Carbohydrate Research, 147 (1986) 101–117
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

COMPARATIVE STRUCTURAL STUDIES OF LIGNIN–CARBOHYDRATE COMPLEXES FROM *Digitaria decumbens* (PANGOLA GRASS) BEFORE AND AFTER CHLORITE DELIGNIFICATION

CLIVE W. FORD
Division of Tropical Crops and Pastures, CSIRO, Cunningham Laboratory, St. Lucia, Queensland 4067 (Australia)

(Received May 25th, 1985; accepted for publication, August 20th, 1985)

ABSTRACT

Lignin–carbohydrate complexes (LCCs) were extracted with methyl sulphoxide from cellulase-digested stem cell-walls of mature pangola grass. Comparative studies, using gel filtration, g.l.c., and spectroscopy, were conducted concurrently on digested cell-walls which had also been treated with sodium chlorite. All the LCCs contained hemicellulose and protein with low contents of hydroxyproline. Gel filtration showed markedly increased polydispersity of the LCCs from chlorite-treated residues. Chlorite treatment caused loss of arabinose and galactose, with a concomitant increase in the proportion of xylose degradable by sodium metaperiodate. The LCCs from untreated cell-walls comprised polysaccharide, esterified *p*-coumaric acid, and a water-insoluble, lignified "hemicellulose A" component (6% carbohydrate) having a low xylose-to-arabinose ratio and a high proportion of hexoses. These components were liberated with alkali, as were, unexpectedly, some free xylose and arabinose. Chlorite-treated walls gave LCCs with ester bonds still present, but *p*-coumaric acid was degraded to lignin-like products, and a "hemicellulose A" xylan (94% carbohydrate) was isolated. The structural relationship of the components in the LCCs from untreated material was complex, and appeared to involve monomer and oligomer pentose units, di-esterified at least, in bridging structures with *p*-coumaric acid. Xylose reducing-units also appeared to be liberated by alkali, suggesting esterification at C-1 in some xylan in the LCC. Thus, chlorite delignification causes significant structural changes in the polysaccharides and non-carbohydrate components of the cell walls, and it is suggested that chlorite treatment be omitted when *in situ* structural data on plant cell-walls are being sought.

INTRODUCTION

Delignification of plant cell-walls is usually performed prior to quantitative determination or isolation of α-cellulose and hemicelluloses. The method most commonly reported for delignification of herbage is based on the Jayme–Wise

0008-6215/86/$ 03.50

procedure[1,2], because of ease of handling of materials and maximising of the amount of carbohydrate in the resulting holocellulose. In addition, this procedure, which uses sodium chlorite in dilute acetic acid, is considered to cause very little degradation of polysaccharides, since polymers with high d.p. have been isolated from the derived holocellulose[3]. However, in addition to degraded lignin, there is some solubilisation of carbohydrates during chlorite treatment[4,5], even at room temperature, and the holocellulose is much more biodegradable than the original cell-walls[6,7]. Hence, significant structural changes in the cell walls, of possible nutritional importance when considering forage species, are produced. Studies of these changes may contribute to an understanding of the variable, but generally high, resistance of cell walls from tropical grasses to microbial degradation.

The effect of chlorite on the structure of cell-wall polysaccharides as evidenced by a comparison of the polysaccharide complexes extracted by methyl sulphoxide from cell-wall materials before and after delignification is now reported.

EXPERIMENTAL

Materials. — Crude cell-walls (CCW) were prepared[6] from 89-day-old pangola grass stems (<1 mm particles).

General methods. — Solutions were concentrated at <40° under reduced pressure. Carbohydrate in solution was determined by the phenol–sulphuric acid method[8], using D-xylose as the standard. Monosaccharide composition of polymers was determined by g.l.c. of trimethylsilyl ethers[7] after hydrolysis with 2M trifluoroacetic acid. Phenolic acids were determined by g.l.c. of their trimethylsilyl ethers, prepared by shaking with a mixture of *N,O*-bis(trimethylsilyl)trifluoroacetamide and chlorotrimethylsilane in pyridine (1:0.1:1). Acetate was measured as acetylpyrrolidine[9] by g.l.c. at 150°, using a nickel column packed with 10% Superox. U.v. and i.r. spectra were recorded for aqueous solutions and potassium bromide discs, respectively.

Fractionation of CCW. — (*a*) *Enzymic hydrolysis.* CCW (40.0 g) was incubated with commercial cellulase[7,10] (100 g) in acetate buffer (0.25M, pH 4.6, 4 L) at 39° for 72 h. The insoluble, digested cell-walls were collected, washed with water until carbohydrate could not be detected in the filtrate[8], and then dried at 50° after solvent exchange using ethanol followed by acetone, to give CCW-CR (29.1 g).

(*b*) *Delignification.* CCW-CR (22.0 g) was treated with sodium chlorite[6] to give a delignified residue CCW-CR-DR (20.5 g).

(*c*) *Grinding of cell walls.* CCW-CR and CCW-CR-DR (7–10 g of each) were pulverised in a Spex Shatterbox mill for 5 min to give residues CCW-CR (Spex) and CCW-CR-DR (Spex), respectively, with particle sizes[7] less than 0.1 mm.

(*d*) *Extraction of lignin–carbohydrate complexes.* Each Spex-ground cell-wall preparation was stirred at 25° with methyl sulphoxide for 38 days. After various times (Fig. 1), the cell-wall residues were centrifuged, and then treated with fresh

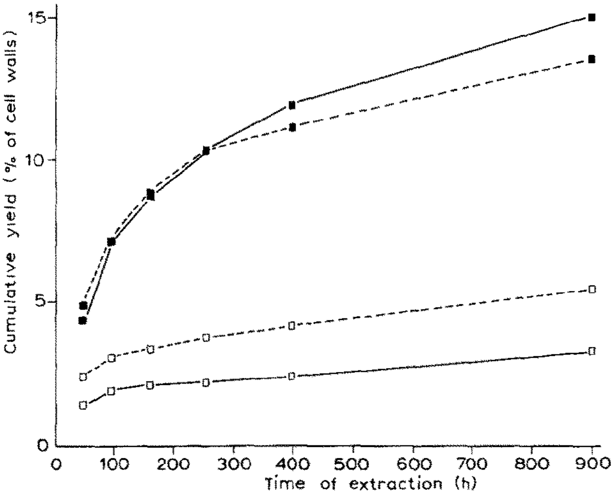

Fig. 1. Cumulative yields of solids and carbohydrate extracted with methyl sulphoxide from cellulase-digested pangola-grass cell-walls before and after treatment with chlorite: ——, CCW-CR, ---, CCW-CR-DR; □, carbohydrate; ■, LCCs.

methyl sulphoxide. The centrifugates, which contained lignin–carbohydrate complexes (LCCs) designated CR and DR (originating from CCW-CR and CCW-CR-DR, respectively), were dialysed against running water for 2 days, followed by several changes of distilled water during 5 h, before being concentrated and freeze-dried. LCCs isolated from methyl sulphoxide after treatment for 48 h were examined in detail.

(*e*) *Gel filtration of LCCs.* In a typical experiment, CR (409 mg) and DR (380 mg) were dissolved as far as possible in 5 mL of water (75 and 97% soluble, respectively). After centrifuging, the solutions were applied to columns (33 × 2.5 cm) of Bio-Gel A-1.5m. The effluent was monitored for carbohydrates and absorbance at 280 nm. Each LCC was fractionated into material of high (CR1 and DR1) and low (CR2 and DR2) molecular weight (Fig. 2a,b).

(*f*) *Treatment with alkali.* Fractions CR1 and DR1 (~20 mg) were dissolved in M sodium hydroxide (1 mL) at 25°. Aliquots (10 μL) were removed at various intervals up to 21 h and the u.v. spectra were recorded; $A_{\max}$ at 280 nm was apparent after 3 and 1 h for CR1 and DR1, respectively. The remaining alkaline solutions were neutralised with 2M acetic acid, and the precipitates were collected by centrifugation and designated CR1(OH)(iii) (brown, 44%) and DR1(OH)(iii) (white, 18%), respectively.

(*g*) *Gel filtration.* The neutral supernatants, containing CR1(OH) and DR1(OH), were applied to a column of Bio-Gel A-1.5m as above. Fractions of high [CR1(OH)(i) and DR1(OH)(i)] and low [CR1(OH)(ii) and DR1(OH)(ii)] molecular weight were isolated (Fig. 3). Fraction CR1(OH)(ii) was further separated on Bio-Gel P-4 (Fig. 4).

Periodate oxidation. — LCCs (15–40 mg) were oxidised in the dark at 25°

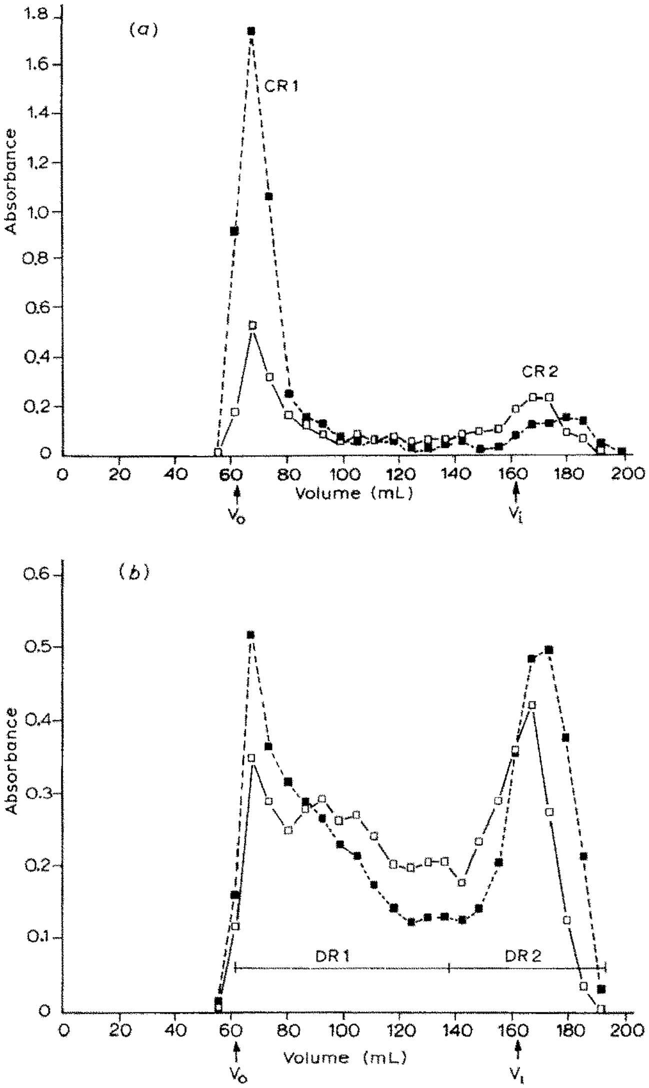

Fig. 2. Fractionation of a methyl sulphoxide extract (48 h) of cellulase-digested pangola-grass cell-walls on Bio-Gel A-1.5m, before (*a*) and after (*b*) chlorite treatment: ——, carbohydrate; ---, A_{280}.

with 15mM sodium metaperiodate (20 mL). The reduction of periodate was measured[11] on aliquots withdrawn after various times (Fig. 5).

RESULTS AND DISCUSSION

Incubation of cell walls (CCW) with a broad-spectrum commercial cellulase left 73% of the starting material undissolved (CCW-CR). CCW and CCW-CR represented 71% and 52%, respectively, of the original dry weight of stems, thus

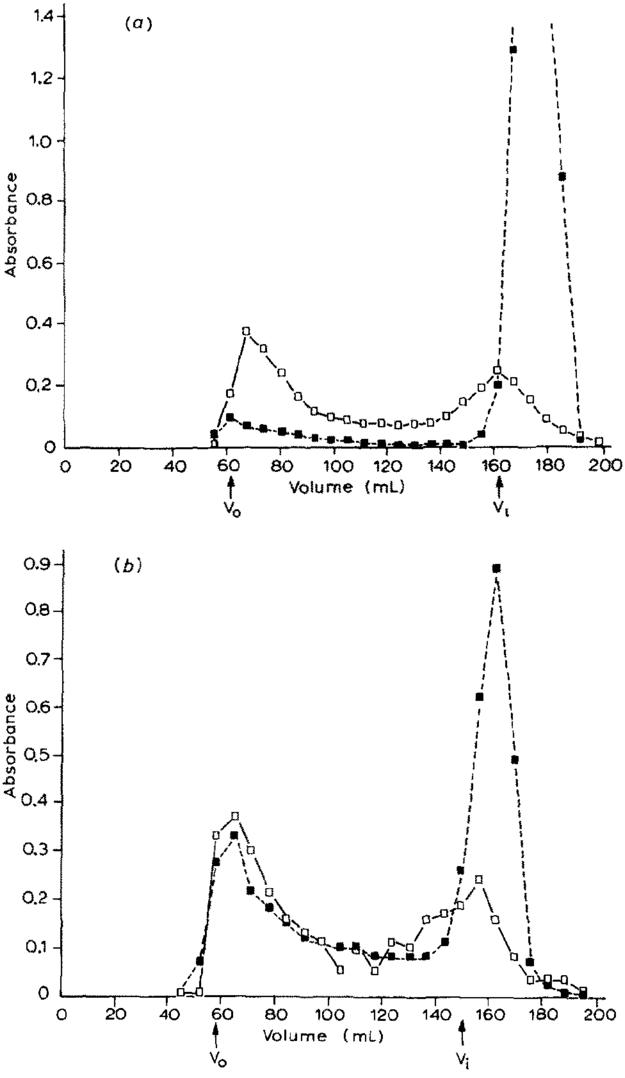

Fig. 3. Fractionation of peak 1 (Figs. 2a and 2b), after alkali treatment, on Bio-Gel A-1.5m before (*a*) and after (*b*) chlorite treatment: ——, carbohydrate; ---, A_{280}.

demonstrating the low biodegradability of the plant material. The cellulase treatment was included to remove easily digested cell-walls, producing a residue (CCW-CR) more relevant to nutritional limitations of the forage than CCW, and from which most of the original ferulic acid, but very little of the acetate or *p*-coumaric acid, had been digested (Table I). Preliminary studies of the hydrolysis of material extracted with methyl sulphoxide showed that the enzyme treatment resulted in a marked decrease (~80%) in the relative glucose content of monosaccharides from CCW-CR compared to extracts from the original CCW. Glucan

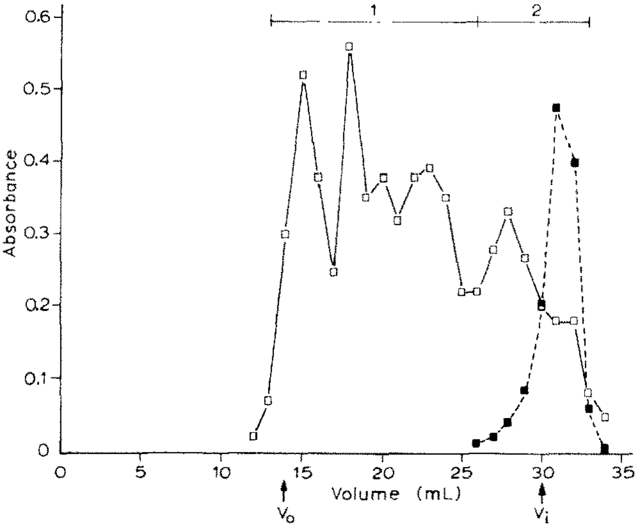

Fig. 4. Fractionation of peak 2 (Fig 3a) on Bio-Gel P-4: ——, carbohydrate, ---, A_{280}

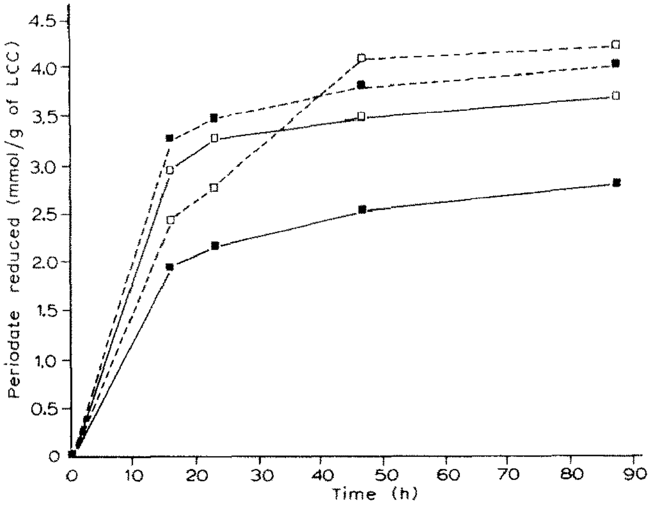

Fig. 5. Oxidation of LCCs from Figs. 2a and 2b with sodium metaperiodate ——, before chlorite treatment; ---, after chlorite treatment, □, peak 1; ■, peak 2.

extracted by methyl sulphoxide from pangola CCW or holocellulose (CCW-DR) has been found to be readily digested *in vitro* by rumen micro-organisms and, from methylation studies, to be largely a (1→4)-linked glucan[12]. High proportions of glucan have also been solubilised by methyl sulphoxide from cell walls of temperate grasses[13,14] and reported to be cellulosic in structure[14].

A portion of CCW-CR was delignified with sodium chlorite at room temperature[6], giving a holocellulose CCW-CR-DR (93%), in which most of the

TABLE I

PHENOLIC ACID AND ACETATE CONTENTS (% OF RESIDUE) OF CELL-WALL PREPARATIONS OF PANGOLA-GRASS STEM BEFORE METHYL SULPHOXIDE EXTRACTIONS

	pCA[a]	*Recovery (%)*	*FA*[a]	*Recovery (%)*	*Ac*[a]	*Recovery (%)*
CCW (100)[b]	1.6	100	0.4	100	1.8	100
CCW-CR (73)	2.0	91	n.d.[c]	0	2.1	85
CCW-CR-DR (68)	n.d.	0	n.d.	0	1.7	64

[a] pCA, *p*-coumaric acid; FA, ferulic acid; Ac, acetate. [b] Figures in parenthesis are percentages of CCW. [c] Not detected.

acetate was retained but none of the *p*-coumaric acid (Table I). The latter was modified to unidentified u.v.-absorbing material, as shown later. Reduction in the particle size of CCW was necessary in order to obtain reasonable yields of LCCs by solvent extraction. A Spex Shatterbox mill[7] reduced the particles to <0.1 mm in 5 min, and was equivalent, in terms of yield of LCCs from methyl sulphoxide extraction (4 days) (Fig. 1), to ball milling for 7 days followed by extraction with solvent[13] for 7 days.

The rate of change in cumulative yield of LCCs from successive methyl sulphoxide extractions decreased with time, particularly after 11 days (Fig. 1). Up to this point, there was little difference between CCW-CR and CCW-CR-DR with respect to yield of LCCs. Thereafter, CCW-CR gave greater amounts of LCCs than CCW-CR-DR, and, after 38 days, non-dialysable recoveries of methyl sulphoxide-soluble material corresponded to 15.0 and 13.4%, respectively, of the cellulase-digested cell walls. However, consistently more carbohydrate material was produced from CCW-CR-DR. Losses during dialysis, determined as xylose equivalent, were greater from CCW-CR than CCW-CR-DR in the first 3 extractions (Table II). The water solubility of LCCs from CCW-CR was high (75–80%) in the first 5 extractions, whereas that of material from CCW-CR-DR decreased consistently from 98 to 47%. The water solubility of the complexes had no relationship with the proportion of carbohydrate present, which was variable but generally higher in LCCs from CCW-CR-DR (Table II). The monosaccharide composition of LCCs from CCW-CR and CCW-CR-DR differed mainly in the generally decreased contents of galactose and glucose and the higher xylose-to-arabinose ratios in the latter (Table III).

Complexes extracted after 48 h, comprising 4.4 and 4.8% of CCW-CR and CCW-CR-DR, respectively, were fractionated by gel filtration on Bio-Gel A-1.5m. Fractions of high and low molecular weight, containing both carbohydrate and u.v.-absorbing substances, were isolated (Fig. 2). Good recoveries were obtained from both fractionations (Table IV), but the elution profiles were different. Carbohydrate from the chlorite-treated walls was much more polydisperse than that from untreated walls, and the strong u.v.-absorbance associated solely with the first peak

TABLE II

DATA ON LIGNIN-CARBOHYDRATE COMPLEXES (LCCs) EXTRACTED BY METHYL SULPHOXIDE FROM CELLULASE-DIGESTED PANGOLA-GRASS STEM CELL-WALLS BEFORE (CR) AND AFTER (DR) CHLORITE TREATMENT

Extraction time (h)	*Recovery[a] after dialysis (%)*		*Solubility in water (%LCC)*		*Carbohydrate[a] content (%LCC)*	
	CR	*DR*	*CR*	*DR*	*CR*	*DR*
47	79	88	75	98	31	49
96	73	91	81	69	18	30
163	81	94	76	65	12	17
256	98	106	74	55	8	29
400	108	98	75	47	10	46
900	103	97	57	41	27	54

[a]Xylose equivalent[8].

TABLE III

MONOSACCHARIDE COMPOSITION OF LIGNIN-CARBOHYDRATE COMPLEXES (LCCs) ISOLATED AFTER INCREASING TIMES OF EXTRACTION WITH METHYL SULPHOXIDE (RELATIVE %)

Extraction time (h)	*Ara*		*Xyl*		*Gal*		*Glc*		*Xyl/Ara*	
	CR	*DR*	*CR*	*DR*	*CR*	*DR*	*CR*	*DR*	*CR*	*DR*
47	16.4	18.3	56.6	69.4	6.3	3.8	20.8	8.5	3.5	3.8
163	19.0	18.7	52.9	64.7	6.5	7.2	21.6	9.4	2.8	3.5
256	15.7	21.8	53.7	65.3	9.3	5.9	21.3	7.1	3.4	3.0
400	18.8	18.4	53.6	64.8	8.9	8.8	18.8	8.0	2.9	3.5
900	22.1	13.0	54.1	78.8	8.2	3.3	15.6	4.9	2.4	6.1

of the latter appeared to be more evenly distributed in the chlorite-treated material. Thus, it appears that chlorite treatment cleaved bonds in the cell-wall matrix to produce complex carbohydrate polymers of molecular size lower than in the original material, although still apparently associated with lignin fragments. Intracarbohydrate linkages need not have been cleaved, since the degradation of non-carbohydrate molecules functioning as cross-linking units between polysaccharide chains[15,16] could explain the observed results. The differences in the gel-filtration elution profiles for CR1 and DR1 were emphasised by refractionation on Bio-Gel A-5m (Figs. 6a,6b). The fraction of high molecular weight CR1 was still eluted as a single peak at the exclusion volume, whereas the polydisperse nature of DR1 was further demonstrated. Gel-filtration elution profiles similar to those of the LCCs from chlorite-treated pangola have been reported for lignin–hemicellulose complexes isolated from spruce chlorite-holocellulose[17]. Carbohydrate contents of the fractions from gel filtration (Fig. 2) were higher in the respective chlorite-treated material (Table V). The fractions of higher molecular weight contained most of the

TABLE IV

RECOVERY OF CR AND DR AFTER GEL FILTRATION ON Bio-Gel A-1.5m

LCC	*Wt. used*[a] *(mg)*	*Recoveries (%LCC)*		
		Peak 1	*Peak 2*	*Total*
CR	307	32.4(47.4)[b]	46.9(18.5)	79.3[c]
DR	373	52.8(61.7)	37.6(40.2)	90.4

[a]Water-soluble part. [b]Figures in parenthesis are carbohydrate contents[8] (%). [c]Does not include discarded zone between peaks (9.9% CR, 59% carbohydrate).

TABLE V

MONOSACCHARIDE COMPOSITION AND PROTEIN CONTENT OF LCCs AFTER FRACTIONATION ON Bio-Gel A-1.5m[a]

LCC	*Ara*	*Xyl*	*Gal*	*Glc*	*Total*	*Protein*[b]
CR1[c]	6.3(14.0)[d]	23.8(52.8)	3.9(8.6)	11.1(24.6)	45.1(100)	9.8
CR2	2.1(9.3)	15.4(68.4)	0.6(2.7)	4.4(19.6)	22.5(100)	n.d.[e]
DR1	6.2(9.9)	44.7(71.5)	2.0(3.2)	9.6(15.4)	62.5(100)	9.0
DR2	4.1(10.5)	27.7(71.0)	1.0(2.6)	6.2(15.9)	39.0(100)	5.7

[a]g.100 g^{-1} LCC; relative percentages in parenthesis. [b]6.25 × N%. [c]See Experimental for explanation of symbols. [d]Includes a trace of rhamnose (*cf.* alkaline degradation of CR1, Table IX). [e]Not determined.

carbohydrate. The complex from the chlorite-treated walls had a higher xylose-to-arabinose ratio, suggesting loss of arabinose residues during delignification, and reduced proportions of galactose and glucose. The fractions of low molecular weight were more similar in their monosaccharide composition. Nitrogen equivalent to 9–10% of protein was present in the fractions of high molecular weight. Amino acid analysis (Table VI) established the presence of protein similar in its proportions of common amino acids to that of other soluble plant-proteins[18]. The gel filtration results suggested that this protein was strongly associated with the carbohydrate complexes, but the relatively low contents of hydroxyproline indicated that it could not be classed as cell-wall extension.

LCCs from chlorite-treated cell walls reduced more sodium metaperiodate per unit weight than LCCs from untreated material (Fig. 5). Xylose was degraded proportionally more than arabinose in all LCCs (Table VII). The high-molecular-weight fraction DR1 from the chlorite treatment had more xylose degraded than CR1, but both LCCs had lost similar proportions of arabinose, galactose, and glucose. Thus, chlorite treatment appeared to have increased the proportion of xylose susceptible to periodate oxidation, possibly by removing substituents at positions 2 or 3. This interpretation is consistent with the observed, increased polydispersity of LCCs from the chlorite treatment, and suggests that it is xylose that is primarily involved in protective cross-linking between polysaccharide chains.

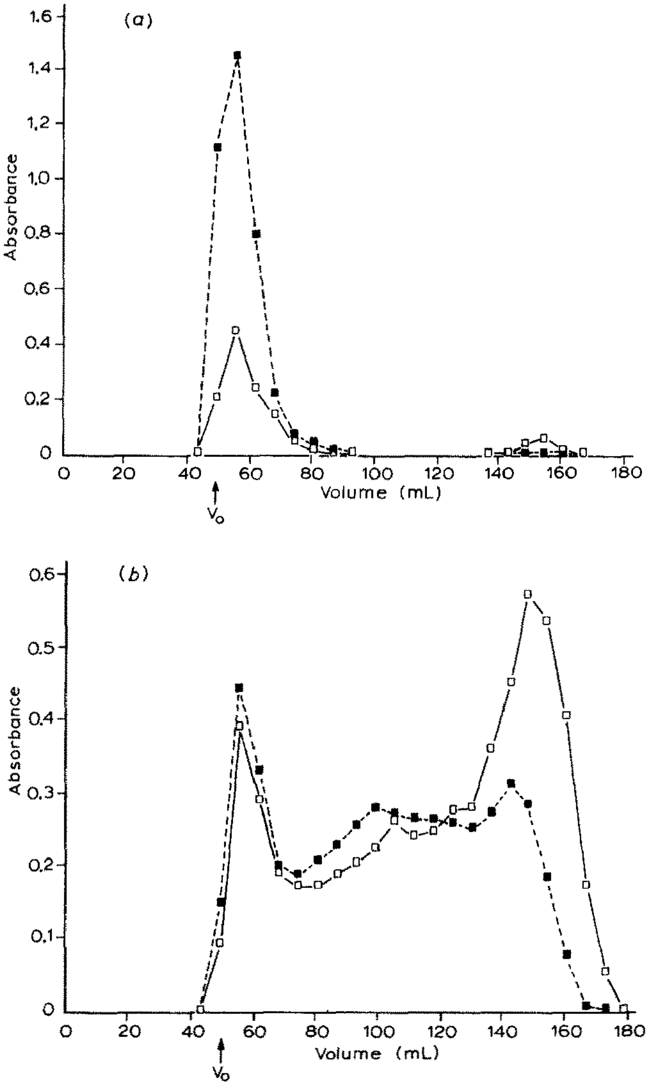

Fig. 6. Refractionation of peak 1 (Figs. 2a and 2b) on Bio-Gel A-5m: (*a*) CR1, and (*b*) DR1; ——, carbohydrate; ---, A_{280}.

Although there is some loss of arabinose during chlorite treatment, many of the residual arabinose residues that are resistant to periodate degradation are not involved with chlorite-degradable cross-linking moieties. These arabinose residues are unlikely to be end groups, and may occur as branch points in the polysaccharides, they may contain non-carbohydrate substituents, or they may be stereochemically hindered towards periodate attack. Little structural information can be deduced about the non-carbohydrate part of the LCCs from the results of periodate oxidation. However, assuming that substituted guaiacol units are present,

TABLE VI

AMINO ACID COMPOSITION OF PROTEIN ASSOCIATED WITH LCCs AFTER FRACTIONATION ON Bio-Gel A-1.5m (RELATIVE %)

LCC	*Lys*	*His*	*Arg*	*Asp*	*Thr*	*Ser*	*Glu*	*Pro*	*Gly*	*Ala*	*Val*	*Ile*	*Leu*	*Tyr*	*Phe*	*Hyp*
CR1	2.9	1.8	2.3	10.8	6.5	6.5	11.9	8.3	9.3	8.8	6.5	4.4	9.5	1.4	6.5	2.4
CR2	2.6	1.2	1.6	8.3	11.2	7.1	10.2	14.4	14.8	8.0	3.3	4.0	7.5	1.4	3.8	0
DR1	2.1	0.3	2.1	12.3	7.7	5.7	13.6	7.4	8.9	8.8	7.0	4.9	10.8	0	6.4	2.0
DR2	5.5	1.0	2.6	11.8	7.8	4.8	13.2	9.3	8.8	11.7	5.8	3.3	9.6	0.1	5.0	0

each of which consumes 2 mol of periodate[19], the amount of periodate reduced in excess of that attributed to carbohydrates corresponded to a guaiacol equivalent content of 10–14% in the LCCs (Table VIII).

Treatment of the high-molecular-weight fractions CR1 and DR1 (Figs. 2a and 2b) with dilute sodium hydroxide yielded insoluble residues CR1(OH)(iii) and DR1(OH)(iii), respectively, from the neutralised solutions of each LCC. The soluble portions [CR1(OH) and DR1(OH)] were further fractionated by gel filtration into materials of high and low molecular weight. The elution profiles (Figs. 3a and 3b) showed that most of the u.v. absorbances previously associated with CR1 and DR1 now appeared in the low-molecular-weight fraction slightly beyond the inclusion volume of the column. It is likely that the alkali-labile linkages were esters, and had thus largely survived delignification. Investigation of the isolated fractions revealed many differences which could be attributed to the chlorite treatment (Table IX). The insoluble precipitates from the neutral solution corresponded to what is frequently referred to in the literature as hemicellulose A, and usually termed xylan. This material is most commonly isolated from chlorite holocellulose. Coincidentally, in the present work, the white, insoluble residue DR1(OH)(iii) of high carbohydrate content had a xylose-to-arabinose ratio similar

TABLE VII

RELATIVE AMOUNTS OF MONOSACCHARIDES OXIDISED AFTER TREATMENT OF LCCs WITH SODIUM METAPERIODATE, AND A COMPARISON OF XYLOSE-ARABINOSE RATIOS BEFORE AND AFTER OXIDATION (*cf.* TABLE V)

LCC	*Ara*	*Xyl*	*Gal*	*Glc*	*Xyl/Ara*	
	(% Sugars)				*(Before)*	*(After)*
CR1	44	46	64	49	3.8	3.7
CR2	71	83	83	75	7.3	4.3
DR1	47	60	65	48	7.2	5.4
DR2	61	70	80	73	6.8	5.3

TABLE VIII

CALCULATED AMOUNTS OF PERIODATE REDUCED BY MONOSACCHARIDE[a] AND NON-CARBOHYDRATE COMPONENTS OF THE LCCs AFTER 88 h (mmol/g OF LCC)

LCC	*Ara*	*Xyl*	*Gal*	*Glc*	*Total*		*Diff.*	*Guaiacol Equiv.*[b]
					Calc.	*Found*		
CR1	0.19	0.73	0.14	0.62	1.68	3.69	2.0	1.0
CR2	0.10	0.85	0.03	0.24	1.22	2.80	1.6	0.8
DR1	0.19	1.79	0.07	0.53	2.58	4.21	1.6	0.8
DR2	0.17	1.29	0.04	0.34	1.84	4.02	2.2	1.1

[a] Assuming an average of 1.0 mol of periodate reduced per mol of degraded monosaccharide [b] 1.0 guaiacol residue consumes ~2 mol of periodate[19].

to that of corn-stalk or wheat-straw xylan[20], whereas CR1(OH)(iii), a brown residue of low carbohydrate content, contained an unusually high proportion of galactose and glucose, with a low xylose-to-arabinose ratio (Table IX). It may be that xylan, as described from holocellulose, is an artifact of the chlorite treatment.

Rhamnose, detected in traces in CR1 (Table V), was concentrated in the high-molecular-weight fraction CR1(OH)(i) (Table IX). Only traces could be detected in DR1(OH)(i). Thus, although a minor component, rhamnose may occupy a structurally significant position in the cell walls, since it appeared to be almost totally removed during the delignification process.

Refractionation of CR1(OH)(ii) (Table IX) on Bio-Gel P-4 confirmed the generally low molecular weight of the u.v.-absorbing material and much of the carbohydrate (Fig. 4). Analysis of fraction 1, before and after reduction, revealed xylitol and a lesser amount of glucitol, but no arabinitol (Table X). These results may point to C-1 of some xylose residues being esterified to cross-linking non-carbohydrate substances in the original LCC. Analysis of the lowest-molecular-weight fraction 2 (Table X) not only confirmed the identity of the major component as *p*-coumaric acid, but also identified free arabinose and xylose, and possibly some

TABLE IX

YIELDS, CARBOHYDRATE CONTENT, AND MONOSACCHARIDE COMPOSITION OF LCCs AFTER ALKALI DEGRADATION

LCC	*Yield (%LCC)*	*Carbohydrate (%)*	*Ara*	*Xyl*	*Gal*	*Glc*	*Rha*
			(Relative %)				
CR1(OH):							
(i)	28.0	66	19	47	17	14	3
(ii)	23.5	42	17	64	5	14	0
(iii)[a]	43.7	6	14[b]	31	21	33	trace
DR1(OH):							
(i)	26.2	45	16[b]	53	12	19	trace
(ii)	56.4	12	11	74	9	7	0
(iii)[a]	17.5	94	6	88	2	5	0

[a]Precipitated from neutral solution. [b]Includes a trace of rhamnose.

TABLE X

MONOSACCHARIDE AND PHENOLIC ACID COMPOSITION[a] OF CR1(OH)(ii) AFTER REFRACTIONATION ON Bio-Gel P-4

Fraction	*Ara*	*Xyl*	*Gal*	*Glc*	*Xylitol*	*Hexitol*	*pCA*[e]
1[b]	18.8	58.5	10.4	12.3	0	0	0
1[c]	20.1	55.3	10.6	11.9	1.6	0.5	0
2[d]	7.6	5.7	tr	tr	0	0	86.8

[a]Relative %. [b]Hydrolysed. [c]Reduced and hydrolysed. [d]Unhydrolysed. [e]*p*-Coumaric acid.

pentose dimers. Thus, the alkali-labile, cross-linking structures that held the LCC CR1 intact appeared rather complex. Coumaric acid could not be viewed as simply directly esterified to polysaccharide chains, but probably also involved pentose monomers and oligomers which perhaps formed diester bridges between polysaccharides and other components of the cell-wall matrix. No *p*-coumaric acid or other aromatic compound could be identified in DR1(OH)(ii). Thus, although chlorite treatment left ester linkages intact, the structure of the phenolic acid had been degraded, possibly to quinone derivatives[21].

Structural changes, attributable to chlorite treatment, in the non-carbohydrate part of the LCCs were also evident from spectroscopic observations. U.v. spectra of complexes CR1 and CR2 each had two distinct maxima (Fig. 7). In contrast, complexes DR1 and DR2, from chlorite-treated material, showed a single maximum in curves typical for polyaromatic lignin[22]. A comparison of i.r. spectra (Fig. 8) showed that bands in the region 1400–1600 cm^{-1}, characteristic of *p*-coumaric acid and evident in CR1, were missing from the DR1 spectrum, which contained a new band in the carboxylate region at 1720 cm^{-1}. Therefore, it is possible that, in degrading phenolic acid esters, chlorite caused the formation of structures susceptible to polymerisation. U.v. spectra of fractions from the alkali-treated complex CR1 indicated two distinct spectral types (Fig. 9). Fraction CR1(OH)(ii) gave a spectrum identical to that of *p*-coumaric acid, confirming the g.l.c. analysis (Table X), and showing that most of the u.v. absorbance in this fraction was due to the phenolic acid. The λ_{max} at 314 nm shown by CR1 had become a shoulder at 302 nm in CR1(OH)(ii) consistent with alkaline hydrolysis of

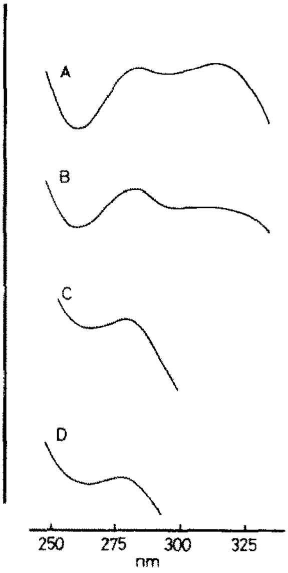

Fig. 7. U.v. spectra of LCCs CR and DR after fractionation on Bio-Gel A-1.5m: A, CR1 (λ_{max} 283 and 314 nm); B, CR2 (λ_{max} 281 and 310 nm); C, DR1 (λ_{max} 277 nm); D, DR2 (λ_{max} 276 nm).

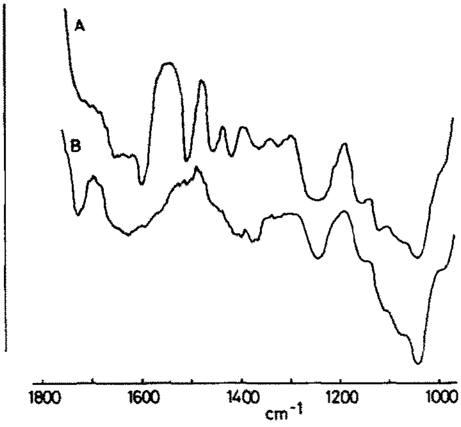

Fig. 8. I.r. spectra of peak 1 of LCCs fractionated on Bio-Gel A-1.5m: A, CR1 (Fig. 2a); B, DR1 (Fig. 2b).

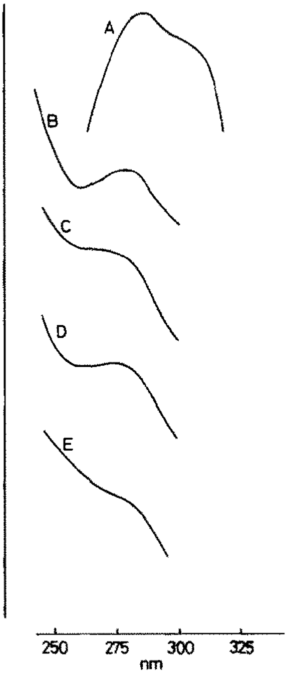

Fig. 9. U.v. spectra of alkali-degraded LCCs CR1 and DR1 after fractionation on Bio-Gel A-1.5m: A, CR1(OH)(ii) [λ_{max} 283 and 302(sh) nm]; B, CR1(OH)(iii) [λ_{max} 277 nm]; C, DR1(OH)(i) [λ_{max} 268 nm]; D, DR1(OH)(ii) [λ_{max} 273 nm]; E, DR1(OH)(iii) [λ_{max} 269 nm (inflection)].

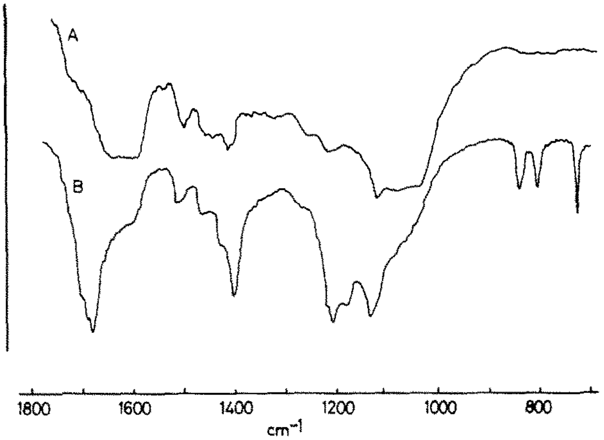

Fig. 10. I.r. spectra of water-insoluble residue CR1(OH)(iii) from alkali degradation of LCC CR1 (Table IX): before (A) and after (B) acid hydrolysis.

an ester[23]. The neutral, insoluble fraction CR1(OH)(iii), which reacted positively with phloroglucinol–HCl, had a u.v. spectrum typical of lignin. I.r. spectra gave further support for the presence of aromatic nucleii (Fig. 10) and, after acid hydrolysis, provided evidence for the possible presence of aryl ketone absorbance at 1680 cm^{-1}. This strong band, produced by acid treatment of a previously alkali-treated residue, is consistent with the presence of acetal bonds in the original material. The existence of acetal or hemi-acetal bonds between lignin carbonyl groups and polysaccharide hydroxyls in hard- and soft-woods has been postulated[24]. However, involvement of carbohydrates with such chemical linkages in the present work remains to be established. The polysaccharide fraction CR1(OH)(i) had very little u.v. absorbance. Thus, the original complex CR1 had been split into three distinct chemical fragments by treatment with alkali, which, together with the g.l.c. and spectroscopic results, suggested that CR1 was a true lignin–carbohydrate complex. Coumaric acid esters may form bridging units between the main polysaccharide chains and the lignin portion, either directly in part, or as bridges involving pentose residues. It was not clear whether or not the small proportion of carbohydrate bound in the lignified fraction CR1(OH)(iii) was also involved in cross-linking structures. Comparison of the u.v. spectra from respective alkali-treated DR1 fractions indicated lignin-type spectra (i) and (ii), but only a weak inflection from the xylan (iii) (Fig. 9) which had a very high carbohydrate content (Table IX). It would appear that the structure of pangola-stem LCC is very different from that of birchwood, for which it has been reported that separation of lignin from carbohydrate could only be achieved after treatment with acid[25].

Thus, it is clear that chlorite delignification modifies the cell-wall structure in many ways. Therefore, for detailed studies of cell-wall polysaccharides, particularly in relation to their environment *in situ*, chlorite treatment should be omitted from the isolation procedure.

ACKNOWLEDGMENTS

The author thanks Mrs. L. Howse for skilful technical assistance, and Mr. R. Court for the amino acid analyses.

REFERENCES

1 G. JAYME, *Cellul.-Chem.*, 20 (1942) 43–49.
2 L. E. WISE, M. MURPHY, AND A. A. D'ADDIECO, *Pap. Trade J.*, 122 (1946) 35–43.
3 R. L. WHISTLER AND C. L. SMART (Eds.), *Polysaccharide Chemistry*, Academic Press, 1953, pp. 112–133.
4 A. J. BUCHALA, C. G. FRASER, AND K. C. B. WILKIE, *Phytochemistry*, 11 (1972) 1249–1254.
5 C. W. FORD AND J. D. BLAKE, *Int. Carbohydr. Symp., XIIth, Utrecht*, 1984.
6 C. W. FORD, *Aust. J. Agric. Res.*, 29 (1978) 1157–1166.
7 C. W. FORD, *Aust. J. Agric. Res.*, 34 (1983) 241–248.
8 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.
9 P. MANSSON AND B. SAMUELSSON, *Sven. Paperstidn.*, 84 (1981) R15.
10 I. GOTO AND D. J. MINSON, *Anim. Feed Sci. Technol.*, 2 (1977) 247–253.
11 G. O. ASPINALL AND R. J. FERRIER, *Chem. Ind. (London)*, (1957) 1216
12 C. W. FORD AND G. N. RICHARDS, unpublished data.
13 I. M. MORRISON, *Phytochemistry*, 12 (1973) 2979–2984.
14 I. M. MORRISON, *Biochem. J.*, 139 (1974) 197–204.
15 R. D. HARTLEY, *Phytochemistry*, 12 (1973) 661–665.
16 H. U. MARKWALDER AND H. NEUKOM, *Phytochemistry*, 15 (1976) 836–837.
17 K. KRINGSTAD, *Acta Chem. Scand.*, 19 (1965) 1493–1494.
18 R. M. ALLISON, in G. W. BUTLER AND R. W. BAILEY (Eds.), *Chemistry and Biochemistry of Herbage*, Vol. 3, Academic Press, 1973, p. 64.
19 E. ADLER AND S. HERNESTAM, *Acta Chem. Scand.*, 9 (1955) 319–334.
20 R. L. WHISTLER AND B. D. E. GAILLARD, *Arch. Biochem. Biophys.*, 93 (1961) 332–334.
21 R. SOILA, O. LEHTIKOSKI, AND N.-E. VIRKOLA, *Sven. Papperstidn.*, 65 (1962) 632–639.
22 R. F. PATTERSON AND H. HIBBERT, *J. Am. Chem. Soc.*, 65 (1943) 1869–1873.
23 Y. NAKAMURA AND T. HIGUCHI, *Cellul. Chem. Technol*, 12 (1978) 209–221.
24 H. I. BOLKER AND N. TERASHIMA, *Adv. Chem. Ser.*, 59 (1966) 110–124.
25 H. I. BOLKER AND P. Y. WANG, *Tappi*, 52 (1969) 920–923.