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METHYLATION ANALYSIS OF CELL-WALL MATERIAL FROM PARENCHYMATOUS TISSUES OF *Phaseolus vulgaris* AND *Phaseolus coccineus*

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ABSTRACT

Cell-wall material (CWM) from parenchymatous tissues of pods of dwarf french and runner beans was prepared by sequentially extracting the wet ball-milled tissues with 1% aqueous sodium deoxycholate, phenol–acetic acid–water, and 90% aqueous dimethyl sulphoxide. The overall monosaccharide compositions of the preparations were similar, and each contained small but significant amounts of hydroxyproline-rich glycoproteins. Methylation analysis of the CWM revealed the following main glycosidic linkages in descending order of concentration: 4-linked galacturonic acid, 4-linked glucose, 4-linked galactose, 5-linked arabinose, 2-linked rhamnose, and 4-linked xylose. The major branch-points were those through C-6 of glucose, C-3 of arabinose, and C-4 of rhamnose. Arabinose, xylose, and galactose constituted the non-reducing end-groups. The general structural features of the CWM are discussed.

INTRODUCTION

Recently, considerable interest has been shown in the physico-chemical characteristics of cell-wall polymers as constituents of dietary fibre[1,2]. To study the properties of these components, it is essential to have well-defined cell-wall preparations[3]. We have described the preparation of starch-free cell-wall material (CWM) from potatoes and its methylation analysis[4]. We now report the analyses of CWM from parenchymatous tissues of the pods of dwarf french and runner beans.

RESULTS AND DISCUSSION

The CWM was prepared by sequential extraction with aqueous inorganic/organic solvents, which prevented co-precipitation of intracellular compounds with the wall material[4,5]. The final products were shown to be free of starch by their negative reaction with I_2/KI.

The SDC-soluble polymers from dwarf and runner beans contained ~70% of cytoplasmic proteins and ~18% of cold water-soluble pectic material. The cyto-

TABLE I

NEUTRAL SUGAR COMPOSITION AND URONIC ACID CONTENT OF SDC[a]-SOLUBLE MATERIAL AFTER AMYLOGLUCOSIDASE TREATMENT

Component[b]	*Dwarf bean*	*Runner bean*
Rhamnose	10.3	10.5
Ribose	3.2	3.0
Arabinose	25.7	32.0
Xylose	3.7	4.1
Mannose	traces	traces
Galactose	87.2	61.8
Glucose	13.8	15.5
Uronic acid	41.3	53.4
Total monosaccharide	185.2	180.3

[a]Sodium deoxycholate. [b]Determined after hydrolysis[7] with M H_2SO_4, and expressed as μg of sugar/mg dry wt. The values for uronic acid were determined by a modified carbazole method[7], and calculated as μg of galacturonic acid/mg dry wt.

TABLE II

CHEMICAL COMPOSITION OF DWARF- AND RUNNER-BEAN CELL-WALL MATERIAL (CWM)

Component	*Dwarf*		*Runner*		*Component*	*Dwarf*	*Runner*
	A[a]	*B*[b]	*A*[a]	*B*[b]			
Rhamnose	17.3	21.8	19.3	18.4	Ala[c]	10.8	18.3
Arabinose	37.8	37.9	35.1	36.0	Gly	11.8	17.3
Xylose	19.2	19.4	17.3	17.3	Val	12.5	21.2
Mannose	9.5	3.7	15.7	8.4	Thr	9.8	14.5
Galactose	70.3	75.6	74.8	75.7	Ser	21.9	30.3
Glucose	324.6	31.7	314.1	24.9	Leu	22.3	30.9
					Ileu	9.9	17.2
					Pro	14.9	22.3
Uronic acid	348.6	—	367.3	—	Hyp	23.6	44.4
					Met	traces	traces
					Asp	26.2	34.8
					Phe	16.1	22.5
Total monosaccharide	827.3	190.1	843.6	180.7	Glu	39.6	52.0
					Lys	30.6	41.6
					Tyr	16.1	14.2
					Arg	10.3	15.3
					His	7.6	7.5
					Yield	284.0	387.1

[a]Estimated by Saeman-hydrolysis[8], and calculated as μg of sugar/mg of dry CWM. Uronic acid was estimated by a colorimetric method[7], and calculated as μg of galacturonic acid/mg of dry CWM. [b]Estimated by hydrolysis[7] with M H_2SO_4, and calculated as μg of sugar/mg of dry CWM. [c]Determined by the method of March[6], and calculated as mg of amino acid/10 g of dry CWM.

plasmic nature of the proteins can be inferred from the fact that they contain very small proportions of hydroxyproline[5]. The carbohydrate compositions of the pectic material after treatment with amyloglucosidase are given in Table I. Arabinose and galactose are the preponderant neutral sugars; the small proportion of ribose could have arisen from ribonucleic acid. The PAW (phenol–acetic acid–water)-soluble polymers from both tissues also contained mainly proteins (~75%), but had smaller proportions of carbohydrate material (~8%); the main neutral sugars obtained on hydrolysis with M H_2SO_4 were arabinose and galactose. The Me_2SO-soluble polymers from both tissues contained ~1.5% of protein and ~80% of carbohydrate. The neutral sugar composition (%) of the material from dwarf beans was as follows: glucose (95.8), galactose (1.6), arabinose (1.2), rhamnose (0.9), and xylose (0.5); the bulk of the glucose was shown to arise from starch[4].

The absence of cytoplasmic proteins in the final preparation is reflected in the relatively high levels of hydroxyproline; the results are shown in Table II. Re-examination of the amino acid composition of the cell-wall proteins is of interest because the earlier investigations used ion-exchange chromatography[5] under conditions that did not separate hydroxyproline and aspartic acid. In the present study, the amino acids were separated by g.l.c. as their heptafluorobutyryl propyl esters[6]. The amino acid values for runner bean compare well with those reported earlier[5].

The neutral sugar composition and uronic acid content of the wall preparations (Table II) show that the carbohydrate compositions are remarkably similar. Hydrolysis was effected with M H_2SO_4[7] and by the Saeman method[8]. The former method hydrolyses the bulk of the neutral sugars from the non-cellulosic polysaccharides of the CWM, whereas the Saeman method also hydrolyses the cellulose component. The uronic acid content of the CWM was determined by a modified carbazole method[7], and the values are in reasonable agreement with those obtained by methylation analysis.

Of the dry matter of CWM, 86–90% could be accounted for as polysaccharides, wall proteins, and ash; the remainder is attributed to "residual water", sugars destroyed during hydrolysis or not measured due to incomplete hydrolysis, and materials lost due to complex formation with amino acids.

Methylation analysis of CWM

Using modifications of the Hakomori procedure, it is possible to methylate CWM from a range of plant tissues[4,9,10]. The bean preparations were methylated by the procedure described[4] for potato CWM, with the exception that the treatment with base was 12 h, as recommended by Jansen *et al.*[11], instead of 6 h. The longer treatment with base seemed to effect almost complete solubilisation (and methylation) of the cellulose component. This is a useful modification, because it makes the comparison with the unmethylated CWM more meaningful. The chloroform–methanol-soluble fraction of the methylated CWM showed very weak i.r. absorption for hydroxyl, indicating methylation of this fraction to be essentially complete.

Preliminary experiments with wet, ball-milled filter paper (particle size, 100–

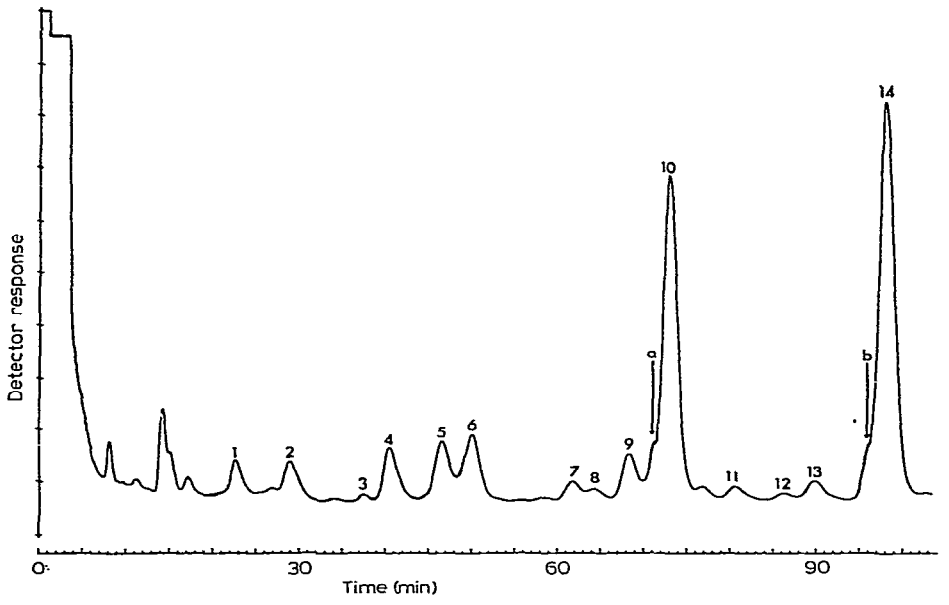

Fig. 1. Separation of the partially methylated alditol acetates (PMAA) from dwarf-bean cell-wall material after carboxyl-reduction. Separation was achieved on OV-225; initial temperature 150°, isothermal for 5 min, and then temperature-programmed at 0.5°/min up to 200°; helium flow-rate 30 ml/min. The unlabelled peaks at the beginning are products derived from the reactants. The PMAA derivatives identified are: (1) 2,3,5-tri-*O*-methylarabinitol, (2) 2,3,4-tri-*O*-methylxylitol, (3) 3,5-di-*O*-methylarabinitol, (4) 3,4-di-*O*-methylrhamnitol, (5) 2,3-di-*O*-methylarabinitol, (6) 2,3- and 3,4-di-*O*-methylxylitol and 2,3,4,6-tetra-*O*-methylgalactitol, (7) 3-*O*-methylrhamnitol, (8) 2-*O*-methylarabinitol, (9) tri-*O*-methylhexitol, (10) 2,3,6-tri-*O*-methylglucitol (the shoulder "a" corresponds to 2,3,6-tri-*O*-methylgalactitol, (11) 2,3,4-tri-*O*-methylgalactitol, (12) 2,6-di-*O*-methylglucitol, (13) 3,6-di-*O*-methylglucitol, (14) 2,3-di-*O*-methylgalactitol (the shoulder "b" corresponds to 2,3-di-*O*-methylglucitol).

300 × 10–30 μm) showed that ~95% of the constituent cellulose could be methylated. Hydrolysis, reduction, and acetylation of the methylated product yielded 2,3,6-tri- (90%), 2,3-di- (3.3%), 3,6-di- (2.9%), 2,6-di- (2.6%), and a trace of 2,3,4,6-tetra-*O*-methylglucitol derivatives. The occurrence of only small proportions of the di-*O*-methylglucitol derivatives, in approximately equal amounts, shows that the methylation of cellulose is virtually complete.

The methylated polysaccharides from the cell-wall preparations were hydrolysed, reduced, and acetylated[12], and the partially methylated alditol acetates were subjected to g.l.c. on OV-225 and ECNSS-M. Their identity was established by combined g.l.c.–m.s., using an OV-225 column as previously described[4]. The gas chromatogram for each preparation contained 18 peaks (Fig. 1), of which 14 were shown to contain carbohydrate derivatives by g.l.c.–m.s.

Using electron-impact mass spectrometry, it is not possible to distinguish between isomers having identical *O*-methyl and *O*-acetyl substitutions[13,14]; such isomers also have similar relative retention times in g.l.c.[11]. Thus, it was difficult to identify peaks 9 and 10 in the tri-*O*-methylhexitol acetate region of the gas chromato-

gram (*cf.* peaks 10 and 11 of Ref. 4). The *T* value (2.04) of peak 9 and the mass-spectral fragmentation pattern of the constituents suggest that it contains mainly 2,3,6-tri-*O*-methylhexitol acetate, together with some 2,4,6-tri-*O*-methylhexitol acetate; these are probably the mannose and galactose derivatives, respectively. The shouldered peak 10 (*T* 2.30) gave the primary and secondary mass-spectral fragments expected for 2,3,6-tri-*O*-methyl-galactitol or -glucitol acetate derivatives[11]. The identification and quantification of these components were obtained from the following investigations.

Preliminary experiments with hot water-soluble pectic substances from CWM of potato [polymers rich in (1→4)-linked galactose residues] and ball-milled filter paper showed that the (1→4)-linked galactose and glucose derivatives were eluted at the leading edge and tail of peak 10 (Fig. 1), respectively. From the experience gained with these products, when chromatographed separately and in combination, it was possible to estimate the 2,3,6-tri-*O*-methyl-galactose and -glucose derivatives in the bean preparations. The results indicated that the bulk of the peak in the present study is due to the glucose derivative and is probably derived from "fully methylated cellulose".

TABLE III

ALDITOL ACETATES OBTAINED FROM METHYLATED CELL-WALL MATERIAL OF DWARF AND RUNNER BEAN

Alditol acetate[a]	T[b]	*Relative amounts*[c]	
		Dwarf	*Runner*
3,4-Di-*O*-methylrhamnitol	0.90	3.9 (3.9)[d]	3.3 (4.3)
3-*O*-Methylrhamnitol	1.69	0.8	0.6
2,3,5-Tri-*O*-methylarabinitol	0.47	2.3 (9.5)	2.5 (8.9)
3,5-Di-*O*-methylarabinitol	0.83	0.4	0.6
2,3-Di-*O*-methylarabinitol	1.09	5.6	5.5
2-*O*-Methylarabinitol	1.90	1.2	0.7
2,3,4-Tri-*O*-methylxylitol	0.61	2.3 (4.9)	3.0 (4.3)
3,4-Di-*O*-methylxylitol	1.19[e]	1.1	1.5
2,3-Di-*O*-methylxylitol	1.19	4.0	4.4
Tri-*O*-methylhexitol[f]	2.04	5.1	4.6
2,3,4,6-Tetra-*O*-methylgalactitol	1.19	1.17 (14.1)	2.1 (15.2)
2,3,6-Tri-*O*-methylgalactitol	2.25	9.0	11.4
2,3,4-Tri-*O*-methylgalactitol	2.79	1.1	1.2
2,3,6-Tri-*O*-methylglucitol	2.30	43.7 (65.5)	45.9 (64.0)
2,6-Di-*O*-methylglucitol	3.26	2.4 [2.6][g]	2.5 [2.6]
3,6-Di-*O*-methylglucitol	3.58	3.0 [2.9]	2.8 [2.9]
2,3-Di-*O*-methylglucitol	4.28	6.6 [3.3]	7.4 [3.3]

[a]Alditol acetates presented in this manner to allow direct comparison with Saeman-hydrolysis values. [b]Retention time relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol on OV-225 at 180°. [c]Calculated as relative mol %. [d]Data obtained by Saeman-hydrolysis, and presented as mol %. [e]Estimated from relative intensities of appropriate ions[4] (*m/e*). [f]See text. [g]Values, mol %, obtained from methylated, ball-milled filter paper.

The results of methylation analysis of the wall preparations (Table III) show that many of the structural features established[4] for potato CWM are also encountered in the bean preparations. The exception being that, in the potato material, the bulk of the cellulose was undermethylated, probably due to the shorter treatment with base, and was therefore not solubilised by chloroform–methanol. A single treatment methylated "completely" all of the non-cellulosic polysaccharides and a large proportion of the cellulose. In addition to i.r. data, the "complete methylation" of the wall polysaccharides was indicated by (*1*) the absence of unmethylated monomers in the hydrolysate of the methylated CWM, (*2*) the presence of only a few types of methylated ethers of each sugar, and (*3*) the correspondence between the amounts of tetra- and tri-methyl ethers representing non-reducing end-groups of hexoses and pentoses, respectively, and dimethyl ethers representing branch points. Item *3* has to be qualified, because the cellulose is not fully methylated and would give rise to a small proportion of di-*O*-methylglucose derivatives. Assuming that the incompletely methylated cellulose of the CWM would yield approximately equal amounts of 2,6-di-, 3,6-di-, and 2,3-di-*O*-methylglucose derivatives (an assumption based on studies with ball-milled filter paper), it would appear that the bean preparations contain a small proportion of non-cellulosic polysaccharides that have 4,6-linked glucose residues (see footnote to Table III).

The formation of appreciable amounts of 2,3,4-tri-*O*-methylxylose and 2,3,5-tri-*O*-methylarabinose derivatives suggests that the xylose and arabinose residues are present in the pyranoid and furanoid forms, respectively. All other component sugars are assumed to be present in the pyranoid form.

The results make it possible to outline the general structural features of the constituent, neutral polysaccharides. It is clear that the bulk of the (1→4)-linked glucose residues arise from cellulose. The number of end groups, represented by tri-*O*-methyl-arabinose (2.3–2.5%) and -xylose (2.3–3.0%), and tetra-*O*-methyl-galactose (1.7–2.1%), corresponds with that required by the number of branching points, as determined by the amount of 2-*O*-methylarabinose (0.7–1.2%), 3-*O*-methylrhamnose (0.6–0.8%), and 2,3-di-*O*-methylglucose (3.3–4.1%). In the above calculation, the presence of ~8.8% of 2,3-di-, 2,6-di-, and 3,6-di-*O*-methylglucose is ascribed to undermethylated cellulose.

Additional evidence for the overall structure of the CWM was obtained by carboxyl-reduction[12] of the methylated CWM with $LiAlD_4$. Hydrolysis of the reduced product gave increased amounts of galactose and rhamnose derivatives, with only slight changes in the proportions of the other sugar derivatives. Only the galactose derivative corresponding to 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methylgalactitol was deuterated at C-6, thus providing quantitative, unambiguous identification of the major uronic acid residues (see Fig. 1). These results are consistent with the occurrence of (1→4)-linked galacturonan chain, common to pectic substances, with interspersed 2-linked rhamnosyl residues. A little branching probably occurs through C-4 of rhamnose. Such residues are present in pectic substances of plant tissues[15–19].

A summary of the identifiable glycosidic linkages after carboxyl-reduction is

TABLE IV

ALDITOL ACETATES OBTAINED FROM METHYLATED CWM OF DWARF AND RUNNER BEAN AFTER CARBOXYL-REDUCTION[a]

Alditol acetate	T	*Relative amount*	
		Dwarf	*Runner*
3,4-Di-*O*-methylrhamnitol	0.90	4.3 (2.4)	3.9 (2.5)
3-*O*-Methylrhamnitol	1.69	1.6	1.4
2,3,5-Tri-*O*-methylarabinitol	0.47	2.9 (5.8)	2.9 (5.5)
3,5-Di-*O*-methylarabinitol	0.83	traces	traces
2,3-Di-*O*-methylarabinitol	1.09	4.3	4.1
2-*O*-Methylarabinitol	1.90	1.1	1.3
2,3,4-Tri-*O*-methylxylitol	0.61	3.7 (3.0)	3.9 (2.5)
3,4-Di-*O*-methylxylitol	1.19	0.9	0.9
2,3-Di-*O*-methylxylitol	1.19	3.9	4.2
Tri-*O*-methylhexitol	2.04	4.2	4.1
2,3,4,6-Tetra-*O*-methylgalactitol	1.19	1.8 (8.0)	2.0 (9.0)
2,3,6-Tri-*O*-methylgalactitol	2.25	4.8	6.4
2,3,4-Tri-*O*-methylgalactitol	2.79	0.8	0.7
2,3,6-Tri-*O*-methylglucitol	2.30	24.2 (39.4)	23.3 (37.8)
2,6-Di-*O*-methylglucitol	3.26	0.9 [1.3]	0.9 [1.3]
3,6-Di-*O*-methylglucitol	3.58	1.7 [1.5]	2.0 [1.5]
2,3-Di-*O*-methylglucitol	4.28	3.9 [1.7]	2.8 [1.7]
2,3-Di-*O*-methylgalactitol[b]	4.32	33.7 (39.4)[c]	35.6 (40.7)

[a]All data presented as in Table III. [b]After carboxyl-reduction and work-up; (1→4)-linked galacturonic acid chromatographs as 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methylgalactitol, and can be distinguished from neutral derivatives by the incorporation of deuterium at C-6. [c]Estimated by a modified carbazole method[7].

shown in Table IV. The overall recoveries obtained from methylation analysis and Saeman-hydrolysis are in broad agreement. It should be borne in mind, however, that the methylation analysis of polysaccharides containing a high proportion of uronic acid residues is difficult. There may be selective losses due to degradation of pectinic acid (pectic acid containing an appreciable proportion of esterified galacturonic acid residues), since prolonged contact with base may result in β-elimination reactions of esterified residues, depolymerization, and an underestimate of the content of galacturonic acid residues. This possibility would account for the lower recovery of galacturonic acid by methylation analysis.

The results indicate that both preparations are chemically very similar. The non-cellulosic polysaccharides contain mainly (1→4)-linked galacturonan chains, probably interspersed with (1→2)- and (1→2,4)-linked rhamnopyranosyl residues. Also present are polymers containing (1→4)-linked galactopyranosyl, (1→5)-linked arabinofuranosyl, (1→4)-linked xylopyranosyl, and (1→2)-linked xylopyranosyl residues. The major branch-points involve C-6 of glucose, C-4 of rhamnose, and C-3 of arabinose residues. Arabinose, xylose, and galactose residues constitute the non-

reducing end-groups. Glucose is not present in the terminal position. The presence of appreciable amounts of pectic substances rich in (1→4)-linked galacturonic acid would account for the bile salt-binding characteristics of the CWM from bean parenchyma[3,21]. The occurrence of appreciable amounts of arabinose residues as end groups and (1→5)-linked arabinose suggests the presence of short arabinose chains, possibly not more than 2–3 units long.

The overall compositions of the CWM from *Phaseolus vulgaris* and *Phaseolus coccineus* are very similar, both analytically and structurally. However, they are different from those of *Solanum tuberosum*[4] and monocotyledonous species[10]. It would therefore be of interest to ascertain if the *Phaseolus* species have very similar cell-wall structure. Such studies may be significant from a chemotaxonomic and physiological point of view.

EXPERIMENTAL

Materials. — Mature, dwarf french beans (*Phaseolus vulgaris* var. Prince) and runner beans (*Phaseolus coccineus* var. Streamline) were obtained from experimental plots near the laboratory. Parenchyma was obtained by scraping the pods (split in half length-wise) up to the parchment layer.

Dimethyl sulphoxide was redistilled from $CaCl_2$ under reduced pressure to remove contaminating phthalates, and kept over molecular sieve 3A. Tetrahydrofuran was distilled from $LiAlH_4$ and stored under argon. All other chemicals were of the highest purity available. Amyloglucosidase (from *Aspergillus niger*) was obtained from Boehringer.

General methods. — Infrared analysis was performed on a Pye–Unicam SP-200G spectrophotometer. Samples were prepared in CCl_4 or as KBr discs. Concentrations were performed under reduced pressure below 40° on a rotary evaporator. Dialysis was performed with continual stirring against distilled water; toluene was added to prevent microbial growth.

Cell-wall preparation. — Wall material of parenchyma was prepared by sequential extraction of fresh, ball-milled tissue with 1% aqueous sodium deoxycholate (SDC), phenol–acetic acid–water (2 : 1 : 1 w/v/v) (PAW), and 90% aqueous Me_2SO[4,5]. Fresh tissue (100 g) gave ~1 g (dry wt.) of wall preparation.

Isolation of polymers. — Comparable amounts of polymers were obtained from the extracts of dwarf and runner beans.

(*a*) *SDC-extract.* The extract was filtered, and dialysed for 48 h against several changes of distilled water. The dialysed material was concentrated and ethanol was added to yield a final concentration of 80% (v/v). The precipitate was collected by centrifugation, freeze-dried, and freed from contaminating starch by treatment with amyloglucosidase. Fresh tissue (100 g) gave ~700 mg of SDC-soluble polymers.

(*b*) *PAW-extract.* The extract was diluted with distilled water and dialysed against 25% (v/v) aqueous acetic acid and then against several changes of distilled

water. The polymers were isolated from the dialysed material as in (*a*). From 100 g of fresh tissue, ~40 mg of polymer was obtained.

(*c*) *Me_2SO-extract.* The extract was dialysed against frequent changes of distilled water and freeze-dried. From 100 g of fresh tissue, ~40 mg of solid was obtained.

Analyses. — Neutral sugars were released from CWM by using Saeman-hydrolysis[8] and hydrolysis with M H_2SO_4 for 2.5 h; the rhamnose figures were corrected for incomplete release[7]. Liberated sugars were analyzed as their alditol acetates by g.l.c.[7]. Uronic acid content was estimated by a modified carbazole method[7]; the values were corrected for interference from neutral sugars.

Amino acids were released from CWM by using 6M HCl at 110° for 24 h in a sealed tube. Corrections for losses over this period were not made. Liberated amino acids were analyzed as their heptafluorobutyryl propyl esters by g.l.c.[6].

Incubation with amyloglucosidase. — SDC-solubilised material (25 mg) was incubated in 10 ml of acetate buffer (pH 5.0) with 1 ml of amyloglucosidase (diluted to 1/10th from stock) for 36 h at 37°. The sample was then dialysed and freeze-dried. The product was analysed by g.l.c. and the carbazole method.

Methylation of cell walls. — Methylation of CWM (50 mg) was performed as previously described[4], except that polyalkoxide formation was allowed to proceed for 12 h[11]. Chloroform–methanol (1:1, 1 vol.) was then added, the mixture was vortexed and allowed to stand for 1 h, and the product was filtered through glass-fibre paper (GF/C). Insoluble material (*I*) accounted for ~5% of the original material and was not analysed further. The filtrate was dialysed against several changes of 50% aqueous ethanol and evaporated to dryness. The product (*S*) was dissolved in 5 ml of chloroform–methanol (1:1).

Analysis of methylated polysaccharides of S. — Aliquots (1 ml) of *S* were evaporated to dryness and the residues sequentially hydrolysed with 90% HCO_2H and 0.25M H_2SO_4. Liberated, partially methylated sugars were reduced with $NaBD_4$ and converted into the corresponding, partially methylated alditol acetates (PMAA). Incorporation of deuterium at C-1 facilitates mass-spectral identification of certain PMAA having similar substitution patterns[12].

The mode of linkage of poly(galactosyluronic acid) residues was determined after carboxyl-reduction of *S* with $LiAlD_4$ in tetrahydrofuran[12]. Sugars liberated by hydrolysis were reduced with $NaBH_4$ and converted into their alditol acetates as before.

Separation of PMAA was carried out as previously described[4]. Combined g.l.c.–m.s. was performed on an AEI MS-30 in the electron-impact mode. The carrier gas was helium at a flow rate of 30 ml/min. Operating conditions: inlet temperature, 250°; ionization potential, 70 eV; ion-source temperature, 200°. Data were acquired by an AEI DS 50 SM data system with continuous scanning at 10 s/decade.

Qualitative identification of each PMAA was made from the following data: (*a*) methoxyl substitution pattern from g.l.c.–m.s. analysis; (*b*) relative retention

time (T) on two columns (3% OV-225 at 180° and 3% ECNSS-M at 170°) relative to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol [components that co-chromatographed (peak 6, Fig. 1) were distinguished by selective ion-monitoring over the area of the peak]; (*c*) sugar composition of the unmethylated CWM. Quantitative analysis was achieved by using peak areas; data were converted into molar values by the methods of Sweet *et al.*[22]. Components that co-chromatographed were quantified by using relative intensities of selected ions[4].

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