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AN ARABINOGALACTOXYLOGLUCAN FROM THE CELL WALL OF *SOLANUM TUBEROSUM*

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Key Word Index—*Solanum tuberosum*; Solanaceae; potatoes; cell-wall material; arabinogalactoxyloglucan; cellulase degradation; oligosaccharides; methylation analysis.

Abstract—Cell-wall material from potatoes was fractionated by successive extractions with water at 80°, 0.2 M $(NH_4)_2C_2O_4$ at 80°, 1 M and 4 M KOH, to leave a residue of α-cellulose. The compositions of the isolated carbohydrate polymers were determined by sugar and methylation analysis. From the 4 M KOH-soluble fraction an arabinogalactoxyloglucan was isolated and (partially) characterized by methylation analysis of the undegraded polymer and partially degraded methylated polymer. Methylation analysis of the oligosaccharides produced on treatment of the xyloglucan with cellulase threw additional light on the structural features of the polysaccharide. The results show that the xyloglucan has a cellulosic backbone which is highly substituted at position 6 with xylopyranose residues, some of which, in turn, carry either arabinofuranose or galactopyranose residues, as a substituent on position 2. The significance of these results is discussed.

INTRODUCTION

Cell walls of higher plants consist largely of polysaccharides which are of various structural types. Recently, considerable interest has been shown in the composition and properties of cell walls from edible plant tissues, because of their dietary significance [1] and also in the structure and properties of 'xyloglucans' (the so-called amyloids) because of their possible role in cell-wall extension [2, 3]. Bauer *et al.* [4] have produced evidence to show that the xyloglucan from suspension-cultured sycamore cells may be covalently linked to pectic polysaccharides and binds non-covalently to cellulose. This property of the xyloglucan is used by the same group to formulate a model for the cell-wall complex [2]. Similar xyloglucans (but without the associated pectic polysaccharides) have been isolated from the seeds of *Tamarindus indica* [5, 6], *Trapaeolum majus* [7], *Annona muricata* L. [8], *Brassica campestris* [9, 10, 11], white mustard [12] and *Simmondsia chinensis* [13], and from tobacco leaves [14, 15], mung bean hypocotyls [16], and also from the extracellular polysaccharides elaborated by suspension-cultured sycamore cells [17]. To clarify the role of xyloglucans in the cell-wall matrix, there is a need to know more about the other carbohydrate-polymers to which they may be attached or closely associated and also the nature of the side-chains linked to the glucan backbone of the xyloglucans.

In an earlier paper [18] we reported a method for preparing gram-quantities of cell-wall material (CWM) from potato tubers. Methylation analysis of the whole CWM revealed the main glycosidic linkages present. Following this work, the CWM was fractionated with aqueous inorganic solvents, and the composition of the isolated carbohydrate polymers was determined by sugar and methylation analysis. From the 4 M KOH-soluble fraction an arabinogalactoxyloglucan was isolated and (partially) characterized by methylation analysis of the undegraded polymer, and partially degraded methylated polymer. Methylation analysis of the oligosaccharides produced on treatment of the xyloglucan with cellulase threw additional light on the structural features of the polysaccharide. The results of these investigations are reported in this paper.

RESULTS AND DISCUSSION

Isolation of CWM

The CWM was prepared by sequentially extracting the wet ball-milled tissue with aq. 1% Na deoxycholate, PhOH–HOAc–H_2O (2:1:1, w/v/v) and aq. 90% DMSO. These solvents minimized co-precipitation of the intracellular compounds with the CWM. All the extractants solubilized a small proportion of pectic polysaccharides, but as the combined weight of these was <6% of the weight of the CWM, they were not looked at further. From 100 g fresh tubers, 1.2 g (dry) CWM were obtained. The CWM was shown to be free of starch by its negative reaction with I_2–KI and by the small amount of glucose released on 1 M H_2SO_4-hydrolysis (Table 1); the glucose released under these conditions arises mainly from xyloglucans and from 5 to 10% of the cellulose.

Sequential extraction of CWM

To obtain a clearer indication of the types of carbohydrate-polymers constituting the cell-wall complex, the CWM was sequentially extracted with hot water, at pH 5.0, hot ammonium oxalate, pH 5.0, 1 M and 4 M KOH containing 10 mM $NaBH_4$ at 20° to leave a residue of α-cellulose. Conditions of extractions were chosen to enable partial fractionation of the main types of pectins and hemicelluloses, and to minimize (1) the degradation of pectins (esterified galacturonic acid residues) [19, 20] and (2) the hydrolysis of furanosidic linkages [18]. The yields of the fractions obtained, their protein content

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Table 1. Products of fractionation of CWM of potatoes

Fractions	Yield (mg/g dry CWM)	Protein content (μg/mg dry fraction)	Monosaccharide composition*						
			Rha	Ara	Xyl	Man†	Gal	Glc	Uronic‡ acid
Purified CWM			20.4	46.1	16.5	T	284	18.6 (315)	270
Hot water-soluble	150	4	20.1	108	—	—	515	T	340
Oxalate-soluble	320	10	14.2	39.4	—	—	192	T	720
1 M KOH-soluble	100	98	33.1	82	32.0	—	429	36.4	90
4 M KOH-soluble	106	22	13.3	71.4	128	T	210	230	70
H_2O-eluate§			—	67.4	190	14.1	75.3	430	—
XG-Cu-precipitatable‖			—	85	235	27.4	88	500	—
XG-Cu-supernatant¶				41.4	110	T	40.1	290	—
α-Cellulose	322	4	11.5	12.5	9.3	20.3	32.6	70.2 (690)	190

*Sugars released on 1 M H_2SO hydrolysis for 2.5 hr at 100°; the figures in parentheses represent glucose released on Saeman-hydrolysis. The results are expressed as μg anhydrosugar/mg dry fraction.

†Some of the mannose probably arises from epimerization of glucose.

‡The values for uronic acid were determined by a modified carbazole method, and calculated as μg galacturonic acid/mg dry fraction.

§H_2O-eluate of 4 M KOH-soluble fraction from DEAE-Sephadex column.

‖Xyloglucan from copper-precipitatable fraction.

¶Xyloglucan (?) from copper-supernatant fraction.

and sugar composition are presented in Table 1. The degree of esterification of the hot water- and oxalate-soluble pectic substances were 56% and 15%, respectively. The cell-wall proteins of potatoes are not rich in hydroxyproline and the bulk of these proteins were extracted with 1 M KOH. Although a large proportion of the pectins were extracted with hot water and oxalate, it is clear that a significant proportion remained, and was only partially extracted with alkali, and some uronic acid containing material was present in the α-cellulose residue. The pectic material associated with α-cellulose could be isolated after treatment with cellulase. A similar pectic fraction has been isolated from the α-cellulose fraction of cabbage CWM [21].

Most of the carbohydrate polymers containing xylose were extracted with 1 M and 4 M KOH. The high glucose and xylose contents of the latter fraction indicated that xyloglucans were present. Further evidence for this was obtained from methylation analysis of the alkali-soluble fractions. The results are summarized in Table 2, columns 1 and 2. There is good agreement between the non-reducing terminal groups (represented by tri- and tetra-*O*-methyl ethers of pentoses and hexoses, respectively) and branch-points (as determined by the amount of mono- and di-*O*-methyl ethers of rhamnose and hexoses, respectively) showing that the methylation of the polysaccharides is virtually complete. Since xyloglucans usually do not contain (1 → 4)-linked xylose residues, the presence of small but significant amounts of this residue, in the 4 M KOH-soluble fraction, showed that the xyloglucans were contaminated with (acidic?) xylans, in addition to some pectic material.

Purification and characterization of the xyloglucan

(a) *Purification.* To purify the xyloglucan(s) it was necessary to remove the acidic components and this was achieved by fractionation on a DEAE-Sephadex column (formate form). The effluent from the column contained ~80% of the original hemicellulose fraction and had no (1 → 4)-linked xylose residues and no detectable uronic acid. Further, it was free of the bulk of the galactose-containing carbohydrate polymers. The neutral polysaccharides from the anion column were further fractionated via copper-complex formation, as described by Aspinall *et al.* [11], to give two fractions of soluble- and insoluble-copper complexes. The ratio of the polysaccharides from the soluble- and insoluble-copper complexes was about 1.1:1, and the molar ratios of the constituent sugars were found to be comparable by sugar and methylation analysis (Tables 1 and 2). However, the neutral sugars released on 1 M H_2SO_4-hydrolysis accounted for 48% and 94% of the dry weights of the carbohydrate-polymers from the soluble- and insoluble-Cu-complexes, respectively. Further structural studies were carried out with only one of them—the copper-precipitatable polysaccharide. The sugar composition of this polysaccharide was unchanged on further copper fractionation and will be referred to as xyloglucan in the rest of the paper.

(b) *Methylation studies.* The potato xyloglucan apparently contains no uronic acid residues and on acid hydrolysis gave glucose, xylose, galactose and arabinose in the molar ratio of 5.6:3.0:1.0:1.1, respectively. Except for the glucose component, this ratio is in good agreement with that found for the methylated polysaccharide (Table 2, column 3). A notable feature of the xyloglucan is the absence of (terminal) fucose residues and the presence of terminal arabinofuranose residues. The results of methylation analysis suggested that the xyloglucan is based on a (1 → 4)-linked glucan backbone which carries substituent groups on C-6 of about 40% of the glucose residues. On the basis of the six methylated sugar derivatives present, in a molecular ratio closely approximating to simple, whole numbers, the repeating unit of the polysaccharide is limited to relatively few structural representations. It is reasonable to assume that the average unit of the polysaccharide is made up of 12 sugar residues consisting of three terminal non-reducing end-groups, comprising one residue each of galactose, xylose and arabinose; the galactose and xylose are present

Table 2. Alditol acetates from methylated polysaccharide fractions isolated from CWM of potatoes

Partially methylated sugar	RR_t*	1 M KOH-soluble	4 M KOH-soluble	XG (Cu-precipitatable)	XG (Cu-supernatant)
		(Relative amounts†)			
3,4-Di-*O*-methylrhamnitol	0.9	1.0	0.7	—	—
3-Mono-*O*-methylrhamnitol	1.69	2.4	1.2	—	—
2,3,5-Tri-*O*-methylarabinitol	0.47	5.2	6.5	7.9	7.7
3,5-Di-*O*-methylarabinitol	0.83	0.8	0.4	—	—
2,3-Di-*O*-methylarabinitol	1.09	7.5	4.2	—	—
2,3,4-Tri-*O*-methylxylitol	0.61	1.0	4.3	9.5	10.1
3,4-Di-*O*-methylxylitol	1.19	1.5	9.9	14.4	14.5
2,3-Di-*O*-methylxylitol	1.19	3.0	5.2	—	—
2,3,4,6-Tetra-*O*-methylgalactitol	1.19	5.3	5.1	9.3	7.9
2,3,6-Tri-*O*-methylgalactitol	2.22	61.2	27.3	—	—
3,6-Di-*O*-methylgalactitol	2.91	3.2	—	—	—
2,6-Di-*O*-methylgalactitol	3.00	0.7	—	—	—
2,3-Di-*O*-methylgalactitol	4.34	1.5	—	—	—
2,3,6-Tri-*O*-methylglucitol	2.30	3.2	19.6	34.0	34.2
2,3-Di-*O*-methylglucitol	4.28	2.5	16.4	24.9	25.5

*Retention time relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol on OV-225 at 180°.

†Data expressed as relative mol %.

in the pyranose form and the arabinose in the furanose form. There are seven residues of glucose and at three of these branching occurs probably through C-6, by analogy with other xyloglucans. The remaining non-terminal residues consist of two (1 → 2)-linked xylose units.

To obtain unambiguous evidence for the presence and nature of the arabinose residues in the xyloglucan, the following experiments were carried out. Advantage was taken of the fact that only the arabinose residues are involved in furanosidic linkages and are therefore far more susceptible to acid hydrolysis. The fully methylated polysaccharide was subjected to mild acid hydrolysis (90% HCO_2H for 40 min at 70°), and the product reduced with $LiBH_4$ and remethylated using CD_3I. Any free hydroxyl groups generated by mild hydrolysis would now be labelled with a deuterated methyl group. The product was hydrolysed, and the mixture of methylated sugars was examined as their alditol acetates by GC/MS. The results are shown in Table 3. Only one of the sugar derivatives was trideuteriomethylated to a considerable extent, i.e. 2,3,4-tri-*O*-methyl xylose derivative at C-2. This was inferred from the relative intensities of the ions at *m/z* 121 and 165. The major changes are: (1) the absence of 2,3,5-tri-*O*-methyl arabinose derivative; (2) a large increase in the percentage of 2,3,4-tri-*O*-methyl xylose derivative; (3) a decrease in the percentage of 2,3,6-tri-*O*-methyl glucose derivative; and (4) the presence of small but significant amounts of tetra- and penta-*O*-methyl glucose derivatives. The last observation is obviously due to some fragmentation of the glucan backbone. From these results it seems reasonable to assume that all, or a large proportion of, the terminal arabinofuranose residues of the xyloglucan are linked to C-2 of the non-terminal xylose.

(c) *Enzymic hydrolysis studies.* To obtain further information on the nature of the side-chains and their mode of attachment to the glucan backbone, the xyloglucan was treated exhaustively with partially purified cellulase from culture filtrates of *Trichoderma viride*. This cellulase contained a mixture of exo- and endo-glucanases and a β-glucosidase. The cellulase was used in this form because if only the endoglucanase is used, the higher oligosaccharides are produced, and these are not amenable to study by GC/MS. The enzyme-treated product was reduced with $NaBD_4$ and then methylated. A portion of the methylated material was hydrolysed, and the resulting methylated sugars were analysed as their alditol acetates by GC/MS (Table 4). The remainder was analysed directly by GC/MS.

From the results given in Table 4, it is clear that the arabinose, galactose, and a major portion of the xylose residues are present as non-reducing end-groups in the oligosaccharides produced on enzymic hydrolysis. Further, the bulk of the β-D-glucan backbone has been hydrolysed, as shown by the absence of 2,3,6-tri-*O*-methyl glucose derivative and the presence of only a small amount of 2,3-di-*O*-methyl glucose derivative. The bulk of the glucose was detected as the pentamethyl derivative, presumably arising from the reducing end of the oligosaccharides produced. The free glucose produced on enzymic hydrolysis would have been converted to the very volatile hexamethyl derivative, and this component was not estimated. The 2,3,4-tri-*O*-methyl glucose derivative arises from oligosaccharides containing (1 → 6)-linked glucose residues.

The ratio of terminal xylose to (1 → 2)-linked xylose in the original xyloglucan is 0.66:1, whereas in the enzymic hydrolysate it is 1.39:1. This observation coupled with the fact that the amount of terminal galactose and arabinose residues in the enzymic hydrolysate are about 50% of the amount of terminal-xylose residues, suggest that the *T. viride* cellulase preparation contains some galactosidase and arabinosidase activities. Enzymic removal of some of the terminal-galactose and -arabinose residues, would result in a higher level of terminal-xylose and a correspondingly lower level of (1 → 2)-linked xylose. This inference is based on the assumption that the terminal-galactose and -arabinose residues are linked to the glucan backbone via a xylose residue. These results, coupled with

Table 4. Partially methylated alditol acetates from cellulase-treated xyloglucan

Partially methylated sugar	Relative mol %
2,3,5-Tri-*O*-methylarabinitol	9.7
2,3,4-Tri-*O*-methylxylitol	20.4
3,4-Di-*O*-methylxylitol	14.6
2,3,4,6-Tetra-*O*-methylgalactitol	9.0
Penta-*O*-methylglucitol	29.9
2,3,4-Tri-*O*-methylglucitol	11.8
2,3-Di-*O*-methylglucitol	4.6

Table 3. Composition of the partially degraded permethylated xyloglucan*

Partially methylated sugar	Relative amounts	Diagnostic fragment ions [*m/z* (rel. int.)]
2,3,4-Tri-*O*-methylxylitol	22.7	117 (22), 118 (13), 121 (10), 161 (3), 162 (2), 165 (1)
3,4-Di-*O*-methylxylitol	10.1	117 (18), 190 (3)
2,3,4,6-Tetra-*O*-methylgalactitol	10.4	118 (17), 205 (3)
Penta-*O*-methylglucitol	1.8	
2,3,4,6-Tetra-*O*-methylglucitol	2.1	
2,3,6-Tri-*O*-methylglucitol	27.8	118 (40), 233 (9), 236 (1)
2,3-Di-*O*-methylglucitol	25.1	118 (62), 261 (6)

*The methylated xyloglucan was subjected to mild acid hydrolysis, reduced with $NaBH_4$ and remethylated with CD_3I.

the results of methylation analysis of the xyloglucan, suggested a tentative structure for a fragment (average unit) of the xyloglucan (Fig. 1—relative proportions of residues are shown, but the sequence is purely arbitrary).

Characterization of oligosaccharides from enzymic hydrolysis

The oligosaccharides formed on enzymic hydrolysis were reduced with $NaBD_4$ and the resulting alditols were methylated and examined by GC on OV-1. Four main peaks (peaks 1–4) were obtained in the ratio 10:1:7:1.6, and these were identified by MS using the principles outlined by Kärkäinen [22, 23] and Kovacik *et al.* [24]. Application of the principles for the identification of oligosaccharides produced on partial acid hydrolysis of CWM from beeswing wheat bran has already been described [25]. Peak 2 contained a mixture of derivatives and the identity of the consistuents present in this peak will be discussed last. The following data were used, (a) RR_t with reference to methylated cellobiitol, (b) the presence of diagnostic fragment ions in the MS, and (c) the oligosaccharides that can be inferred to be present from the above study, i.e. the ones suggested by the tentative structure of the xyloglucan. In Table 5 the relative intensities of the pertinent ions in the MS of the peaks are given. The nomenclature for the degradation of a permethylated trisaccharide alditol (**abc**) is as follows: **abc** $\mathbf{J}_1$ denotes the ion arising by fission of ring **a** following the pathway **J**, and being substituted by rings **b** and **c**.

Peak 1 (*max RR*$_t$ 0.85). This component eluted in the methylated disaccharide alditol region. The origins of some pertinent fragments are shown in Fig. 2. The MW can be calculated from the *m/z* values of the fragments $\mathbf{aA}_1$ and the alditol moiety: MW = $\mathbf{aA}_1$ + 236 + 16 = 427. Ions of the **aA** series at 175 and 143 indicate that a pentose is the non-reducing end-group. The absence of the ion at *m/z* 219 shows that this peak is not contaminated with a methylated disaccharide alditol having hexose as the non-reducing end-group. The ions at 236, 178, 134 and 46 show that the reducing end is a hexose linked through position 6 to the pentose. From these results and those of the preceding section the structure of the compound in this peak could be inferred to be *O*-(2,3,4-tri-*O*-Me-xylopyranosyl)-(1 → 6)-1,2,3,4,5-penta-*O*-Me-glucitol. The parent compound is therefore Xylp-(1 → 6)-Glcp.

Peak 3 (*max RR*$_t$ 3.40). This component eluted in the methylated tetrasaccharide alditol region. The origins of some pertinent fragments are shown in Fig. 3. Ions of the

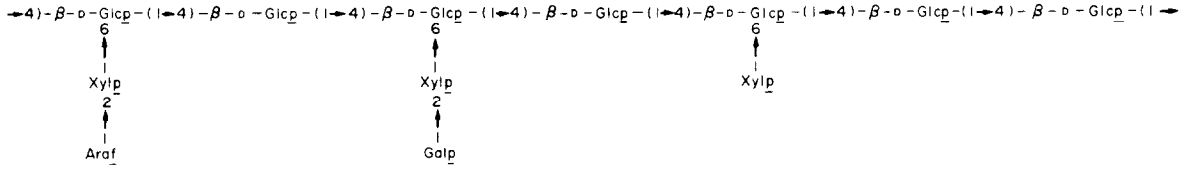

Fig. 1. The main structural features of potato arabinogalactoxyloglucan.

Table 5. Partial MS of permethylated oligosaccharide alditols from cellulase-treated xyloglucan

m/z of diagnostic ions	Rel. int. Peak 1	Peak 2	Peak 3	Peak 4	Symbol
46	42.1	14.1	7.1	6.8	
88	61.9	100.0	81.8	82.7	$\mathbf{H}_1$
90	36.2	10.5	6.5	5.3	
101	100.0	75.6	100.0	58.6	$\mathbf{F}_1$
134	12.6	7.4	6.7	4.8	
143	34.6	31.6	41.7	12.3	$\mathbf{aA}_2$
146	16.3	1.1	4.4	1.4	
175	49.0	37.9	50.8	6.5	$\mathbf{aA}_1$
178	8.2	0.3	0.2	0.2	
187	0.6	1.7	2.3	29.4	$\mathbf{aA}_2$ (*Pk* 4)
219	—	0.6	0.0	8.0	$\mathbf{aA}_1$ (*Pk* 4)
236	8.3	17.0	51.9	36.5	
296	1.6	0.7	5.2	2.6	**bc** $\mathbf{J}_1$ (*Pk* 2); **cd** $\mathbf{J}_1$
303	—	1.7	49.3	1.9	**ba** $\mathbf{A}_2$
335	—	0.5	3.8	0.3	**ba** $\mathbf{A}_1$
347	—	0.6	0.3	22.4	$\mathbf{baA}_2$ (*Pk* 4)
379	—	0.1	0.1	0.6	$\mathbf{baA}_1$ (*Pk* 4)
408	—	0.4	1.5	1.3	$\mathbf{bcA}_2$ (*Pk* 2); $\mathbf{cdA}_2$
440	—	0.1	0.6	0.4	$\mathbf{bcA}_1$ (*Pk* 2); $\mathbf{cdA}_1$
500	—	0.2	1.2	0.3	**abc** $\mathbf{J}_1$ (*Pk* 2); **bcd** $\mathbf{J}_1$
660	—	—	1.1	2.2	**abcd** $\mathbf{J}_1$
746	—	—	0.1	—	**abcd** $\mathbf{E}_1$

Pk—Peak.

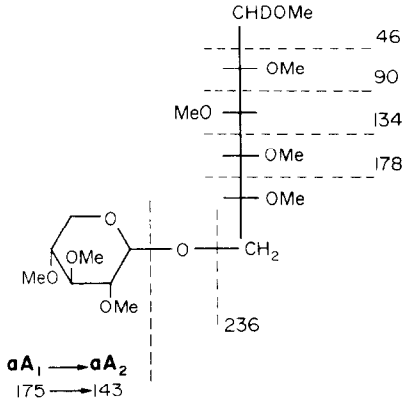

Fig. 2.

aA series at 175 and 143 indicate that a pentose is the non-reducing end-group. The absence of the ion at *m/z* 219 shows that this peak is not contaminated with a methylated tetrasaccharide alditol having hexose as the non-reducing end-group. Ions of the **baA** series at 335 and 303 indicate that the sequence from the non-reducing end is pentose–pentose. The relatively high intensity of the $\mathbf{baA}_2$ ion compared with the $\mathbf{baA}_1$ ion shows that the **a** → **b** linkage is C-1 → C-2. An ion at 236 indicates that the reducing end-group is a hexose, and the ions at 134 and 90, and the virtual absence of the ion at 178 show that the hexose is 4-linked. The **cdA** ions at 440 and 408 show that the sequence from the reducing end is hexitol–hexose. Ions of the **J** series at *m/z* 500 and 660, namely $\mathbf{bcd\,J}_1$ and $\mathbf{abcd\,J}_1$, confirm that the sequence from the non-reducing end is pentose–pentose–hexose–hexitol. The presence of a small but detectable amount of the ion at *m/z* 746 ($\mathbf{abcd\,E}_1$) is consistent with the view that a furanose sugar is present at the non-reducing end. Based on these results and those of the preceding section, the structure of the compound in this peak could be inferred to be *O*-(2,3,5-tri-*O*-Me-arabinofuranosyl)-(1 → 2)-*O*-(3,4-di-*O*-Me-xylopyranosyl)-(1 → 6)-*O*-(2,3,4-tri-*O*-Me-glucopyranosyl)-(1 → 4)-1,2,3,5,6-penta-*O*-Me-glucitol. The parent compound is therefore Araf-(1 → 2)-*O*-Xylp-(1 → 6)-Glcp-(1 → 4)-Glcp.

Peak 4 (*max* RR_t 3.95). This component also eluted in the methylated tetrasaccharide alditol region. The origins of some pertinent fragments are shown in Fig. 4. Ions of the **aA** series at *m/z* 219 and 187 indicate that a hexose is the non-reducing end-group. Ions of the **baA** series at 379 and 347 indicate that the sequence from the non-reducing end is hexose–pentose. The relatively high intensity of the baA_2 ion compared with the $\mathbf{baA}_1$ ion shows that the **a** → **b** linkage is C-1 → C-2. Otherwise the structure is very similar to the compound in peak 3. Using similar arguments the sequence from the non-reducing end is hexose–pentose–hexose–hexitol. As before, the structure of the major component in this peak could be inferred to be *O*-(2,3,4,6-tetra-*O*-Me-galactopyranosyl)-(1 → 2)-*O*-(3,4-di-*O*-Me-xylopyranosyl)-(1 → 6)-*O*-(2,3,4-tri-*O*-Me-glucopyranosyl)-(1 → 4)-1,2,3,5,6-penta-*O*-Me-glucitol. The parent compound is therefore Galp-(1 → 2)-*O*-Xylp-(1 → 6)-Glcp-(1 → 4)-Glcp.

Peak 2 (*max* RR_t 2.50). This peak was rather small and eluted in the methylated trisaccharide alditol region. Mass resolved chromatography showed that this peak was composed of at least two components. Since the components had very similar mass spectra, the partial mass spectra of only one of the components (the slightly faster moving one) are given in Table 5. A careful study of the mass spectra showed that two or more compounds were present. The major component appears to be derived from a trisaccharide (**A**) having the empirical structure: non-reducing pentose–hexose–(1 → 4)-linked hexose. This structure is supported by the following evidence. Ions of the **aA** series at 175 and 143 indicate that a pentose is the major non-reducing sugar. An ion at 236 indicates that the reducing end-group is a hexose, and the ions at 134 and 90 and the relatively small amount of the ion at 178 show that most of the hexose is 4-linked. The presence of small but significant amounts of the ions of the **baA** series at 379 and 347 indicate that the sequence from the non-reducing end is pentose–hexose. The **bcA** ions at 440 and 408 show that the sequence from the reducing end is hexitol–hexose.

However, the ions of the **baA** series at 335 and 303 suggest the presence of a compound derived from a trisaccharide (**B**) having the partial structure non-reducing pentose–pentose. Further, the presence of small but significant amounts of the ions of the **aA** series at 219 and 187 indicate the presence of a trisaccharide (**C**) having a non-reducing hexose as the end-group.

It is probable that **A**, **B** and **C** are: Xylp-(1 → 6)-Glcp-(1 → 4)-Glcp, Araf-(1 → 2)-Xylp-(1 → 6)-Glcpand Galp-(1 → 2)-Xylp-(1 → 6)-Glcp, respectively. The expected ions from the methylated alditols of all three trisaccharides are

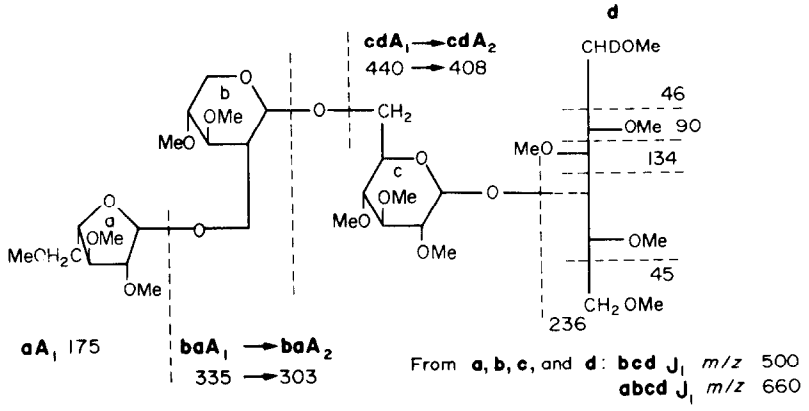

Fig. 3.

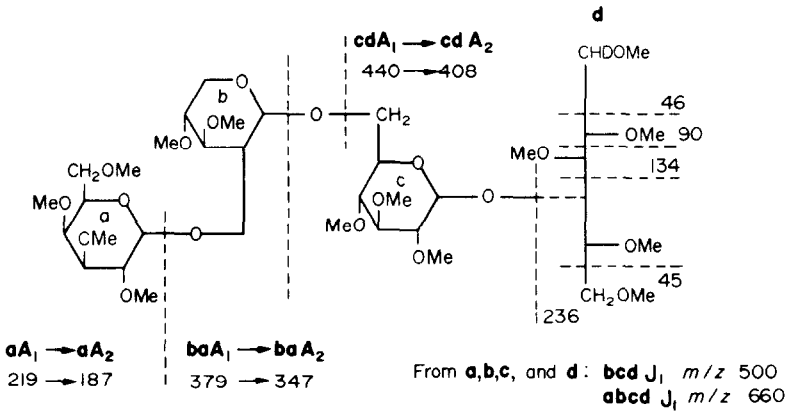

Fig. 4.

present, but unambiguous identification is not possible. It may be useful to note that **B** and **C** would be the expected products from β-glucosidase action on the tetrasaccharides described earlier. Compound **A**, of course, would be expected to be present, because it is xylosyl–cellobiose.

A note on the enzymic degradation of the xyloglucan

Treatment of cellulose (ball-milled filter paper) with cellulase from *T. viride* in acetate buffer, pH 5.0, at 37° for 3 days, resulted in the production of glucose and cellobiose in the approximate ratio 10:1. In the case of potato xyloglucan, it would appear from the above results, especially from the amounts of oligosaccharides produced, that the nature of the side-chains influences the amount and type of oligosaccharides produced. From regions of the xyloglucan where the glucose residues carry xylose side-chains, the main product is xylosylglucose (isoprimeverose) with little xylosyl cellobiose. However, when the side-chain is a disaccharide, Araf-(1 → 2)-*O*-Xylp-(1 →, the main product is a tetrasaccharide (Ara-Xyl-Glc-Glc), with very little trisaccharide (Ara-Xyl-Glc). This may be due to steric effects, the longer side-chain inhibiting the cleavage of the cellobiose unit. When the side-chain is Galp-(1 → 2)-*O*-Xylp-(1 →, the main product appears to be a penta- (or higher) oligosaccharide, with little tetrasaccharide (Gal-Xyl-Glc-Glc), and still less trisaccharide (Gal-Xyl-Glc). The last inference is based on the fact that the amounts of oligosaccharides, as estimated by the GC method, do not account for all the linkages present in the enzyme-treated product. There is a particular short-fall in the amount of galactose-containing oligosaccharides. It must be assumed that the terminal galactose is present mainly in oligomers where *n* (the number of sugar residues) > 4.

GENERAL DISCUSSION

The xyloglucan fractions from potato CWM are similar to those of other members of the xyloglucan family. The highly branched character of the Cu-precipitatable potato xyloglucan was shown, and the nature of the linkages was established. It has a cellulosic backbone which is highly substituted at position 6 with D-xylopyranose residues, some of which, in turn, carry either L-arabinofuranose or D-galactopyranose residues, as a substituent on position 2. The main structural difference between potato xyloglucan and other members of this family is the presence of arabinofuranose as a non-reducing end-group, terminating some of the side-chains. Arabinose has been reported as being a component of some xyloglucans but it has often been thought of as being from a contaminating polysaccharide. The work reported here confirms its presence as an integral part of the xyloglucan constituting a Araf-(1 → 2)-*O*-Xylp-(1 → sidechain.

From the sequential extraction studies, it appears that the potato xyloglucan fractions are not covalently linked to pectic polysaccharides by *O*-glycosidic linkages through their reducing ends. These results are in contrast to the work on xyloglucan from suspension-cultured sycamore cells [4]. It can be argued that strong alkali could cleave some of the linkages between the xyloglucan(s) and pectic polysaccharides. If this were the case then the xyloglucan should contain additional sugar (galactose?) residues in the form of a chain at the reducing end. In the structural studies of potato xyloglucan no such additional sugar residues were found. However, the fact that the xyloglucans are strongly associated with α-cellulose, and require treatment with 4 M KOH to release the bulk of them, suggests the involvement of strong H-bonds, although the possible involvement of phenolic-crosslinks (which may be degraded by strong alkali) should not be overlooked. The (Cu-precipitable) potato xyloglucan once isolated from the CWM becomes water-soluble like most other polysaccharides of this group. The parent polysaccharide, cellulose, is water-insoluble, as is a xyloglucan of intermediate structure from *Annona muricata* L. [8]. With increasing substitution of the glucan backbone the polymers become more water-soluble, the side-chains decreasing the strength of non-covalent interactions with other xyloglucan chains.

In common with some other members of this group potato xyloglucan interacts strongly with cellulose. If the xyloglucan is adsorbed onto cellulose powder it cannot be desorbed with water. Urea can release some of the xyloglucan but aqueous alkali is the best extractant (Ring and Selvendran, unpublished results). Cellulose swells in alkali, but generally the alkali concentration has to be > 12% (w/w), before changes in the spacing between the individual cellulose chains occur. The extent of swelling also depends upon the crystallinity of the cellulose [26]. The swelling is considered to be due to the ionization of the hydroxyl groups involved in the H-bonding of the cellulose chains [27]. The swelling of cellulose in alkali may give an insight into the nature of the association between xyloglucan and cellulose in the cell wall, and may

help to explain why aqueous alkali is a good extractant for cell-wall xyloglucans. The fact that in the case of potato, runner bean [28] and cabbage [21] cell walls, 4 M alkali is a better extractant than 1 M indicates the strength of the association. The interaction between xyloglucan and cellulose has been seen as one of the functions of the xyloglucan in the cell wall. Keegstra *et al.* [2] have suggested that xyloglucan 'creep' along the cellulose microfibrils may be a critical wall-loosening step. Thus our studies are relevant in understanding the association and possible role of cell-wall polysaccharides.

EXPERIMENTAL

Chemicals. $LiAlD_4$, $NaBD_4$ and CD_3I were purchased from Fluka, Switzerland; DMSO, tetrahydrofuran, NaH, $LiAlH_4$, $NaBH_4$ were obtained from BDH. DMSO was vacuum-distilled over CaH_2 and stored over molecular sieve 3A. Tetrahydrofuran was distilled over $LiAlH_4$ and stored under Ar. All other chemicals were of the highest purity available.

Cellulase from culture medium (300 ml) of *Trichoderma viride*, grown on ball-milled filter paper as described in ref. [29], was prepared by precipitation with $(NH_4)_2SO_4$ between the limits of 20% and 80% saturation, at 2° [30]. The ppt. was dissolved in 20 ml of 0.1 M acetate buffer (HOAc–NaOH) containing 5 mM NaN_2, pH 5.0, and stored at 1°.

Plant material. Potatoes (var. Desiree) were obtained from plants grown in experimental plots near the laboratory. From a batch of mature potatoes harvested 10 September 1975, those between 100 and 150 g were selected and stored for 3–4 months at 6° before analysis. The composition of the cell-wall material did not change appreciably during this period.

General methods. IR: in CCl_4 or as KBr discs. Evaporations were carried out under red. pres. at 40° or less. Dialysis was performed with continual stirring against distilled H_2O; toluene was added to inhibit microbial growth. GC was carried out using columns (2.8 m × 2.2 mm) containing JJ's diatomite CQ coated with (a) 3% OV-225, and (b) 3% ECNSS-M as described in ref. [18], and (c) 3% OV-1, which was temp. programmed from 220 to 320° at 2°/min [25]. Columns a and b were used for the separation of partially methylated alditol acetates and column c for the separation of methylated olisaccharide alditols. RR_t of partially methylated alditol acetates are given relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-Me glucitol and those of the methylated oligosaccharide alditols relative to permethylated cellobiitol. GC/MS: Pye 104 gas chromatograph interfaced to a medium resolution spectrometer (AEI MS 902); inlet temp. 250°; ionization potential 70 eV; ion source temp. 230°; data acquired on an AEI DS 50 SM computer system.

Preparation of CWM. CWM of potatoes was prepared by sequential extraction of the fresh wet ball-milled tissue with aq. 1% Na deoxycholate containing 5 mM $Na_2S_2O_5$, PhOH–HOAc–H_2O (2:1:1, w/v/v) and aq. 90% DMSO [18, 31]. The material solubilized by the solvents included low MW intracellular compounds, cytoplasmic proteins, the starch present in the tissues and some pectic polysaccharides which are rich in galactose. As the combined weight of the pectic polysaccharides was < 6% of the weight of the CWM, they were not looked at further. Fresh tissue (100 g) gave about 1.2 g (dry wt) of CWM containing about 2% wall protein.

Sequential extraction of CWM. CWM (1 g) was extracted with 100 ml distilled H_2O at 80° for 2 hr and the mixture was filtered through a sintered glass funnel and the residue washed with 20 ml warm H_2O. The filtrate was freeze-dried and the residue extracted with 200 ml of aq. 1% (w/v) $(NH_4)_2C_2O_4$, pH 5.0, at 80° for 2 hr and filtered as before. The polymers in the filtrate were isolated after dialysis by freeze-drying. The depectinated residue was sequentially extracted with 200 ml each of 1 M and 4 M KOH containing 10 mM $NaBH_4$ under Ar for 2 hr at 20°, to leave a residue of α-cellulose. The alkaline extracts were acidified to pH 5 with HOAc, dialysed to remove salts, and freeze-dried. Note that in the above procedure, the delignification stage was omitted because potatoes contain negligible amounts of lignin.

Isolation and purification of potato xyloglucan. Preliminary expts had indicated that polysaccharide fractions rich in xyloglucans were best isolated by extraction of the CWM with 4 M KOH after prior removal of pectic materials and 1 M KOH-soluble polymers. Accordingly, the xyloglucan fractions were isolated from the 4 M KOH-soluble carbohydrate polymers. The above polymers gave a positive amyloid type reaction with I_2/KI. The polymers (200 mg) were chromatographed on a column of DEAE-Sephadex A-50 (34 × 2.5 cm, formate form) to remove acidic polysaccharide contaminants, and the aq. eluate was freeze-dried to yield 160 mg crude xyloglucans. The crude xyloglucans (150 mg) were fractionated further by precipitation from H_2O (10 ml) by the addition of Fehling's solution (1.5 ml), dispersal of the resulting gelatinous precipitate in H_2O (15 ml), and dropwise addition of 0.2 M HCl, and precipitation with EtOH (Cu-precipitatable xyloglucan, yield 60 mg). A carbohydrate-polymer was also isolated from the Cu-supernatant fraction (yield 68 mg). Sugar and methylation analysis revealed that both fractions were very similar, although their carbohydrate content was different. The Cu-precipitatable xyloglucan fraction contained about twice as much carbohydrate. Repetition of the Cu-fractionation procedure gave no further change in sugar composition (directly or after methylation).

Cellulase degradation of the xyloglucan. The Cu-precipitatable xyloglucan (15 mg) was dissolved in 0.1 M acetate buffer, pH 4.7, and incubated with 0.1 ml *T. viride* cellulase preparation at 37° for 3 days in the presence of toluene (0.1 ml of enzyme will release 10 μmol of glucose from cellulose/hr at 37°). At the end of this period the polymeric material was precipitated by the addition of EtOH (final concn 80% v/v). The ppt. was removed by centrifugation and the supernatant was rendered free of EtOH, dil. with H_2O and passed through a column of Dowex AG-50 (8% cross-linked; H^+ form) to remove Na^+ and freeze-dried. The product was shown by PC, using EtOAc–HOAc–H_2O (3:1:3), to contain glucose, a disaccharide (R_f relative to cellobiose 1.26) and some higher oligosaccharides.

Methylation analysis. Polysaccharide fractions: Methylation analysis of the polysaccharide fractions was carried out as previously described [18, 32]. Methylation of the polysaccharides was judged to be virtually complete from the very weak IR absorption owing to hydroxyl groups. The sugar components of the methylated polysaccharides were analysed as their partially methylated alditol acetates by GC/MS [18, 32, 33].

Partial acid hydrolysis of methylated xyloglucan: The methylated xyloglucan from the Cu-precipitatable fraction (5 mg) was hydrolysed with 90% HCO_2H (5 ml) for 40 min at 70°. The acid was removed by co-distillation with H_2O and the residue was dissolved in dry tetrahydrofuran (2 ml) and reduced with $LiBH_4$ (10 mg). After 4 hr, excess $LiBH_4$ was removed by addition of 10 ml H_2O containing 3 ml cation exchange resin (Dowex AG-50, H^+ form). The resin was filtered off and the filtrate evapd to dryness under vacuum. The boric acid liberated was removed by co-distillation with dry MeOH (4 × 5 ml). The product was remethylated with CD_3I and the sugar components analysed as before. The incorporation of $-CD_3$ into the partially methylated alditol acetates was recognized by a shift of some of the fragments in the MS to a mass three units higher.

Methylation studies on enzymic hydrolysate. The mixture of oligosaccharides produced on treatment with cellulase was reduced with $NaBD_4$ and methylated as described in ref. [25]. One half of the methylated product was hydrolysed and derivatized to produce a mixture of partially methylated alditol acetates which were analysed by GC/MS, using column (a). The remainder was analysed directly by GC/MS using column (c).

Sugar analysis. Neutral sugars were released from the polysaccharide fractions using 1 M H_2SO_4 and Saeman hydrolysis for 2.5 hr, and the liberated sugars analysed as their alditol acetates by GC [34]. Uronic acid content was estimated by the modified carbazole method and the values were corrected for interference from neutral sugars [34]. In the earlier paper [18], the bulk of the uronic acid present in the CWM of potatoes was shown to be galacturonic acid, by methylation analysis.

Protein and amino acid analysis. Nitrogen was determined by the micro-Kjeldahl digestion procedure and expressed as 'protein' after multiplication by the factor 6.25. Amino acids were released by hydrolysis with 6 M HCl at 110° for 24 hr in a sealed tube. Liberated amino acids were analysed as their heptafluorobutyric *n*-propyl derivatives by GC [35].

Degree of esterification of pectic substances. This was determined by IR spectroscopy as described by Bociek and Welti [36].

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