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Cell-Wall Carbohydrates of the Endosperm of the Seed of *Gleditsia triacanthos*[1]

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ABSTRACT

The endosperm of the seed of *Gleditsia triacanthos* L. contains 18.55% of its dry weight as nonreserve, cell-wall carbohydrates. Of this carbohydrate material, comprising mainly mannose, galactose, and glucose, 76.1% was of low-molecular weight or highly hydrophilic. Mannose, galactose, and glucose were also the major sugar components of the polysaccharides extracted with alkali (23.1% of the cell-wall), while the same sugars, with minor amounts of arabinose, form the residues. Methylation analysis of the polysaccharides and the borate-sodium hydroxide residue indicate that the cell walls are built up on a network of galactomannans, with high Man/Gal ratios, reinforced with minor amounts of cellulose.

Gleditsia triacanthos is a dicot of the family Leguminosae, subfamily Caesalpinioideae. The seeds are albuminated, and the endosperm consists of a parenchymatic tissue of branched cells with thick primary walls containing as an incrustant, water-soluble reserve, galactomannans (8). These galactomannans consist of a β-(1 → 4)-linked D-mannosyl backbone with α-(1 → 6)-linked stubs of D-galactose (4). Fine structural details modulate this gross structure in each case with possible consequences on the secondary structure, and on the formation of aggregates (8).

To the best of our knowledge, no studies have been carried out on the composition of these primary cell walls, although the nature of its water-extractable polysaccharides and the specialized function of the endosperm suggest that this composition could be very different from those previously known (1, 10).

We now report studies on the composition of the carbohydrates from the cell walls of the endosperm of the seeds of *G. triacanthos*.

MATERIAL AND METHODS

Plant Material

The seeds of *Gleditsia triacanthos* L. were obtained from ripe pods collected at the Ciudad Universitaria (Buenos Aires). The separation of the endosperm was performed as previously described (8).

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Microscopical Examinations

The endosperm tissue as well as that remaining after the different extraction procedures were examined by optical and scanning electron microscopy (SEM).

Extraction

The fractionation is shown in Scheme 1; products of each extraction and their yields are given in Table I. The endosperm (30 g) was treated with water (5 L) at 95°C for 24 h, three times immediately after milling (8). Further extraction in the same conditions (1 L) did not produce any carbohydrate material.

The residue (EE) remaining after the aqueous extractions (18.5% based on dry weight) was exhaustively extracted with 7 M urea at room temperature with constant mechanical stirring for 8 h. The new residue (EU) was centrifuged off, and the extracts were combined and dialyzed against distilled water in a closed system for 48 h (DU). Presence of carbohydrates in the dialysis water was checked by the phenol-sulfuric acid method. The dialyzed extract was concentrated to 300 mL and submitted to stepwise addition of ethanol. Only traces precipitated at 30 to 40% ethanol concentration (0.05% of the exhausted endosperm), which were discarded. The 85% ethanol-soluble products were recovered by concentration and freeze-drying of the solution (SU).

The remaining residue (EU) was exhaustively extracted with 1% ammonium oxalate solution at boiling temperature for 2 h with constant mechanical stirring. After centrifugation of the residue (EO), the combined extracts were worked as before, the addition of ethanol gave no precipitate, and the loss of carbohydrates by dialysis was also checked (DO). The 85% ethanol-soluble products were obtained as above (SO).

The residue of the previous extraction (EO) was submitted to exhaustive extractions with 10% potassium hydroxide solution containing 1% sodium borohydride at room temperature, with constant mechanical stirring and nitrogen bubbling, for 6 h. The insoluble (EK) was centrifuged off and washed several times with water, which was added to the second extract. Each extract was immediately dialyzed up to neutrality (about 72 h) and a little precipitate appeared, which was discarded. The dialyzed extracts were combined and concentrated to 150 mL when a precipitate appeared (PCK) which was separated. Ethanol was added stepwise to the supernatant liquor, and a precipitation occurred between 40 and 50% ethanol concentration (PK). Further addition of ethanol did

not produce precipitate, and the 85% ethanol-soluble products were recovered by concentration and freeze-drying (SK).

Exhaustive extraction for 24 h with 25% sodium hydroxide containing 5% sodium borate and 1% sodium borohydride (5) was attempted over the latter residue (EK). The extracts and the new residue (EN) were treated as above, and similar insoluble products were obtained: PDN (which precipitated during the dialysis), PCN (which precipitated during concentration), and PN (which precipitated between 40–50% ethanol concentration); 85% ethanol-soluble products were recovered as usual (SN). Loss of carbohydrates by dialysis was also checked for the alkaline extractions.

The exhaustively extracted, endosperm (EA) (0.8 g) was dried in the usual way (8).

Analytical Methods

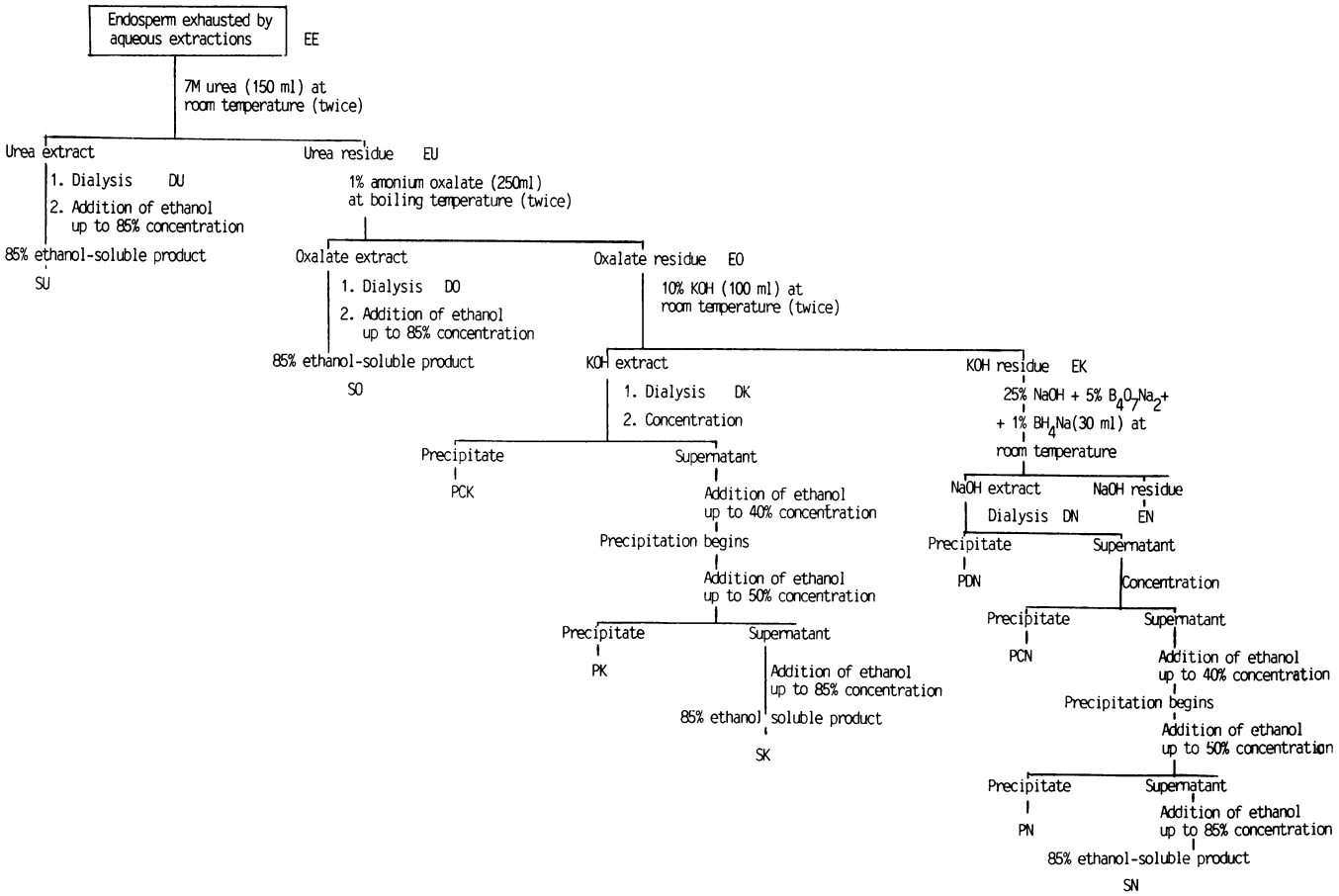
General procedures have been described (8). Amino acid analyses were performed using a Beckman 119 CL Amino-Acid Analyser, following hydrolysis in the usual conditions (8).

The hydrolysis of the polysaccharides was carried out with 2 *N*-trifluoroacetic acid for 2 h at 121°C. Sugar analysis, through the alditol acetate derivatives, was performed by the gas-liquid chromatographic method of Talmadge *et al.* (15) using a glass column (0.2 × 180 cm) of 3% ECNSS-M on Gas Chrom (100–200 mesh) at 190°C, with a nitrogen flow rate of 24 mL/min. The extracted endosperm and its methylation residue were hydrolyzed with 72% (w/v and w/w) sulfuric acid at 30°C for 2 h with magnetic stirring. The solutions were diluted 10 times and were treated at 98°C for 4 h. The hydrolysis products were then submitted to a second hydrolysis with 2 M TFA as above.

Hydroxyproline content was analyzed by the spectrophotometric method of Leach (6). Uronic acids were determined by the method of Bitter and Muir (3).

Methylation Analysis

Alkali-soluble products were methylated by a two-step sequence of Haworth and Hakomori procedures as previously described (8). Exhaustively extracted endosperm was submitted to the methylation procedure described by Taldmadge *et al.* (15). Permethylated polysaccharide samples were hydrolyzed for 2 h at 121°C with 2 M TFA. The resulting partially methylated aldoses were converted into the corresponding alditol acetates and analytical GLC was conducted in the same column as before, but at 170°C. Differentiation of tetra-*O*-methyl derivatives of glucose and mannose was afforded by the procedure of Bebault *et al.* (2), using GLC-MS of the partially *O*-acetylated, partially *O*-methylated acetates.



Scheme 1

Table I. *Yields of the Products Extracted with 7 M Urea, 1% Ammonium Oxalate, and Alkali, and Obtained by Fractionation of These Extracts, from the Endosperm of the Seed of Gleditsia triacanthos*[a, b]

Extraction Solvent	Total Yield[c]	Weight				
		Lost by dialysis[d, e]	Precipitated by neutralization[d]	Precipitated by concentration[d]	Precipitated by addition of ethanol[d]	Soluble in 85% ethanol[d, f]
	%			%		
7 M Urea	32	65 (DU)			tr	35 (SU)
1% Ammonium oxalate	21	37 (DO)				63 (SO)
10% KOH	32	46 (DK)	tr	34.3 (PCK)	11.1 (PK)	8.6 (SK)
25% NaOH/5% sodium borate	14.2	16 (DN)	9.0 (PDN)	28 (PCN)	23.4 (PN)	23.6 (SN)
Insoluble	0.8 (EA)					

[a] Previously exhausted with water at 95°. [b] For nomenclature see the text or Scheme 1. [c] Relative to water-exhausted endosperm. [d] Relative to the total amount of material extracted with each solvent. [e] The total amount of material extracted from the water-exhausted residue with the different solvents and lost by dialysis was 45.6% of the residue. [f] The total amount of material extracted from the water-exhausted residue with the different solvents and that, after extraction, remained soluble in 85% ethanol was 30.5% of the residue.

RESULTS

Microscopical Observations

The cells of the ripe endosperm of *Gleditsia triacanthos* are substellate with thick walls and wide intercellular spaces. The protoplast is reduced to the small cell-lumen left by the reserve-walls (Figs. 1A; 2, A and B). Microscopical examination shows the absence of starch grains. The endosperm was exhaustively treated with hot water (95°C) to solubilize the water-extractable galactomannans and to inactivate enzymes. After the extraction, the cells were not broken but the thickening of the walls was remarkably diminished and the wall appeared less compact (Figs. 1B; 2, C and D).

The extraction of the cells with 7 M urea (Figs. 1C and 2E) and further with 1% oxalate (Figs. 1D and 2G) produced contraction and walls notably thinner. The spatial relationship between the cell shape and the intercellular spaces did not change. SEM shows that the wall is more porous (Fig. 2, F and H).

The extraction with 10% KOH and afterward with 25% NaOH plus 5% borate produced different results. In the first case, the cell was contracted but its shape was maintained and the walls were rendered thin and frail (Figs. 1E; 2, I and J). On the other hand, after the NaOH 25% extraction, the cells were broken and all the tissue formed an amorphous mass with occasionally visible cellular contours (Figs. 1F; 2, K and L).

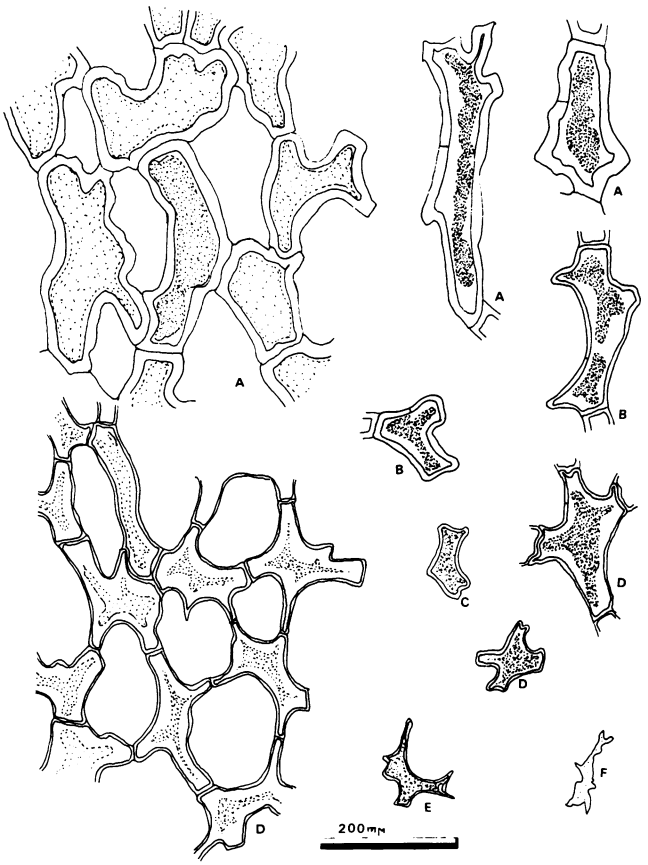


Figure 1. A–E, walls of endosperm cells of *G. triacanthos* (drawings). A, Nonextracted endosperm; B, endosperm extracted with hot water; C, endosperm extracted with 7 M urea; D, endosperm extracted with boiling 1% ammonium oxalate; E, endosperm extracted with 10% potassium hydroxide; F, endosperm extracted with 25% sodium hydroxide/5% sodium borate.

Chemical Studies

The composition of the residue remaining after the hot water extraction (EE) (18.5% yield) was 82% carbohydrate and 18% protein. The main component sugars were mannose (74.0%) and galactose (16.3%) with minor amounts of arabinose (6.2%) and glucose (2.3%) and traces of rhamnose, xylose, and *N*-acetylglucosamine (Table II). The molar ratio Man/Gal was 4.5.

When EE was extracted with 7 M urea and further with 1% oxalate, 53% of the cell wall dissolved, but the composition of the final residue was similar to that of the starting material with major amounts of mannose and galactose (80.8 and 11.6%, respectively). The molar ratio Man/Gal increased to 7.0. The two extractions yielded 32 and 21% material (calculated from the weight of the residues, EU and EO), respectively. About 65 and 37% (DU and DO, respectively) of the extracts were lost by dialysis, and the remaining materials (SU

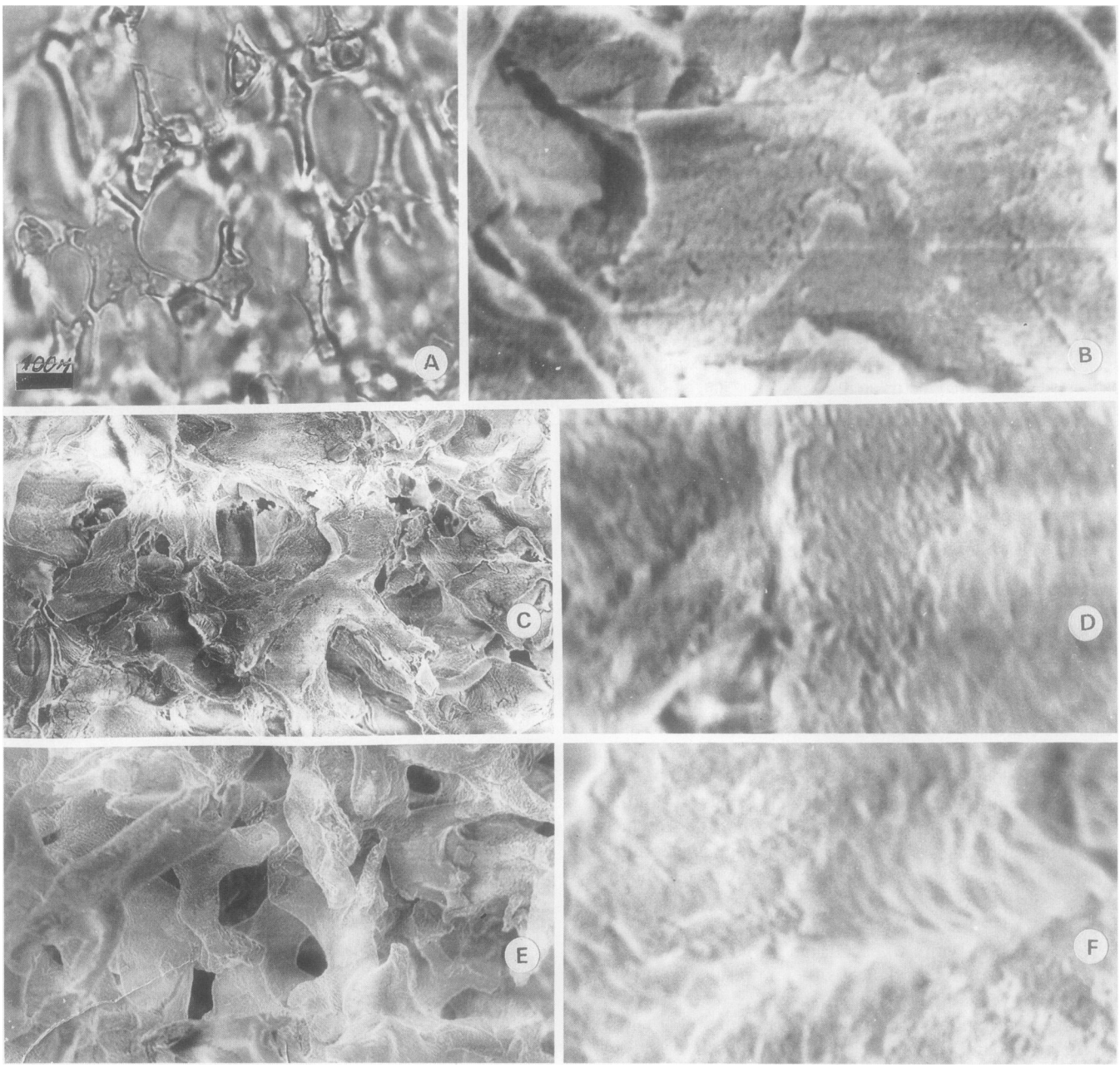


Figure 2. A–L, walls of endosperm cells of *G. triacanthos* observed with OM (optical microscopy) and SEM (scanning electron microscopy). A, Nonextracted endosperm (OM); B, nonextracted endosperm (SEM); C and D, endosperm extracted with hot water (SEM); E and F, endosperm extracted with 7 M urea (SEM); G and H, endosperm extracted with boiling 1% ammonium oxalate (SEM); I and J, endosperm extracted with 10% potassium hydroxide (SEM); K and L, endosperm extracted with 25% sodium hydroxide/5% sodium borate (SEM).

and SO) were soluble in 85% ethanol (Table I). The carbohydrate composition of the 85% ethanol-soluble extracts SU and SO (Table III) follow a similar pattern. Both extracts have the same Man/Gal ratio (2.0–2.1). Uronic acid was not found in SU or SO.

The extraction with 10% KOH produced a residue (EK) composed of mannose plus galactose (91.1%), but the Man/Gal molar ratio increased to 10.3. There were also minor amounts of arabinose (3.9%) and glucose (5.0%) (Table II).

The extraction with 25% NaOH/5% borate gave a residue (EN) the composition of which is given in Table II. Treatment with 2 *N*-TFA or 72% (w/v) sulfuric acid left a small residue, but total solubilization was obtained with 72% (w/w) sulfuric acid. In this case the major sugar was glucose (62.7%) and the molar ratio Man/Gal was 9.6, similar to that found in the residue (EK) after the extraction with 10% KOH (Table II).

Precipitation of polysaccharides occurred during the neutralization of the sodium hydroxide extract (PDN) and during the concentration of both alkaline extracts (PCK and PCN) (Table I). The remaining solutions were fractionated by stepwise addition of ethanol, and in both cases precipitates appeared at 40 to 50% ethanol concentration (PK and PN)

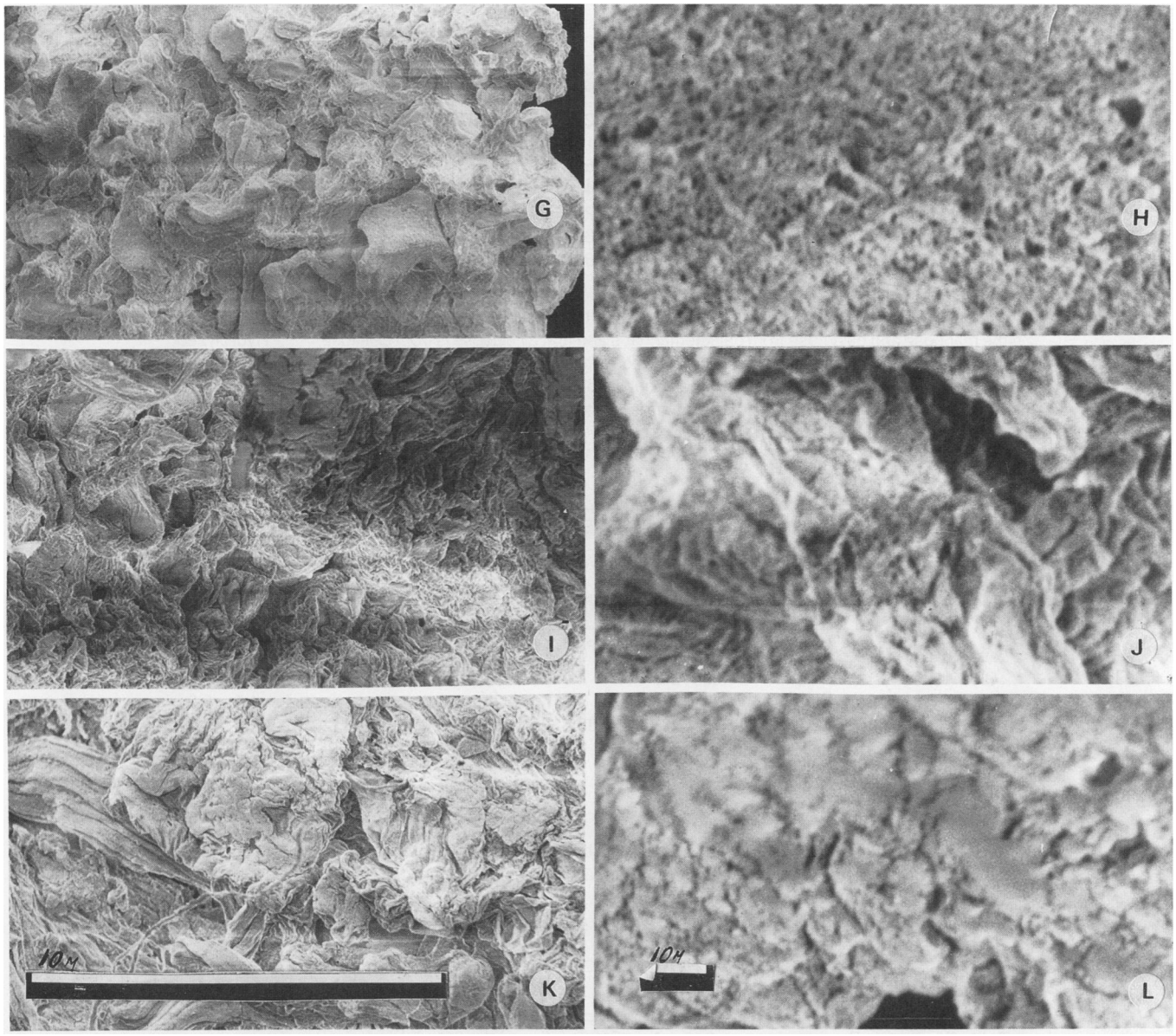


Figure 2. *Continued*

(Table I). The mother liquors yielded the 85% ethanol-soluble products. The carbohydrate composition of the 85% ethanol-soluble extracts SK and SN shows a similar pattern with some quantitative differences (Table III). They have the same Man/Gal ratio (17.0–17.5). No uronic acids were found in SK and SN.

The yields of the extractions as well as those obtained in the fractionations proved to be difficult to reproduce. The materials extracted from the residue (EE) with the different solvents that were lost by dialysis represented 45.6% of that residue, and the ones extracted that remained soluble in 85% ethanol were 30.8% of the residue (Table I).

The composition of the polysaccharide fractions isolated is given in Table III. The polysaccharides contain major amounts of mannose, together with significant quantities of galactose and glucose. Glucose becomes the major component of the sodium-hydroxide/borate residue (EA). The fractions contain also traces of xylose, rhamnose, and/or glucosamine.

All the fractions and residues contained protein. The percentages and composition of the protein associated with soluble fractions, residues, and PK is given in Table IV; they contain little hydroxyproline, if at all. The major amino acids are glutamic acid-glutamine, aspartic acid-asparagine, alanine, and leucine. Nevertheless, in all the samples but EK, no amino acid predominates clearly.

Some polysaccharide fractions (PCK, PCN, and PN) were submitted to methylation analysis, and the permethylated derivatives were obtained through a two-step sequence of a Haworth and Hakomori procedure. Their relative proportions of partially methylated monosaccharides are shown in Table V. These three products yield the mannose and galactose derivatives obtained after the permethylation of reserve galactomannas, namely: 2,3,4,6-tetra-*O*-methylgalactose, 2,3,6-tri-*O*-methylmannose, and 2,3-di-*O*-methylmannose. A small amount of 2,6-di-*O*-methylmannose was found in the meth-

Table II. *Carbohydrate Composition of the Residues (mol %)*

Fraction[a]	Sugar[b]				Man/Gal Ratio	Galactomannan/Cellulose[c] Ratio
	Ara	Man	Gal	Glc		
EE	6.2	74.0	16.3	2.3	4.5	39.3
EO	3.8	80.8	11.6	1.3	7.0	71.1
EK	3.9	83.0	8.1	5.0	10.3	18.2
EN[d]		77.0	8.6	14.4	9.0	5.9
EN[e]		33.7	3.5	62.7	9.6	0.6
EN[f]	6.2	38.9	6.4	46.2	6.1	1.0
ENI[g]		24.6	5.8	56.0	3.6	0.7

[a] For nomenclature, see text or Scheme 1. [b] Minor amounts of xylose, rhamnose, and/or 2-acetamido-2-deoxy-D-glucose were detected in all the fractions. [c] Calculated on the basis that all the mannose and galactose from galactomannans and that all glucose is from cellulose. [d] Hydrolyzed with 72% (w/v) sulfuric acid. [e] Hydrolyzed with 72% (w/w) sulfuric acid. [f] Hydrolyzed with trifluoroacetic acid. [g] Chloroform-methanol insoluble fraction obtained after methylation of EA, hydrolyzed with 72% (w/w) sulfuric acid. This fraction also contains partially methylated sugars (see text).

Table III. *Carbohydrate Compositions of the Alkali-Extracted Polysaccharides and of the 85% Ethanol-Soluble, Nondialyzable Products*[a]

Sugars	Alkali-extracted polysaccharides[b]								85% ethanol-soluble, non-dialyzable, products[b,c]						
	P[d]	P^d_{50}	P^d_{95}	PCK	PK	PDN	PCN	PN	S[e]	S^e_{50}	S^e_{95}	SU	SO	SK	SN
Glc				6.8	12.2	30.7	4.9	6.3	24.4	8.8	3.5	12.0	29.3	11.5	2.2
Gal	26.1	18.9	21.9	17.4	14.5	6.5	9.3	6.3	24.7	27.4	15.4	21.3	21.2	4.5	5.3
Man	66.5	78.0	73.7	75.5	73.2	63.4	85.5	85.1	38.1	26.6	26.0	43.6	43.0	76.4	92.5
Xyl	1.0								2.3	5.3	3.7	4.2			
Ara	5.4	3.1	4.0						5.6	18.9	46.7	12.0	4.0	4.7	
Fuc									1.5		2.0	7.0	2.0	3.0	
Man/Gal	2.6	4.1	3.4	4.3	5.0	9.8	8.9	13.5	1.5	1.0	1.7	2.1	2.0	17.0	17.5

[a] Those products (S, S_{50} and S_{95}) extracted with water were not submitted to dialysis. [b] For nomenclature see text or Scheme 1. [c] Minor amounts of 2-acetamido 2-deoxy D-glucose were detected in all fractions. [d] Composition of the galactomannans extracted with water at room temperature, 50 and 95°C, see ref. (8). [e] Composition of the 85% ethanol-soluble products extracted with water at room temperature, 50 and 95°C (8).

Table IV. *Protein Percentages and Amino Acid Composition of the 85% Ethanol-Soluble Extracts, Residues, and Polysaccharides Isolated from the Endosperm Cell Walls of G. triacanthos*

Fraction[a]	Protein	Amino Acids[b]															
		Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	Lys	His	Arg	Hyp[c]
	%	*g/16 g N*																
PE[d]		13.2	4.5	7.0	14.3	4.0	3.6	7.8	6.1		7.3	8.4	5.9	4.8	5.2	1.6	6.2	
SU	2.9	17.2	3.3	5.2	16.8		5.8	4.1	4.6	1.7	2.2	7.4	2.0	2.4	2.6	3.7	3.6	
SO	4.5	11.6	5.2	7.4	15.4	4.1	7.4	8.1	5.4	0.6	7.2	7.5	3.0	3.0	3.4	4.5	5.3	0.5
SK	16.0	3.7	10.6	4.4	12.7	5.0	4.1	10.2	7.1	0.8	5.4	13.2	4.6	5.5	2.3	1.9	8.9	
SN	0.3	11.6	5.8	11.7	8.3	4.4	3.5	9.2	6.3	1.0	5.8	7.6	2.5	4.2	8.3	3.2	5.8	0.8
EE	17.7	2.8	5.0	7.5	18.2	3.5	7.5	7.1	5.0		5.1	8.5	4.1	4.2	4.5	0.8	6.3	
EO	24.6	10.0	4.6	7.9	14.3	3.3	10.9	6.2	6.4	0.6	6.4	8.3	3.7	4.0	4.4	2.0	6.9	
EK	23.6	9.8		16.8	5.9	2.5	24.6	10.1	3.5		2.6	5.3	3.0	2.8	5.9	3.7	3.4	
EN	3.8	8.2	6.2	5.9	10.9	4.8	3.5	8.2	9.3	0.9	6.8	13.1	3.4	7.4	4.3	1.1	5.5	0.5
PK	0.2	13.1	5.3	11.1	16.6	4.2	9.8	9.2	4.4	3.1	2.9	6.2	3.8	3.3	4.7	2.2		

[a] For nomenclature, see text or Scheme 1. [b] Percentages lower than 0.5% have not been considered. [c] The lack of hydroxyproline was shown not only with the amino acid analyser but also through the Leach's spectrophotometric method (6). [d] Amino acid composition of a fraction of reserve galactomannans extracted with water at room temperature (8).

Table V. *Relative Proportions of Methylated Sugars from the Products Obtained by Extraction with Alkali from the Endosperm[a] of the Seeds of G. triacanthos, and of the Totally Exhausted Endosperm (mol %)*

Fraction	Deduced Glycosidic Linkages	PCK	PCN	PN	EA
Sugar residues					
Mannose					
2,3,4,6-tetramethyl	Terminal	2.2	3.3	1.8	4.3
2,3,6-trimethyl	4-	60.9	74.9	76.8	48.0
2,3-dimethyl	4,6-	9.9	5.1	8.9	2.8
2,6-dimethyl	3,4-	1.9	1.9	–	2.2
Galactose					
2,3,4,6-tetramethyl	Terminal	19.0	9.8	6.9	7.5
Glucose					
2,3,6-trimethyl	4-	6.0	4.6	5.6	35.3
Minimum d.p. of the galactomannan moiety[b]		46	30	56	23

[a] Previously exhausted with water at 95°C 7 M urea and 1% ammonium oxalate. [b] d.p., Degree of polimerization. Determined on the basis of the nonreducing, end chain mannose (see text).

ylated derivatives of the polysaccharides PCK and PCN suggesting that some lateral chains were linked through (1→3) linkages. The molar ratio of tetramethylated galactose to dimethylated mannoses is about 1.4 to 1.6 in PCK, PCN, and EA suggesting a slight undermethylation. In addition, the presence of 2,3,6-tri-*O*-methylglucose together with small amounts of a tetramethyl derivative was observed. The latter was isolated by preparative paper chromatography and unambiguously identified as 2,3,4,6-tetra-*O*-methylmannose by GLC-MS of the acetate. The proportion of tetra-*O*-methyl derivative of mannose allows us to calculate, on the basis that it is produced by the nonreducing end-chain of the mannose backbone, the minimum degree of polymerization (d.p.) of these polysaccharides (Table V). Neither nonreducing, end-chain glucose nor methylated derivatives of xylose, arabinose, or rhamnose were found. The molar ratio Man/Gal determined in the methylated polysaccharides is similar to that obtained from the hydrolysis of the nonmethylated ones.

As the residue (EN) was insoluble in 60% sodium hydroxide, its methylation was attempted by performing a partial methylation using the Hakomori conditions. The product of the first methylation was extracted with chloroform-methanol which solubilized 10.5% of material. Analysis of this fraction indicated undermethylation, and the permethylated derivative was obtained after a second Hakomori methylation (Table V). This product showed the same partially methylated derivatives found in the above mentioned polysaccharides but now the molar ratio Man/Gal changed to 7.6 and the 4-linked glucose became one of the major sugars. The insoluble fraction was composed of 86.4% nonmethylated sugars, namely: glucose (56.0%), mannose (24.6%), and galactose (5.8%) together with minor amounts (13.6%) of incompletely methylated derivatives of the same sugars: 2,3,6-tri-*O*-methylmannose (0.9%), 2,6-di-*O*-methylgalactose (2.7%), 2,3-di-*O*-methylglucose (0.7%), 6-*O*-methylglucose (3.9%), and 2-*O*-methylmannose (5.5%).

DISCUSSION

The morphology of the endosperm cells of *Gleditsia triacanthos* (Fig. 1A) is similar to that of the carob seed (14) having a clear division into aleurone layer and storage tissue, and a living protoplast. In the endosperm of fenugreek seed most of the cells appear to be completely filled with polysaccharides and must be considered nonliving (12). The walls of the endosperm cells of *G. triacanthos* contain 81.5% of water-extractable carbohydrates, mainly galactomannans (4, 8) and 18.5% of cell-wall material. The yield of cell-wall material which is much higher than that (≃1%) obtained from growing cells (15) is in agreement with the idea of an extremely expanded tissue (11).

The carbohydrates in the residue (EE) were mainly (90.3%) the two sugars which constitute the galactomannans (Table II) with only minor amounts of glucose and arabinose. The amount of glucose (2.3%) (Table II) indicates a lower percentage of cellulose than that usually found in primary cell walls (20–30%) (15).

Nondegrading solvents extract important quantities of products (53%) of the residue EE. The products have low mol wt or high hydrophilicity (Table III). The composition of these products is similar to that of the 85% ethanol-soluble material extracted with water (7, 9) (Table III). It is noteworthy that the 85% ethanol-soluble material extracted with nonalkaline solvents contains glucose suggesting the presence of noncellulosic glucose-containing polysaccharides. Again, the residue EO contains major amounts of mannose and galactose (92.4%, Table II) but with its molar ratio increased to 7.0.

The cell shape is still maintained after the use of KOH 10%, about one-third of the original residue EE (32%, Table I) is solubilized and from this nearly half (46%) has a low mol wt (SK). Its composition is similar to that of the other 85% ethanol-soluble products (Table III). Two galactomannans were obtained with similar Man/Gal ratios (4.3 and 5.0, respectively) (Table I and III).

The residue (EK) is again composed mainly (91.1%) of galactomannans but with the ratio Man/Gal increased to 10.3. This increasing Man/Gal ratio indicates that the extent of galactose substitution determines the association of the galactomannan molecules within the cell wall.

The cell walls were broken by the more aggressive 25% NaOH/5% borate reagent. The three galactomannans (PDN, PCN, and PN, Tables I and IV) isolated from the supernatant can be considered as mannans due to the high Man/Gal ratios. The supernatant also contains highly hydrophilic products (SN, Tables I and III) composed mainly of mannose and low mol wt material (DN, Table I).

The structure of the isolated polysaccharides, the composition of the residues, and the characteristics of the soluble fractions suggest that the cell walls are composed mainly of galactomannans with different degrees of polymerization and branching. The degree of polymerization of the alkali-soluble galactomannans is lower than that of the reserve ones. This may reflect some decomposition caused by the alkali but also can be a way to build up a cell wall easily hydrated and decomposed by enzymatic hydrolysis during germination.

The fractionation of the galactomannans is difficult to reproduce and this variability appears to be associated with temperature-, time-, and composition-dependent molecular associations, as is the case of the reserve galactomannans (8).

The endosperm cell walls contain about twice the amount of protein as the walls of growing plants (15), but the polysaccharides isolated retain only trace amounts of proteins, which remain in the residues and soluble fractions (Table V). The composition of the proteins suggests that they are not extensin-like, being similar to those precipitated with the reserve galactomannans (8). The amino acid composition was also similar to that of the protein associated with barley endosperm cells (13). This is compatible with a nontightly cross-linked cell wall. The Hakomori treatment of EN yielded 10.5% of chloroform-methanol soluble derivatives of galactomannans (65%) and cellulose (35%) (Table V). The insoluble fraction contained undermethylated galactomannans and 86.4% of nonmethylated sugars, chiefly glucose (56%). These results show that the fractionation is due to the different accesibility of the residue to the methylating reagents. Therefore, part of the galactomannans are not extracted with alkali, indicating a strong association of galactomanns with high Man/Gal ratio with the cellulose.

The swelling properties of the endosperm require that the cell walls be constructed on the basis of a few strong bonds and many groups of weak bonds (12). The first ones are obviously formed between the β-mannan, ribbon-like molecules and reinforced through the hydrogen-bonding with a small amount of cellulose. Little is known about the kind of weak bonds but the low mol wt and/or high hydrophilicity of most of the cell-wall components must play some role in their formation.

It is likely that the galactomannans bind not only to cellulose but also to themselves and that in regions of the molecules containing a high proportion of side chains the aggregation is disrupted. Hence, a single galactomannan molecule may contain regions which bind to cellulose, regions which bind to other galactomannan molecules, and regions that do not form aggregates at all.

The Gramineae *Triticum aestivum* is taxonomically very far from the leguminose *Gleditsia triacanthos* (Dicots). Moreover, the endosperm tissue of the Gramineae contains cytoplasmic starch grains as reserve polysaccharides, whereas the endosperm of *G. triacanthos* has the galactomannans as reserve polysaccharides in their thick cell walls. In spite of these differences, several similarities between both types of endosperm walls are noteworthy, namely: (a) the major polysaccharide components, arabinoxylans (9) and galactomannans, are essentially hydrophilic molecules, building up endosperms which are hard only in the noninhibited state. These polysaccharides with different chemical structures, present similar bonding patterns producing aggregates with comparable mechanical properties. (b) Cellulose, which is an important component of the walls of plant cell, is reduced to trace amounts in both endosperm walls suggesting that their microfibrils network is not so strong. (c) Both endosperm walls contain comparable amounts of protein which composition is similar to that of the protein extracted with water. This protein, which is not extensin, is probably not cross-linked to polysaccharides.

The composition and organization of endosperm walls may reflect an adaptation to their function. During development, while the reserve material is accumulating, some compactness is required but at germination the highly hydrophilic components absorb, and retain, large amounts of water. This allows access of enzymes from the aleurone layer which further solubilize and depolymerize the wall, reaching the incrusting reserve galactomannans in the case of Leguminosae or the cytoplasmic starch granules and protein bodies in the Gramineae.

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