Nutrition and Selection/Breeding. The fact that (1) the first limiting amino acid may vary even between roots from the same plant and (2) it varies between three different amino acids (leucine, lysine, and the S-containing amino acids) has important nutritional consequences. Thus, the well-known complementarity of amino acids that exists in a diet of rice (lysine limiting), combined with legumes (limiting in S-containing amino acids), is attained within a diet of sweet potato, because of the variability between the different roots. Clearly, the combination of a reasonably good chemical score (0.60–0.73) with internal compensation of three first limiting amino acids within different roots (even from the same plant) means that there is less imbalance of essential amino acids than is indicated from the chemical score. Unfortunately, the quantity of protein present in sweet potato (0.5–3%) (and other tropical root crops) is greatly inferior to that of rice (~6%) and legumes (peas, beans) (Paul and Southgate, 1978).

For a particular amino acid, results over all samples in Table III differ by about 2–3-fold which agrees with previous results (Bradbury et al., 1984). This range is very much less than the 5.6-fold range of protein contents (0.5–2.81%) in Table I and the 167-fold range of trypsin inhibitor contents in Table II. Because of (1) the greater variability in protein content than in protein quality and (2) the likely small loss of essential amino acids due to amino acid imbalance (see above), improvements may be made in protein quantity rather than protein quality by selection/breeding. As indicated earlier and shown in Figure 1, the best cultivars identified in this work for high and only moderately variable protein content over different environments in the Highlands of Papua New Guinea are Simbul Sowar and Takion.

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Characterization of Polysaccharides from White Lupin (*Lupinus albus* L.) Cotyledons

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Cell wall material (CWM) was isolated from defatted white lupin cotyledon flour by Pronase and α-amylase treatment. The main sugar component of CWM was galactose, followed by arabinose and uronic acids. Small quantities of rhamnose, fucose, xylose, and glucose were also present. Methylation analysis of CWM showed that galactose units were mainly (1→4) linked. Only small numbers of branched points were present on galactose units. Arabinose was included in a highly branched structure. Xylose was present mainly as nonreducing terminal units and glucose as (1→4)-linked units. CWM was fractionated into pectic substances, hemicelluloses A and B, and α-cellulose. Pectic substances (the largest fraction) were further separated, by ion-exchange chromatography, into a neutral fraction and several acidic polysaccharides. Gel filtration of the neutral fraction produced a polysaccharide primarily composed of galactose.

The development of alkaloid-free mutants of several lupin species (*Lupinus albus*, *Lupinus angustifolius*, *Lupinus luteus*) has allowed the exploitation of their pulses as a protein source both for animals and for humans (Gladstones, 1970; Pompei and Lucisano, 1976a,b). In particular, the white lupin (*L. albus*), which has seeds rich in both protein and oil, appears to have considerable potential as a crop for circummediterranean countries (Hill, 1977). However, the cotyledons of white lupin seeds have a high cell wall material (CWM) content, and this might limit their nutritional value. The CWM content of lupin seed cotyledons varies greatly among species, ranging from

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7.1 to 32.1% (Brillouet and Riochet, 1983). The CWM content of white lupin cotyledons is about 20%.

The microbial fermentation of plant cell walls in the hindgut may play an important role in the digestion physiology (Rérat, 1978; Nyman and Asp, 1982). Since it has been demonstrated, in both pigs and rats, that volatile fatty acids may be absorbed from the hindgut (Friend et al., 1964; Yang et al., 1970), it seems likely that microbial fermentation can produce available energy. But, microbial fermentation can also influence the fecal nitrogen excretion (Rérat, 1978) and the generation of intestinal gases (Fleming, 1981) in nonruminants. It is pertinent to point out here that the poor growth rates of pigs fed on diets containing lupin is thought to be partly attributable to flatulence (Bourdon et al., 1980).

The extent to which plant cell walls are broken down by microbial fermentation probably depends to some extent on their structure (Nyman and Asp, 1982). It is therefore clear that any assessment of the nutritional properties of white lupin cotyledon CWM must include extensive biochemical investigations of their composition and structure.

The presence of β-(1→4)-linked galactose in alkali extracts of white lupin cotyledons was first reported by Hirst et al. (1947). However, the amount present appeared to be very low ($\simeq$0.25%/cotyledons) relative to the total cell wall galactose content ($\simeq$10%/cotyledons) (Brillouet and Riochet, 1983). The presence of terminal arabinosyl residues was also reported by Hirst et al. (1947). The aim of this study was to obtain further information on the nonstarchy polysaccharides present in white lupin cotyledons. Cell wall material (CWM) was isolated from white lupin cotyledons by an enzymatic procedure. Individual sugars occurring in CWM and in water-soluble nonstarchy polysaccharides (WSP) were analyzed. Structural characteristics of polysaccharides were investigated by methylation analysis (Hakomori, 1964) of whole CWM. CWM was also fractionated into pectic substances (hot EDTA extract), hemicelluloses (alkali extract), and α-cellulose, and the pectic substances obtained were separated into neutral and acidic fractions by ion-exchange chromatography.

As a comparison with the CWM isolation procedure, the neutral detergent fiber (NDF) residue was isolated by using the Van Soest's procedure (Van Soest and Wine, 1967). Then, the NDF residue was analyzed for neutral and acidic sugars.

EXPERIMENTAL SECTION

White Lupin Cotyledons. Sweet white lupin seeds (*L. albus* L., cv. Kalina) were harvested in 1980 at the Station d'Amélioration des Plantes Fourragères, Lusignan (France). Cotyledons were obtained from hand-dissected seeds and milled to pass through a 0.5-mm screen.

Chemicals. Purified dimethyl sulfoxide (Me_2SO) (<0.03% H_2O; stored over a molecular sieve) and sodium hydride (~80% in oil), used for methylation analysis, were purchased from Merck (West Germany) and Fluka (Switzerland), respectively. Oligosaccharides and polysaccharides used for synthesis of partially methylated alditol acetates standards were obtained from the following sources: larchwood xylan and microcrystalline cellulose from Sigma (USA); stachyose from Serva (West Germany). Kraft lignin was purchased from Westvaco (USA). *m*-Phenylphenol (MHDP) was from Eastman Kodak Co. (USA). Pronase from *Streptomyces griseus* (6 units/mg) and α-amylase (EC 3.2.1.1) from *Bacillus subtilis* (1350 units/mg) were obtained from Boehringer (West Germany). Amyloglucosidase (EC 3.2.1.3) from *Aspergillus niger* (50 units/mg) was obtained from Merck.

Preparation of Cotyledon Cell Wall Material (CWM). Lipids and pigments were extracted from native flour (100 g) in a Soxhlet apparatus, by successive treatments with chloroform–methanol (2:1) and methanol–water (80:20). After air-drying, the defatted flour (74 g) was suspended in 2 L of 0.1 M phosphate buffer, pH 7.5, containing 0.02% sodium azide as bactericide, and the suspension was homogenized with Polytron. Proteolysis was then started by the addition of Pronase (400 mg). Peptide release was monitored by measuring 10% TCA soluble amino acids and oligopeptides (Saunders and Kohler, 1972). After a 9-h hydrolysis at 30 °C, the medium was centrifuged and the residue retreated with Pronase (200 mg) for a further 6-h treatment. After centrifugation, the residue was heated in 1 L of water for 5 min at 95 °C to allow starch gelatinization, and the temperature was then adjusted to 50 °C before the addition of α-amylase (1.25 mg in 100 mL of 0.2 M acetate buffer, pH 5.6). Amylolysis was allowed to proceed for 3 h. The course of the hydrolysis was monitored by measuring reducing end groups by using the Somogyi–Nelson procedure (Nelson, 1944). The residue was recovered by centrifugation, extensively washed with water, and dried by solvent exchange through ethanol, acetone, and diethyl ether. The resulting white powder was considered as purified CWM.

Supernatants from Pronase and α-amylase treatments were pooled, extensively dialyzed against distilled water, and poured into 5 volumes of ethanol. The resulting precipitate, considered as water-soluble polysaccharides (WSP), was recovered by centrifugation, washed, and dried as above.

Analytical Methods. Moisture contents were determined by drying at 130 °C for 2 h. Proteins ($N \times 5.7$) in CWM were determined by a micro-Kjeldahl procedure. Proteins occurring in WSP were measured by the Folin–Lowry method (Lowry et al., 1951), with bovine serum albumin (BSA) as the standard. Starch was determined in defatted flour and corresponding CWM as follows: 100 mg of material was stirred overnight in 95% aqueous Me_2SO (2 mL) at ambient temperature, diluted to 40% Me_2SO, and then treated with amyloglucosidase (1 mg) for 2 h at 50 °C. Prior to glucose analysis, the digestion medium from defatted flour was diluted to 8% Me_2SO and treated with charcoal to prevent the action of interfering substances. Glucose was measured by the glucose oxydase–peroxydase–ABTS system (Colonna et al., 1981).

Minerals were determined by X-ray dispersion microanalysis (Colonna et al., 1980). Lignin in CWM was estimated by a modified acetyl bromide method (Morrison, 1972; Monties and Rambourg, 1978): CWM (150 mg) was treated with 20 mL of 1 M NaOH for 24 h at 20 °C to remove phenolic acids and the residue rinsed with water and dried. Lignin content was determined on the alkali-treated residue by using a kraft lignin as the standard. Uronic acids were measured by the *m*-phenylphenol (MHDP) method with galacturonic acid as the standard (Blumenkrantz and Asboe-Hansen, 1973); the CWM was dispersed in concentrated sulfuric acid (El Rayah Ahmed and Labavitch, 1977) prior to the uronic acid analysis.

Polysaccharides were hydrolyzed with 2 M trifluoroacetic acid (TFA) for 1 h at 120 °C (Albersheim et al., 1967) or by pretreatment with 72% sulfuric acid for 1.5 h at 20 °C followed by dilution to 1 M and heating at 100 °C for 2.5 h (Saeman et al., 1954). Liberated neutral sugars were analyzed as their alditol acetate derivatives (Sawardeker et al., 1965) by GC on a glass column (180 × 0.2 cm) packed with 3% SP 2340 coated on Supelcoport (100–120 mesh),

Table I. Yield and Composition of Water-Soluble Polysaccharides (WSP) from White Lupin Cotyledons

		relative sugar distribution[c]								
yield[a]	polysaccharides[b]	rhamnose	fucose	arabinose	xylose	mannose	galactose	glucose	uronic acids	protein[d]
6.9	24.2	0.8	0.8	13.2	2.5	18.2	49.2	7.8	7.4	33.0

[a] Percent of cotyledon dry matter. [b] Percent of WSP dry matter. [c] Percent of polysaccharides, expressed as anhydropolymeric sugars. [d] Percent of WSP dry matter, determined by the Lowry procedure with BSA as the standard.

at 225 °C. Inositol was used as the internal standard.

Methylation of CWM. Methylation of CWM (40 mg) was carried out according to Hakomori (1964) as described by Ring and Selvendran (1978). Following methylation of CWM, 1 volume of chloroform–methanol, 1:1, was added. The mixture was then dialyzed against 50% aqueous ethanol and evaporated to dryness. The resulting product was remethylated twice by the same procedure. The final dry product was then blended with chloroform–methanol, 1:1, filtered on a glass crucible and washed. The methylation yield (80%) was calculated by weighing the chloroform–methanol-insoluble fraction, which represented 20% of the starting CWM. The methylated polysaccharides present in the filtrate were successively hydrolyzed with 90% formic acid (1 h, 100 °C) and 0.25 M H_2SO_4 (12 h, 100 °C) (Jansson et al., 1976). The partially methylated sugars produced were derivatized as their alditol acetate form (Sawardeker et al., 1965) and analyzed by GC and GC–MS on two glass columns (280 × 0.2 cm) packed with 3% OV-225 and 3% ECNSS-M, respectively, each one coated on Gas-Chrom Q (100–120 mesh). Separations were performed isothermally at 170 °C. The mass spectrometer, operating with the OV-225 column, was run under the following conditions: 250 °C inlet temperature, 70-eV ionization potential, and 220 °C ion source temperature (Ring and Selvendran, 1978). Mass spectra were recorded on several portions of peaks. Partially methylated alditol acetates were identified on both columns by relative retention times (RR_t) with reference to 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol, and by their related mass spectra. The data obtained were compared with previously reported results (Talmadge et al., 1973; Jansson et al., 1976; Ukai et al., 1978) and with GC–MS chromatograms of standards obtained from methylated larchwood xylan, microcrystalline cellulose, and stachyose.

Fractionation of CWM. CWM (5 g) was extracted with 2% aqueous disodium ethylenediaminetetraacetate (EDTA) (500 mL), pH 5.0, for 4 h at 100 °C (Aspinall et al., 1967a). After centrifugation the residue was retreated 3 times under the same conditions. Supernatants from successive extractions were pooled, concentrated, extensively dialyzed against water, and precipitated by adding 2.3 volumes of ethanol. The precipitate was recovered by centrifugation, washed by 70% ethanol, dried by solvent exchange, and thereafter called the pectic substance (PS) fraction.

Hemicelluloses were extracted from the EDTA-insoluble residue (660 mg) with 10% potassium hydroxide (100 mL) under nitrogen, for 16 h at 20 °C. Hemicellulose A (14 mg) was precipitated from supernatant by acidifying to pH 5.0 with glacial acetic acid, recovered by centrifugation, and washed and dried by solvent exchange. Five volumes of ethanol were added to the acidified supernatant to allow precipitation of hemicellulose B (216 mg). The cellulosic residue (α-cellulose) (306 mg) from the alkali extraction was also washed and dried. All fractions were analyzed for acidic and neutral sugars by the methods described above. The final cellulosic residue was hydrolyzed according to Saeman et al. (1954).

Fractionation of Pectic Substances (PS). A sample (180 mg in 15 mL of 0.05 M acetate buffer, pH 4.8) of the total pectic substances (PS) fraction was applied to a column (16 × 5.0 cm) of DEAE-Sepharose CL-6B (acetate form) (Pharmacia). After the column was washed with 0.05 M acetate buffer, pH 4.8 (unbound fraction), a linear gradient (0.05–1 M) of acetate buffer, pH 4.8, was applied to the column (bound fraction) (Barbier and Thibault, 1982). Fractions (21 mL) were collected and analyzed automatically for uronic acids and neutral sugars by the MHDP (Thibault, 1979) and orcinol (Tollier and Robin, 1979) methods, respectively, using galacturonic acid and galactose as standards. Orcinol responses were corrected for uronic acid interferences. The unbound fraction and bound polysaccharides (eight fractions) were extensively dialyzed against water, concentrated, and analyzed for monosaccharide by GC as described above.

The unbound fraction (5 mg in 5 mL of water) was submitted to gel filtration on a column (82 × 2.2 cm) of Sepharose CL-6B (Pharmacia) and eluted with distilled water. Fractions (4.8 mL) were collected and analyzed automatically for uronic acids and neutral sugars as described above. Material eluted within the fractionation range (excluding the void volume fraction) was concentrated and analyzed by GC.

Yields in different pectic fractions were calculated from colorimetric measurements.

RESULTS AND DISCUSSION

Composition of Water-Soluble Polysaccharides (WSP). Some polysaccharides went into solution during both enzymatic treatments used for cell wall isolation, the greatest quantity being liberated during Pronase treatment. Both supernatant from proteolysis and amylosysis were pooled and considered as a whole. WSP yield was 6.9% of the starting native flour. It contained 24.2% polysaccharides and 33% proteins (Table I). The remaining portion was essentially minerals originating from buffers, as shown by X-ray dispersive microanalysis. Proteins of WSP were resistant to an additional Pronase treatment, possibly due to linkages with polysaccharides, lipids, or phenolic compounds. The soluble polysaccharide moiety, which represented only 1.6% of the cotyledons, was mainly composed of galactose, mannose, arabinose, and uronic acids (Table I). The relatively high mannose content might be related to the presence of minute amount of galactomannan in the lupin native flour, as such polymers are of wide occurrence in legume seeds (Bailey, 1971). Rhamnose and uronic acids probably corresponded to rhamnogalacturonan chains (Mc Neil et al., 1980). It have been already mentioned that Pronase treatment could lead to the release of a small portion of pectic substances, this being mainly due to the action of the buffer (Stevens and Selvendran, 1980a). A similar qualitative sugar distribution has been observed in WSP from *L. luteus* cotyledons (Matheson and Saini, 1977).

Composition of Cell Wall Material (CWM). Cell wall material (CWM) was obtained by Pronase and α-amylase treatments of the defatted flour, as described by Brillouet and Carré (1983). The first Pronase treatment released 97% of total proteins ($N \times 5.7$) and the second one only

Table II. Yield and Composition of Cell Wall Material (CWM) Isolated from White Lupin Cotyledons

yield[a]	20.2
polysaccharides[b]	
rhamnose[c]	1.4
fucose[d]	0.6
arabinose[d]	12.0
xylose[d]	3.6
mannose[d]	tr[i]
galactose[d]	48.5
glucose (noncellulosic)[c]	0.9
glucose (cellulosic)[e]	5.0
uronic acids[f]	11.6
noncarbohydrates	
proteins ($N \times 5.7$)[b]	5.6
lignin[b,g]	1.7
minerals[b,h]	0.5
total	91.4

[a] Percent of cotyledon dry matter. [b] Percent of cell wall dry matter. Expressed as anhydropolymeric sugars. [c] Determined from TFA hydrolysate. [d] Determined from Saeman hydrolysate. [e] Determined as difference between Saeman and TFA hydrolysate values. [f] Determined colorimetrically by using the MHDP method. [g] Determined by the acetyl bromide method with kraft lignin as the standard. [h] Determined by X-ray dispersion microanalysis. [i] Traces.

0.7%. By light microscope examination after Fast Green staining, it appeared that the remaining proteins (5.6%/CWM) were not of cytoplasmic origin but were cell wall components, since only the middle lamella area was stained green. Lupin seeds are known to contain minute amounts of starch (Gross and Von Baer, 1977). However, a destarching treatment was carried out to prevent any ambiguous results concerning the glucose determinations. By light microscope examination (polarized light), some residual starch granules were observed even after the gelatinization (95 °C)–α-amylolysis sequence. However, the starch content of purified CWM, measured by the Me_2SO–amyloglucosidase method (Boehringer, 1978), was only 0.1% and was then considered as negligible.

The neutral sugar composition was obtained from analyses of TFA (Albersheim et al., 1967) and Saeman hydrolates. The Saeman hydrolysate provided the highest values except for rhamnose. Noncellulosic glucose was determined by analysis of TFA hydrolysate, and cellulosic glucose was estimated by the difference from total glucose measured from Saeman hydrolysis.

As already reported (Brillouet and Riochet, 1983), the yield of CWM was strikingly high (20.2%) as compared to values reported for other legume seeds: yellow lupin (12.3%), pea (7%), broad bean (7%), soybean (9%), and groundnut (10%) (Brillouet and Carré, 1983; Reichert, 1981; Tharanathan et al., 1975). A similar CWM content (22%) was obtained by Crawshaw and Reid (1984) on blue lupin flour.

The sugar composition of CWM (Table II) is in agreement with previously reported results (Brillouet and Riochet, 1983): the main components were galactose (48.5%), arabinose (12.0%), and uronic acids (11.6%), glucose (5.9%) and xylose (3.6%) being minor constituents. This figure is characteristic of "primary" cell walls rich in pectic substances (Albersheim, 1976).

Cellulose and lignin contents (5.0 and 1.7% of CWM, respectively) were low and of same magnitude as in other legume seeds when expressed in percent of cotyledons: 1.0 and 0.3% of lupin native flour vs. 0.8 and 0.3% for soybean vs. 0.8 and 0.2% for broad bean (Brillouet and Carré, 1983).

It is likely that cell wall proteins are partly connected with some polysaccharidic moieties: in a nutritional experiment where a large amount of CWM was prepared by using a far lower amount of Pronase than in this experiment (10 times less) and antibiotics instead of sodium azide, a tremendous decrease (~75%) of galactose was observed, with a concomitant fall in Pronase-resistant protein (~73%); other wall polysaccharidic constituents remained unchanged, exhibiting similar molar ratios. This phenomenon may be related to the galactose–serine *O*-glycosidic links described by Lamport et al. (1973) in structural protein. The partial degradation of galactose polymers could be due to endogenous depolymerases, as already observed during germination of yellow lupin and blue lupin seeds (Matheson and Saini, 1977; Crawshaw and Reid, 1984).

As a comparison with our CWM isolation procedure, the NDF residue (Van Soest and Wine, 1967) was isolated and analyzed. The very low NDF content of the flour (4.3%) indicates that the cell walls were considerably degraded during the NDF preparation. Analyses of NDF polysaccharides revealed that all components were affected except glucose. Losses relative to CWM were as follows: rhamnose, 95%; fucose, 80%; arabinose, 80.7%; xylose, 70.1%; galactose, 85.8%; uronic acids, 86.0%. This observation is in agreement with the results of Bailey and Ulyatt (1970), which indicate that the bulk of pectic substances are removed during the NDF preparation. As Reichert (1981) has pointed out, NDF content cannot be used for the estimation of cell wall content in material containing pectic polysaccharides.

Structural Analysis of CWM Polysaccharides. The interglycosidic links between neutral sugars were investigated by using the Hakomori (1964) methylation procedure. Three successive methylations were performed on CWM to improve the yield of methylated polysaccharides that represented, as the chloroform–methanol-soluble fraction, 80% of CWM. After hydrolysis and alditol acetate derivatization, partially methylated alditol acetates were analyzed by GC and GC–MS. Fifteen compounds were identified on the OV-225 column (Figure 1). Most of the peaks were homogeneous except peak 7 for which variations of fragmentation pattern were observed along its plotting. On the rising portion of peak 7, the mass spectrum was as follows (peak mass numbers and their relative intensities in brackets): 43 (100), 45 (17), 71 (9), 87 (18), 99 (6), 101 (19), 113 (4), 117 (37), 129 (27), 145 (10), 161 (9), 173 (1.2), 189 (7), 205 (3). On the descending portion it was 43 (100), 45 (25), 71 (7), 87 (14), 99 (4), 101 (27), 113 (4), 117 (27), 129 (19), 145 (17), 161 (13), 173 (1.3), 189 (2), and 205 (7). All fragments except m/e 189, belonged to 2,3,4,6-tetra-*O*-methylhexitol. Fragment m/e 189 corresponded to 2,3- (or 3,4) di-*O*-methylpentitol. The bulk of this last derivative was eluted in the rising portion of peak 7, where the mass spectrum exhibited a higher intensity of peak 189 and lower intensities of peaks corresponding specifically to the terminal hexitol derivative (peaks 145 and 205). With respect to the $RR_t = 1.18$, hexitol and pentitol derivatives of peak 7 corresponded to galactitol and xylitol methyl ethers, respectively. Separation and quantitation of 2,3,4,6-tetra-*O*-methylgalactitol and 2,3- (or 3,4) di-*O*-methylxylitol derivatives were achieved by GC on ECNSS-M column. Identification of 2,3,4,6-tetra-*O*-methylgalactitol ($RR_t = 1.22$ on ECNSS-M) was confirmed by comparison with an authentic standard of terminal galactose prepared from methylated stachyose.

With respect to the yield of the chloroform–methanol-soluble fraction (80% of starting CWM), it appeared that the bulk of cell wall polysaccharides was methylated. That was reflected in the relatively good agreement between molar ratios of methylated alditol acetates and that of their

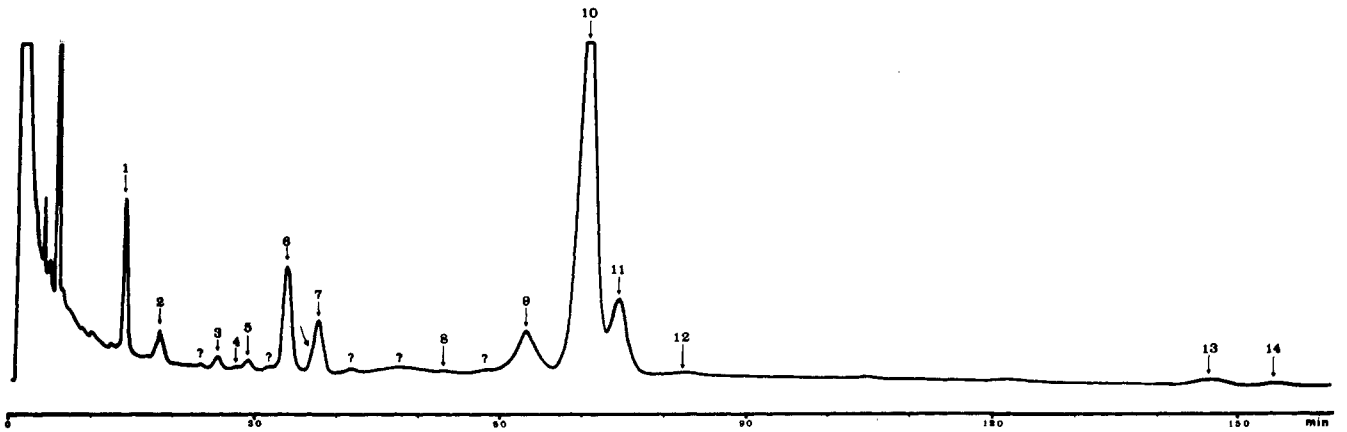

Figure 1. Chromatogram of partially methylated alditol acetates obtained from three successive methylations of white lupin cotyledon cell wall material. An OV-225 column was used at 170 °C. The identified peaks corresponded to (1) 2,3,5-tri-*O*-methylarabinitol, (2) 2,3,4-tri-*O*-methylxylitol, (3) 3,5-di-*O*-methylarabinitol, (4) 3,4-di-*O*-methylrhamnitol, (5) 2,3-di-*O*-methylrhamnitol, (6) 2,3-di-*O*-methylarabinitol, (7) 2,3,4,6-tetra-*O*-methylgalactitol and 2,3- (or 3,4-) di-*O*-methylxylitol (indicated by the arrow on the rising portion of the peak), (8) 3-*O*-methylrhamnitol, (9) 3-*O*-methylarabinitol, (10) 2,3,6-tri-*O*-methylgalactitol, (11) 2,3,6-tri-*O*-methylglucitol, (12) arabinitol, (13) 2,3-di-*O*-methylglucitol, and (14) 2,3-di-*O*-methylgalactitol.

Table III. Methylation Analysis of Cell Wall Polysaccharides from White Lupin Cotyledons

O-methyl ether[a]	T_1[b]	T_2[b]	rel mol %	rel mol % of parent sugars	rel mol % of parent sugars, by direct analysis[c]
2,3-Me_2Rha	0.91	0.98	0.7		
3,4-Me_2Rha	0.87	0.91	tr[d]	1.4	1.8
3-MeRha	1.66	1.88	0.7		
2,3,5-Me_3Ara	0.44	0.46	5.1		
2,3-Me_2Ara	1.06	1.30	7.3		
3,5-Me_2Ara	0.79	0.90	0.6	21.4	19.6
3-MeAra	1.98	2.51	7.7		
Ara	2.59	3.51	0.7		
2,3,4-Me_3Xyl	0.57	0.63	1.3		
2,3-Me_2Xyl } 3,4-Me_2Xyl }	1.18	1.48	0.5	1.8	5.9
2,3,4,6-Me_4Gal	1.18	1.22	3.5		
2,3,6-Me_3Gal	2.22	2.48	59.6	63.7	64.8
2,3-Me_2Gal	4.75	6.09	0.6		
2,3,6-Me_3Glc	2.34	2.60	10.1	11.6	7.9
2,3-Me_2Glc	4.50	5.63	1.5		

[a] 2,3-Me_2Rha = 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl rhamnitol, etc. [b] Retention times relative to 2,3,4,6-Me_4Glc on OV-225 (T_1) and ECNSS-M (T_2) columns at 170 °C. [c] Determined from Saeman hydrolysate of CWM. [d] Traces.

parent sugars measured by direct analysis (see Table III). However, a major discrepancy was observed in the case of xylose, the relative amount of methylated xylose derivatives being very low (1.8%) in comparison to the relative amount of the parent sugar measured by direct analysis (5.9%) (Table III). This observation might be due to the remethylation procedure used in the present experiment. It is possible that most of the xylose residues occurred as xylosyl short side chains branched on the rhamnogalacturonan backbones as in the case of pectin from soybean cotyledons (Aspinall et al., 1967b). Thus, cleavages inside rhamnogalacturonan chains, induced by β-elimination due to remethylation (Mc Neil et al., 1980), could involve formation of oligogalacturonides containing xylosyl residues, which are lost during the dialysis following the methylation. This hypothesis is supported by the high proportion of xylose present as an integral component of acidic pectic substances (51%, see below).

It should be noted that the 2,3-di-*O*-methylrhamnitol derivative represented a high proportion of the rhamnosyl residues (Table III). This partially methylated derivative is very unusual and could be an artifact produced by remethylations. Mc Neil et al. (1980) observed that repeated alkylations of a pectic fraction from sycamore primary cell walls led to the production of a GC-undetectable unsaturated residue from 2-linked rhamnose and emergence of a free unsubstituted hydroxyl on C_2 from 2,4-linked rhamnose with the subsequent appearance of 2,3-di-*O*-alkyl ether. These authors attributed this phenomenon to the cleavage of glycosyl–uronic acid linkages, due to the effect of the very strongly basic dimsyl ion on the methylated acidic polysaccharides (Aspinall and Rosell, 1977). In a previous experiment (Carré and Brillouet, 1982) where the methylation was carried out in a single step, the 2,3-di-*O*-methylrhamnitol derivative was present only as traces, whereas the 3,4-di-*O*-methyl- and 3-*O*-methylrhamnitol derivatives represented 30 and 70% of the total rhamnosyl derivatives, respectively. These observations would confirm that rhamnose is linked to galacturonic acid through C_1 and C_2 as in pectic substances derived from sycamore primary cell walls (Mc Neil et al., 1980). However, the methylation yield of the single step procedure was only 20%. Therefore, in order to obtain a proper structural analysis of neutral associated polymers (arabinan, galactan, and glucan), the three-step methylation procedure was preferred, even if it appeared that this procedure would not be entirely conclusive for the investigation of rhamnosyl and xylosyl links. It must be pointed out that no noticeable degradation of neutral pectic polysaccharides is observed when the remethylation procedure is used (Aspinall and Rosell, 1977; Mc Neil et al., 1980).

The difference between the proportion of branching

Table IV. Yield and Composition of Pectic Substances, Hemicelluloses, and α-Cellulose, Isolated from White Lupin Cotyledon Cell Wall Material (CWM)

	hot EDTA soluble pectic substances	ETA-insoluble residue	hemicelluloses A	hemicelluloses B	α-cellulose
yield[a]	71.0	13.1	0.3	4.3	6.1
$N \times 5.7$	1.5	17.0	ND[g]	ND[g]	ND[g]
polysaccharides[b]	88.0	64.1	20.8	74.2	84.3
relative sugar distribution[c]					
rhamnose	2.0	0.6	1.0	1.5	0.4
fucose	0.5	1.4		2.4	0.8
arabinose	14.0	5.9	10.6	8.6	3.2
xylose	3.6	8.3	13.9	14.0	4.5
mannose	tr[h]	0.6		0.3	0.5
galactose	66.3	23.1	26.9	42.0	15.7
glucose					
noncellulosic[d]	1.1	3.0	47.6	22.0	4.5
cellulosic[e]		48.7	g		67.9
uronic acids[f]	12.5	8.4	ND	8.9	2.5

[a] Percent of cell wall dry matter. [b] Sum of sugar constituents, as percent of the fraction. [c] Monosaccharide composition in percent of polysaccharides, expressed as anhydro polymeric sugars. [d] Determined from TFA hydrolysate. [e] Calculated as the difference between Saeman and TFA values. [f] Determined colorimetrically. [g] Not determined. [h] Traces.

points (11.9%) and the proportion of terminal nonreducing ends (9.9%) probably arose from undermethylation of cellulose (Ring and Selvendran, 1978; O'Neil and Selvendran, 1980) and possibly of other polymers.

The high proportion of methylated glucose derivatives (11.6%) as compared to the proportion of glucose measured by direct analysis (7.9%) might be a consequence of an overestimation of the area of peak 11 (2,3,6-tri-*O*-methylglucitol) (Figure 1), which is located on the tail of the large 2,3,6-tri-*O*-methylgalactitol peak (peak 10). In conclusion, taking into account certain reservations about the remethylation procedure, it is clear that the white lupin cotyledon CWM contained a very high proportion of 4-linked galactosyl residues bearing only a few branching points (Table III), which may be indicative of the presence of (1→4)-galactan chains. Hirst et al. (1947) have already demonstrated the presence of 4-linked galactosyl residues in white lupin cotyledons. However, these authors detected only 0.25% 4-linked galactose, whereas it can be deduced from the present study that the 4-linked galactose represents at least 9% of white lupin cotyledons. Such an amount of galactose is unusual in plant cell walls. The starchy parenchyma cell walls of potatoes also contain large quantities of 4-linked galactose (Ring and Selvendran, 1978) but in lower proportions (~20%) than found here (~45%). It seems likely that most of the galactosyl residues are included in a structure similar to that proposed for β-(1→4)-galactan (Toman et al., 1972; Mandal and Das, 1980) rather than in the structure proposed for type I arabinogalactan (Aspinall, 1980). It has been stated previously (Brillouet and Riochet, 1983) that cotyledon cell walls from different lupin species show large variations in galactose: uronic acid molar ratios (from 1.2 to 8.2) and contrastingly low variations in arabinose:uronic acid ratios (from 1.1 to 2.3). This last figure suggests that most of the arabinose and galactose units are not closely associated in a well-defined polymeric structure, i.e., a large part of arabinosyl units are probably not bound to galactan backbones. This is in agreement with the present finding that galactosyl units are poorly branched. Arabinose was present mainly as branched units as well as nonreducing terminal ends (Table III), suggesting a highly branched structure of pectic arabinan type (Aspinall, 1980). The main branching point was located on C_2 as shown by the high proportion of the 3-*O*-methylarabinitol derivative produced. The distribution of different links in which arabinose was involved is comparable to structural features of Azuki bean arabinan (Ukai et al., 1978) in which the major branching point is also located on C_2. Azuki bean and white lupin arabinans seem to be different from the arabinans of other plants such as soybean (Aspinall and Cotrell, 1971), mustard seed (Hirst et al., 1965), rapeseed (Siddiqui and Wood, 1974), and cabbage (Stevens and Selvendran, 1980b) in which arabinose is mainly branched on C_3.

Occurrence of xylose as nonreducing ends and the presence of glucose branched on C_6 could correspond to xyloglucan, a hemicellulosic polymer typically found in the primary cell walls of dicotyledonous plants (Bauer et al., 1973).

Fractionation of Cell Wall Polysaccharides. The major fraction was pectic substances (71% of CWM), hemicelluloses A (0.3%) and B (4.3%), and α-cellulose (final residue 6.1%) representing smaller amounts. This figure is in agreement with the results of the methylation analysis, which showed that most of the links were characteristic of pectic substances.

The composition of isolated pectic substances (Table IV) was very similar to cell wall composition (Table II), with the exception of glucose, the bulk of this compound being recovered as cellulose in the final residue (α-cellulose). The occurrence of glucose and xylose as major monosaccharides in hemicellulosic fractions (Table IV) suggests the presence of xyloglucan (the same suggestion was put forward in the discussion of the results of the methylation analysis). But, it is pertinent to point out that, even if all xylose and noncellulosic glucose from hemicellulosic fractions and α-cellulose were considered as xyloglucan, this polymer could not represent more than 1.6% of CWM. Fucose: xylose molar ratios were almost identical (~0.16) in most fractions (Table IV), suggesting that most of the fucosyl residues are closely related to xylose-containing polymers. The occurrence of fucose in xyloglucans is well-known (Bauer et al., 1973), but it may also be present in xylose-containing chains branched on rhamnogalacturonan (Aspinall, 1980).

The presence of rhamnose and uronic acids in hemicellulosic fractions and α-cellulose (Table IV) indicates that it is likely that these fractions still contained residual pectic polymers that were not fully removed by EDTA extraction. The significance of the presence of arabinose and galactose in hemicelluloses and α-cellulose is uncertain, but arabinose:galactose:uronic acid molar ratios were almost identical in hemicellulose B (25:100:20), α-cellulose (25:100:15)

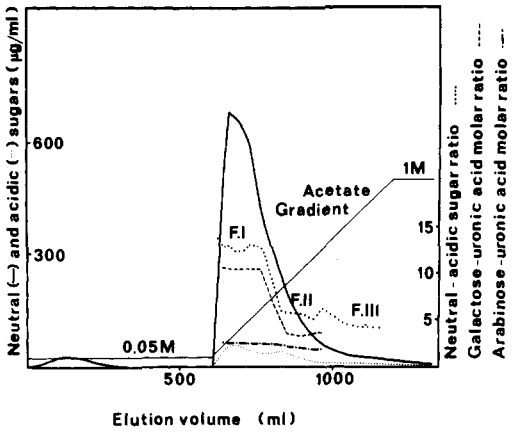

Figure 2. Ion-exchange chromatography of EDTA-soluble pectic substances from white lupin cotyledon CWM on DEAE-Sepharose CL-6B (16 × 5.0 cm). After sample loading (~180 mg), the column was extensively washed with 0.05 M acetate buffer, pH 4.8. Bound polysaccharides were eluted by a linear sodium acetate gradient (0.05–1 M). Collected fractions were analyzed for neutral (—) and acidic (···) sugars by orcinol and *m*-phenylphenol, respectively. Arabinose and galactose contents were obtained by GC after acid hydrolysis of eight fractions.

Table V. Yield and Composition of Pectic Fractions Extracted from White Lupin Cotyledon Cell Wall Material (CWM)

	neutral galactose-rich fraction	acidic fractions		
		I	II	III
yield[a]	1	39	9	4
rhamnose[b]	0	1.3	1.9	ND[e]
fucose	0.2	tr[d]	tr	
arabinose	5.7	14.7	24.4	
xylose	0.3	2.9	8.0	
mannose	0.2	0.3	0.2	
galactose	91.3	72.5	48.3	
glucose	2.3	0.9	1.9	
uronic acids[c]	0	7.4	15.2	
neutral:acidic sugar ratio[c]		12.5	5.6	4.1

[a] Expressed as percent of polysaccharidic material in CWM. [b] Percent of the polysaccharides. [c] Obtained by automatic colorimetric analyses. [d] Traces. [e] Not determined.

and EDTA-soluble pectic substances (26:100:17), suggesting that arabinose and galactose moieties present in hemicellulose B and α-cellulose are components of pectic polymers.

It would not be correct to equate the cellulose content of the CWM with the yield of α-cellulose residue, since this fraction was contaminated by noncellulosic polymers (Table IV). The CWM cellulose content was 3.5% as measured by the α-cellulose glucose content and 5.0% when calculated from the difference between CWM glucose contents obtained from Saeman and TFA hydrolysates. This discrepancy could arise from partial solubilization of cellulose-like material by the alkali treatment (Tharanathan et al., 1975).

A neutral pectic fraction was isolated, as unbound material, after ion-exchange chromatography of pectic substances on DEAE-Sepharose CL-6B (Figure 2; Table V). Contaminating uronic acids (2.9%) were still present in this neutral fraction and were eliminated by further gel filtration (Sepharose CL-6B) (Figure 3). A strictly neutral fraction eluted in the fractionation range (K_{av} = 0.51) corresponding to a mean molecular weight (M_r) of 65 000, calculated from a dextran calibration curve; this neutral material (1% of CWM) contained 91.3% galactose, 5.7% arabinose, and 2.3% glucose. It is likely that this galac-

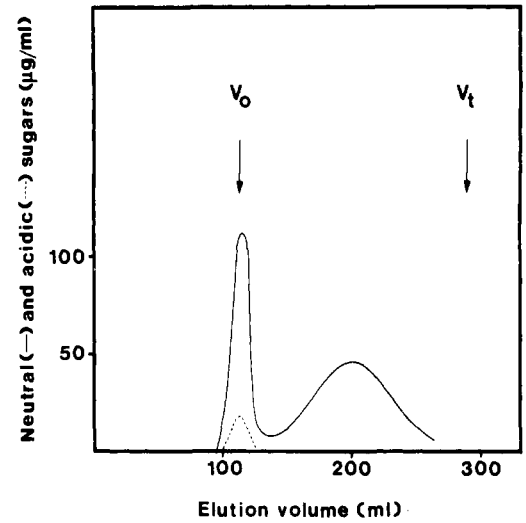

Figure 3. Isolation of a galactose-rich neutral fraction by gel filtration on Sepharose CL-6B (82 × 2.2 cm), of the neutral unbound fraction obtained from ion-exchange chromatography of lupin pectic substances (Figure 2). Collected fractions were analyzed for neutral (—) and acidic (---) sugars by orcinol and *m*-phenylphenol, respectively.

tose-rich fraction (Table V) is not produced by chemical degradation due to hot EDTA extraction at pH 5.0 since, in a preliminary experiment (Carré and Brillouet, 1982), a comparable neutral pectic fraction, containing traces of uronic acids, was isolated in a similar way from pectic substances extracted by cold EDTA (20 °C). However, the yield of the neutral fraction was lower at 20 °C (0.4% of CWM) than at 100 °C. In both cases, the arabinose: galactose molar ratios were in the same order: 0.13 at 20 °C and 0.08 at 100 °C. Yellow lupin CWM exhibits a similar yield of cold EDTA soluble neutral fraction (0.3%) (Matheson and Saini, 1977), but a significantly higher yield of hot EDTA-soluble neutral one (7%) (Matheson and Saini, 1977). The arabinose: galactose ratio of the hot EDTA soluble neutral fraction from yellow lupin (0.19) (Matheson and Saini, 1977) is higher than that of white lupin (0.08). This could be related to differences in the composition of whole polysaccharides that exhibit a higher arabinose:galactose molar ratio in yellow lupin (0.58) (Matheson and Saini, 1977) than in white lupin (0.30). The composition of our galactose-rich neutral fraction (Table V) was quite close to that of purified galactan isolated from *Strychnos nux-vomica* seeds (Andrews et al., 1954) or from leaves of *Aloe barbadensis* Miller (Mandal and Das, 1980). The mean M_r (65 000) of the present fraction was similar to that of galactan isolated from the midrib of leaves of *Nicotiana tabacum* (Eda and Kato, 1978). The occurrence of a high M_r fraction, in which 91.3% of sugars was represented by galactose, is in agreement with our hypothesis that most of CWM galactose units occurred as poorly branched long chains and should be compared with the finding of Stoddart and Northcote (1967) that neutral pectic polysaccharides are precursors of labile neutral moieties present in weakly acidic pectic polysaccharides.

Bound acidic pectic polysaccharides, which were progressively eluted from an ion-exchange column by a linear acetate gradient (0.05–1 M) (Figure 2), exhibited variations in their neutral:acidic sugar ratio. In fact, three levels corresponding to three pectic fractions (I, II, III) were observed, as in broad bean, smooth pea, and soybean pectic substances (Brillouet and Carré, 1983). Polysaccharide (unbound plus bound fractions) recoveries were 87 and 77% for neutral moieties and uronic acids, respectively, as previously reported (Brillouet and Carré, 1983) for the above-mentioned legume seeds. Poor uronide recovery

might be due to strong adsorption of uronic acid rich fragments on the exchanger (Brillouet and Carré, 1983).

Pectic fractions I, II, and III were eluted at ionic strengths of 0.16, 0.48, and 0.85 M, respectively. Some typical differences appeared, when compared to acidic pectic substances of other lugume seeds. The neutral: acidic sugar ratios of white lupin acidic fractions (13.0, 5.8, 4.2) were approximately twice as much as those of broad bean (5.5, 3.6, 1.4), smooth pea (5.9, 2.8, 0.8), and soybean (4.3, 2.0, 1.4). This is due to the superimposition of galactose units in the white lupin acidic pectic fractions (Table V). Another characteristic of white lupin acidic pectic fractions is a high variability in the galactose: uronic acid ratio (fraction I vs. II) without noticeable changes in the arabinose:uronic acid ratio (Figure 2). The galactose change was the main factor responsible for the neutral: acidic sugar ratio variations (fraction II vs. I). This phenomenon was not observed in the broad bean, smooth pea, or soybean (Brillouet and Carré, 1983) in which galactose:arabinose ratios remained practically unchanged (fraction II vs. I). The high variability of the galactose: arabinose ratio (Figure 2) suggests that most of galactosyl chains are not closely related to arabinosyl units and occur essentially as poorly branched galactan chains. This is supported by the fact that, in soybean, which is known to contain type I arabinogalactan (Aspinall and Cottrell, 1971), there is little variability in the galactose:arabinose ratio of acidic pectic fractions (Brillouet and Carré, 1983).

The rapid drop in cell wall galactose content but only moderate drop in arabinose, which occur during the first days of germination in yellow lupin (Matheson and Saini, 1977) and blue lupin seeds (Crawshaw and Reid, 1984), might be indicative of the occurrence of rather linear galactan chains. Crawshaw and Reid (1984) claim that galactose present in the lupin cotyledon cell wall is a storage component; our data tend to indicate that this storage galactose is likely in the form of linear galactan chains of a high degree of polymerization, linked to rhamnogalacturonan backbones (fraction I).

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Registry No. rhamnose, 3615-41-6; fucose, 2438-80-4; arabinose, 147-81-9; xylose, 58-86-6; mannose, 3458-28-4; galactose, 59-23-4; glucose, 50-99-7.

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