-type" structure (Fig. 4) which is commonly encountered in plant polysaccharides[24]. The structure was derived from the relative amounts of the oligosaccharides that were structurally characterised, and the results of previous studies[1]. Of the xyloglucan, ~70% corresponds to regions substituted with mono-, di-, and tri-saccharide side-chains and regions substituted with xylose only. The remaining portion of the polymer would have a relatively low degree of substitution with various types of side chains.

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