

## POLYSACCHARIDE COMPOSITION IN RELATION TO EXTENSIBILITY AND POSSIBLE PEPTIDE LINKED ARABINO-GALACTAN OF LUPIN HYPOCOTYL CELL WALLS

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**Abstract**—The relationship between extensibility, growth rate and carbohydrate composition in different sections of lupin hypocotyl has been investigated. Although significant differences in extensibility were found, the carbohydrate composition of elongating and non-elongating regions was similar. The nature of a possible arabino-galactan in hypocotyl cell walls was studied with particular reference to its insolubility in pectin solvents and solubilization by well-established delignification procedures or dilute acid. Barium hydroxide hydrolysis also preferentially released the cell wall arabinose and galactose.

### INTRODUCTION

THE PHYSICAL properties of the plant cell wall are known to be an important factor governing the rate of cell elongation<sup>1</sup> but the way in which extensibility is structurally controlled is as yet unknown. Cell wall carbohydrates could exert such control by either a change in the level of some structural polysaccharide or by the production of a specific controlling polymer or linkage.

Although many studies of cell wall structure have been made,<sup>2</sup> no correlations between extensibility,<sup>3</sup> growth rate, and total carbohydrate composition have been reported. The structural polysaccharide composition of elongating and non-elongating sections of lupin hypocotyl have, therefore, been measured to discover possible correlations between extensibility and polysaccharide composition. The possibility that the glycoprotein extensin, which is rich in hydroxyproline linked arabinose and galactose,<sup>4</sup> controls extensibility by cross-linkage to polysaccharides has been suggested by Lamport.<sup>5</sup> Levels of galactose and arabinose in the various depectinated cell wall preparations were therefore also measured. The results are considered in relation to extensin and the more conventional pectin and hemicellulose polysaccharides of the plant cell wall.

### RESULTS AND DISCUSSION

#### *Growth and Extensibility of Lupin Hypocotyl*

When lupin hypocotyls were divided into lower (L), middle (M) and upper (U) regions the various extensibilities measured (plastic and elastic compliance, and creep, Table 1) showed differences between the three sections which were significant at the 1% level using

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<sup>1</sup> R. CLELAND, *Planta* **74**, 197 (1967).

<sup>2</sup> D. H. NORTHCOTE, *Essays in Biochemistry* **5**, 89 (1969).

<sup>3</sup> R. CLELAND, *Ann. Rev. Plant Physiol.* **22**, 197 (1971).

<sup>4</sup> D. LAMPORT, *Biochem.* **8**, 1155 (1969).

<sup>5</sup> D. LAMPORT, *Ann. Rev. Plant Physiol.* **21**, 235 (1970).

the method of paired comparisons. Most elongation (76%) was in the upper region (Table 1). The turgor pressure was found to vary only slightly between the three regions.<sup>6</sup>

TABLE 1. COMPOSITION AND PROPERTIES OF THREE REGIONS OF 6 CM LUPIN HYPOCOTYL

Properties	Hypocotyl region		
	Lower	Middle	Upper
Length (cm)	2	2	2
Elongation rate (% of total for hypocotyl)	0	24	76
Total compliance (DT) (mm <sup>2</sup> /Newton)	1.17 ( $\pm 0.06$ )	1.43 ( $\pm 0.08$ )	1.68 ( $\pm 0.07$ )
Plastic compliance (DP) (mm <sup>2</sup> /Newton)	0.36 ( $\pm 0.024$ )	0.53 ( $\pm 0.03$ )	0.66 ( $\pm 0.03$ )
Elastic compliance (DE) (mm <sup>2</sup> /Newton)	0.81 ( $\pm 0.04$ )	0.90 ( $\pm 0.05$ )	1.0 ( $\pm 0.05$ )
Ratio (DE/DP)	2.27	1.71	1.55
Creep (% increase in length)*	1.21 ( $\pm 0.15$ )	1.91 ( $\pm 0.30$ )	4.35 ( $\pm 0.39$ )

\* Measured between 1 and 100 min after adding 100 g weight.

#### *Carbohydrate Composition of Cell Wall Preparations from Lupin Hypocotyl*

Acid fractionation of the cell walls from the three regions showed that they did not differ significantly in terms of pectic polyuronide, total hemicellulose or cellulose (Table 2a) or in the monosaccharide composition of the fractions (Table 2b). Alkaline fractionation of the cell wall holocellulose also showed no major difference in polysaccharide content or in monosaccharide content of the polymer between elongating and non-elongating sections (Table 3). On the above evidence the differences in growth rate or extensibility observed in the three regions of the lupin hypocotyl could not be correlated with gross quantitative changes in polysaccharide composition of the cell wall. If structural polysaccharides in any way exert control over extensibility then it must be by some means such as cross linkage which is not necessarily reflected in the amount of polysaccharide.

#### *Acid Solubility of Cell Wall Fractions Containing Arabinose and Galactose*

Comparison of the monosaccharide compositions of the polysaccharides obtained by the acid and alkaline fractionations respectively showed a large discrepancy in the quantities of arabinose and galactose present. More than 90% of the arabinose and galactose of Table 2a is unaccounted for in the alkali fractionation (Table 3). The fact that this loss arose during delignification was confirmed by the following experiment: Liberated monosaccharides were measured in *N*-acid hydrolysates of depectinated hypocotyl cell wall material before and after delignification with chloramine *T*-acetic acid.<sup>7</sup> The results showed that the following percentages of total cell wall monosaccharides were removed by the delignification: arabinose 95+%, galactose 70%, xylose 30%, mannose 20% and glucose 15%. Acetic acid of the strength and pH (3.6) encountered in the chloramine *T* treatment caused a similar, although lower, preferential solubilization of arabinose and galactose, as did the chlorite-acetic acid (pH 4.5) delignification of Whistler and BeMiller.<sup>8</sup>

<sup>6</sup> D. PENNY, P. PENNY, J. MONRO and R. W. BAILEY, *Proc. 7th Int. Conf. on Plant Growth Regulators* (in press).

<sup>7</sup> B. D. E. GAILLARD, *J. Sci. Food Agric.* **9**, 170, 346 (1958).

<sup>8</sup> R. L. WHISTLER and J. M. BEMILLER, *Methods in Carbohydrate Chemistry* **3**, 21 (1964).

TABLE 2. CARBOHYDRATE COMPOSITION BY ACID FRACTIONATION OF CELL WALL OF THREE REGIONS OF HYPOCOTYL

Fraction	Hypocotyl region					
	Lower		Middle		Upper	
(a) Polysaccharide fraction (% of hypocotyl tissue dry wt)						
Pectin	5.6		5.1		6.2	
Hemicellulose	4.3		4.7		3.6	
Cellulose	9.5		8.2		8.0	
Lignin	1.0		0.6		0.6	
(b) Monosaccharide content of fraction (% of fraction)						
	Hc*	C	Hc	C	Hc	C
Glucose	33.9	85.9	33.4	79.8	36.4	80.8
Galactose	10.1	—	11.8	—	12.9	—
Mannose	2.6	10.2	3.1	15.2	4.1	13.5
Xylose	23.2	3.8	25.9	5.0	23.9	5.7
Arabinose	30.1	—	25.8	—	22.7	—

\* Hc and C stand for hemicellulose and cellulose respectively.

The solutions from all acid and delignification treatments were dialysed against tap water for 48 hr, hydrolysed with  $\text{NH}_2\text{SO}_4$  at  $100^\circ$  and analysed by paper chromatography. The chromatograms showed the presence of galactose and arabinose and in largest amounts from the delignification treatments.

TABLE 3. COMPOSITION OF DELIGNIFIED HOLOCELLULOSE BY ALKALINE EXTRACTION. THE POLYSACCHARIDIES EXTRACTED AND MONOSACCHARIDE COMPOSITION OF THE POLYSACCHARIDES

Polysaccharide fraction	Polysaccharide composition of hypocotyl region			Monosaccharide composition*				
	Lower	Middle	Upper	Glucose	Galactose	Mannose	Xylose	Arabinose
	(% of total polysaccharide)			(% of total anhydro-sugar in fraction)				
Branched B <sub>10</sub> †	2.5	3.7	2.4	40	10	5	40	5
Linear B <sub>10</sub>	8.0	9.5	12.0	39	7	3	50	1
Hemicellulose B <sub>24</sub> †	7.3	8.2	6.7	55	2	33	10	0
Hemicellulose A <sub>10</sub>	0	0	0	—	—	—	—	—
Cellulose	82.2	78.5	79.0	94	0	6	0	0

\* Monosaccharide composition same for each polysaccharide from all three hypocotyl regions.

† Subscripts 10 and 24 refer to 10% and 24% alkali solubility.

All of these treatments involved heat ( $75$ – $100^\circ$ ) at a pH 3.6–4.5 suggesting the possibility of cleavage of acid-labile, furanoside-linked sugar units from the hemicellulose xylan. While this possibility might apply to the arabinose it is unlikely to explain the release of galactose. In any case in all of the above delignification and acetic acid treatments the released sugars were largely non-dialysable suggesting that they were still present in polymer form. If the polymer is an arabino-galactan it appears to be bound to the cell wall in a way that renders it insoluble in hot water or oxalate, but by links which are very acid labile. In this connection the hydroxyproline-arabinose links of extensin are apparently acid labile.<sup>5</sup>

High arabinose and galactose contents have been reported for other acid hydrolyses of total unextracted hemicellulose from non-delignified immature legume tissue, e.g. pea (*Pisum sativum*) epicotyl<sup>9</sup> and mung bean (*Phaseolus aureus*) hypocotyl.<sup>10</sup> The present results indicate that such tissues should be delignified with caution as the conventional delignification procedures can cause very large losses of polysaccharide compared with the much smaller losses reported for other tissues such as *Avena*.<sup>11</sup>

TABLE 4. BARIUM HYDROXIDE EXTRACTION OF DEPECTINATED HYPOCOTYL CELL WALL

Treatment of wall	Hemicellulose monosaccharides* (relative to glucose)			
	Galactose	Arabinose	Xylose	Glucose
Depectinated	4.03	3.23	3.75	1
Ba(OH) <sub>2</sub> extracted	0.64	0.25	3.0	1

\* Mannose present but not measured.

*Possible Relationship of Acid Soluble Arabinose and Galactose with the Glycoprotein Extensin*

Although extensin has been shown to contain acid-labile hydroxyproline-arabinose links, evidence for the nature of this compound is indirect and based on partial degradations as the glycoprotein has not so far been extracted intact from plant cell walls. Treatment with hot barium hydroxide does not break the hydroxyproline-arabinose bonds but does break peptide links to release hydroxyproline-(arabinoxyl)<sub>1-4</sub> compounds,<sup>12</sup> apparently without dissolving the conventional hemicellulose polymers. Lupin hypocotyl was therefore

TABLE 5. CARBOHYDRATE COMPOSITION OF BARIUM HYDROXIDE HYDROLYSATE OF DEPECTINATED LUPIN HYPOCOTYL CELL WALLS

Fraction	Reducing sugars (glucose equivalent, mg/g of cell wall)	Arabinose: galactose
Hydrolysate	5.14	—
Hydrolysate after acid hydrolysis	43.98	6:1
Hydrolysate after dialysis followed by acid hydrolysis	14.2	2.5:1

homogenized, depectinated by extraction with neutral detergent and ammonium oxalate and finally subjected to the barium hydroxide hydrolysis. Analysis of the walls before and after this hydrolysis showed that nearly all of the arabinose and galactose had been dissolved, as shown in Table 4. In contrast, the barium hydroxide does not appear to have solubilized the normal cell wall xylan.

<sup>9</sup> M. C. PROBINE, *Proc. Roy. Soc.* **161B**, 526 (1965).

<sup>10</sup> R. W. BAILEY, S. HAQ and W. Z. HASSID, *Phytochem.* **7**, 2037 (1968).

<sup>11</sup> J. S. G. REID and K. C. B. WILKIE, *Phytochem.* **8**, 2045 (1969).

<sup>12</sup> D. T. A. LAMPORT, *Nature, Lond.* **216**, 1322 (1967).

When the hydrolysate from the barium hydroxide treatment was examined, results given in Table 5 were obtained. These show that very little of the released sugar was present as monomer and that much of it (33 %) was present as non-dialysable (48 hr against tap water) polymer. The change in arabinose to galactose ratio as a result of dialysis suggests that most of the arabinose is in short chain oligomers, consistent with Lamport's<sup>12</sup> hydrolysis results, and the galactose in the non-dialysable polymer. The release of the bulk of the galactose by barium hydroxide does not appear to have been recorded previously.

Hydroxyproline was used as an index of cell wall protein or extensin as this amino acid appears to be unique to cell wall protein.<sup>5</sup> Measurements showed that, in agreement with Lamport,<sup>12</sup> the barium hydroxide hydrolysates contained liberated hydroxyproline (5 mg/g of depectinated cell wall).

The almost complete removal of the cell wall galactose and arabinose by either barium hydroxide or dilute acid and delignification suggests that the same polymer is involved and that most of the cell wall arabinose and galactose not removed by depectination of lupin hypocotyl could be present as glycoprotein. Conventional carbohydrate fractionation could lose this carbohydrate moiety during delignification, at least in immature tissue. When depectinated undelignified legume cell wall material is extracted with alkali much of the galactose and arabinose appears in a water soluble hemicellulose B fraction.<sup>13</sup> It is possible that this fraction owes its initial insolubility in oxalate and solubility in alkali to association with protein in the wall rather than to some other difference from conventional, oxalate-soluble, pectic arabino-galactan.

Recently Rayle<sup>14</sup> has shown with cell walls of *Avena* coleoptile that low pH elicits an extensibility change similar to that induced by indolyl-3-acetic acid in coleoptiles. This has focused attention on acid-labile bonds in cell walls, especially the hydroxyproline-arabinose linkage known to exist in all higher plants so far investigated.<sup>5</sup> It becomes increasingly important, therefore, to understand how the carbohydrate moiety of this possible glycoprotein is structurally and metabolically related to the remainder of the cell wall.

## EXPERIMENTAL

**Plant tissue.** Seeds of blue lupin (*Lupinus angustifolius*) were germinated and grown under previously described conditions.<sup>15</sup> Hypocotyls ( $6 \pm 0.2$  cm in length) were harvested and cut into three 2-cm sections where comparison of lower, middle and upper regions was required: otherwise whole hypocotyls were excised. Tissue was deep frozen within 30 min of cutting. However, in the case of sections for extensibility measurements tissue was boiled in methanol.

**Extensibility, elongation and creep measurements.** Extensibility was measured with an Instron extensometer as described by Penny *et al.*<sup>16</sup> At least 18 sections per region were measured. Elongation was calculated from the photographically recorded separation of 2 mm markings on hypocotyls as they elongated through 6 cm. A modified beam balance<sup>17</sup> was used for the measurement of creep<sup>18</sup> on rehydrated tissue prepared as for the Instron measurements.

**Cell wall preparation.** (1) For fractionations tissue sections (60 g) were freeze-dried (5 g) and ground in a Wiley mill. In the case of acid fractionation the freeze-dried tissue was extracted with boiling 80 % EtOH for 3 min while for alkaline fractionation it was refluxed with the neutral detergent of Van Soest<sup>19</sup> for 2 hr. (2) In investigating the solubilizing effect of delignification on polysaccharides, tissue (1 g) was prepared by grinding whole fresh hypocotyls in a Wareing blender with 1 % 'Nonidet 90' detergent (Shell) in cold H<sub>2</sub>O, and extracting them with EtOH and acetone prior to refluxing for 2 hr with 0.5 % ammonium oxalate

<sup>13</sup> B. D. E. GAILLARD, *Phytochem.* **4**, 631 (1965).

<sup>14</sup> D. L. RAYLE, P. M. HAUGHTON and R. CLELAND, *Proc. Natl Acad. Sci. U.S.* **67**, 1814 (1970).

<sup>15</sup> P. PENNY, *N.Z. J. Bot.* **7**, 290 (1969).

<sup>16</sup> P. PENNY, D. PENNY, D. C. MARSHALL and J. K. HEYES, *J. Exptl Bot.* **22** (in press).

<sup>17</sup> D. PENNY, (in preparation).

<sup>18</sup> M. C. PROBINE and R. D. PRESTON, *J. Exptl Bot.* **13**, 111 (1962).

<sup>19</sup> P. J. VAN SOEST, *J. Ass. Off. Agr. Chem.* **46**, 825 (1963).

to remove pectin. (3) Where hydroxyproline determinations were to be made the tissue was refluxed successively for 1 hr with neutral detergent and then in 0.5% ammonium oxalate.

*Delignification.* The method of Gaillard<sup>7</sup> involving chloramine *T* and ethanolamine extraction was used. In this method the tissue is subjected to a pH of 3.6 for 6 hr at 100°.

*Carbohydrate extractions.* (1) *Acidic fractionation.* After removal of pectin by refluxing the cell wall preparation for 1 hr with ammonium oxalate the residue was hydrolysed successively with 1 N H<sub>2</sub>SO<sub>4</sub> under reflux for 2 hr, and 72% H<sub>2</sub>SO<sub>4</sub>.<sup>20</sup> (2) *Alkaline fractionation.* Hemicelluloses were extracted essentially as described by Gaillard and Bailey<sup>21</sup> from the delignified tissue, with 10% KOH and 24% KOH; 4% H<sub>3</sub>BO<sub>4</sub>. The extracted polysaccharides were sub-fractionated into linear and branched species by iodine precipitation in CaCl<sub>2</sub>. (3) *Barium hydroxide treatment.* Oxalate extracted tissue (ca. 1 gm) or a water extract of this tissue was treated with 0.43 M Ba(OH)<sub>2</sub> (150 ml) for 8 hr at 90° and filtered.<sup>12</sup> The filtrate was neutralized with H<sub>2</sub>SO<sub>4</sub> and filtered.

*Hydroxyproline determination.* Hydroxyproline was determined by the method of Bergman and Loxley<sup>22</sup> in Ba(OH)<sub>2</sub> hydrolysates of tissue or extracts.

*Carbohydrate analyses.* Polysaccharide fractions were hydrolysed with acid and neutralised with BaCO<sub>3</sub>. All methods for carbohydrate analysis, except uronic acids are given in Gaillard and Bailey<sup>21</sup> except that butylacetate-pyridine-H<sub>2</sub>O-EtOH (8:2:1:2) was used as the chromatography solvent. Uronic acids were measured by the carbazole method of Dische<sup>23</sup> as described by Montreuil and Spik.<sup>24</sup>

<sup>20</sup> R. W. BAILEY, *N.Z. J. Agric. Res.* **7**, 496 (1964).

<sup>21</sup> B. D. E. GAILLARD and R. W. BAILEY, *Phytochem.* **7**, 2037 (1968).

<sup>22</sup> I. BERGMAN and R. LOXLEY, *Analyt. Chem.* **35**, 1961 (1963).

<sup>23</sup> Z. DISCHE, *J. Biol. Chem.* **167**, 189 (1947).

<sup>24</sup> J. MONTREUIL and G. SPIK, *Microdosage des Glucides* (Publ. Faculte des Sciences de Lille, France) **1**, 59.

*Key Word Index*—*Lupinus angustifolius*; Leguminosae; polysaccharide; cell walls; extensibility; arabino-galactose hydroxyproline.