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Structural Investigations on the Lignin–Carbohydrate Complexes of *Lolium perenne*

By IAN M. MORRISON
The Hannah Research Institute, Ayr KA6 5HL, U.K.

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1. Lignin–carbohydrate complexes isolated from leaf blade, leaf sheath and stem tissue of ryegrass by extraction with dimethyl sulphoxide were examined by fractionation procedures. Although the complexes are heterogeneous, heterogeneity is shown only in the ratio of the individual monosaccharide residues and not in the ratio of lignin to carbohydrate. 2. The molecular weight of the complexes is high ($\geq$150000), but chemical modification by alkaline hydrolysis, borohydride reduction or lead tetra-acetate oxidation does not drastically decrease it. Low-molecular-weight fragments released by alkaline treatment were shown to contain acetic acid, ferulic acid and *p*-coumaric acid. 3. On the basis of the chemical stability of the complexes, it is postulated that at least three types of bonding may be present between lignin and carbohydrate, namely one cleaved on borohydride reduction, another cleaved by alkali and a linkage resistant to alkali. 4. The carbohydrate portion of the complexes is composed of β-(1$\rightarrow$4)-linked D-glucose residues (cellulose) and β-(1$\rightarrow$4)-linked chains of xylose residues. Side chains involving arabinose and galactose residues are linked to C-3 of some of the xylose residues. 5. How the components of the complexes are held together is not certain, but it is suggested that the phenolic acids may act as cross-linking agents.

There is considerable uncertainty about the nature of the bonding that occurs between lignin and the carbohydrates of the plant cell wall. It is possible that some lignin is free and the remainder is chemically bonded to cell-wall polysaccharides (Pearl, 1967). Most information to date has been concerned with woods and very little is known about lignin–carbohydrate bonding in the *Gramineae*. Many types of linkage have been postulated (e.g. Merewether *et al.*, 1972; Bolker & Wang, 1969; Brownell, 1965; Bolker, 1963; Hayashi, 1961) and it is very likely that several types could occur in lignin–carbohydrate complexes from the same plant. All the above reports suggest, however, that the carbohydrate moiety of the linkage region involves residues from the hemicellulosic group of polysaccharides and not from cellulose.

Methods for the isolation of lignin–carbohydrate complexes from grass cell walls have been reported (Morrison, 1973) and the yield and monosaccharide residues found in these complexes from different plant tissues have been investigated (Morrison, 1974). In the present paper, the nature of the carbohydrate portion of the complex extracted by dimethyl sulphoxide from grasses is discussed and results are given suggesting that there are different types of bond in this complex.

Materials and Methods

Starting materials

Cell-wall preparations from leaf blade, leaf sheath and stem tissue of S23 ryegrass (*Lolium perenne*) were ball-milled until they had lost their fibrous nature. The resultant buff-coloured powder was extracted with dimethyl sulphoxide for 7 days at 25°C and the lignin–carbohydrate complexes were precipitated by the addition of 4vol. of ethanol (Morrison, 1973).

Gas–liquid chromatography

The equipment used was a Pye 104 model fitted with dual flame-ionization detectors. Alditol acetates, aldose acetates and methylated alditol acetates were separated on a glass column (1.5m) of 3% (w/w) ECNSS-M organosilicone polymer on Gas-Chrom Q (100–120 mesh). Methyl glycosides were separated on a column (1.5m) of 5% (w/w) neopentyl glycol adipate on Gas-Chrom Q (100–120 mesh). Trimethylsilyl aldonolactones were separated on a column (1.5m) of 10% (w/w) neopentyl glycol sebacate on Chromosorb W (80–100 mesh). Phenolic acids were separated on a column (2.75m) of 10% (w/w) cyclohexane–dimethanol succinate on Chromosorb W (80–100 mesh). All column packings were

purchased from Phase Separations Ltd., Queensferry, Flintshire, U.K. Retention times were compared with those of authentic standards.

Iodine colour reaction for xyloglucans

The procedure followed was essentially that of Kooiman (1960) as described by Gould *et al.* (1971). The extinction of the solutions was measured at 640nm on a Pye–Unicam SP. 600 spectrophotometer. For quantitative analysis, it was assumed that the relationship between extinction and weight was linear and that 1mg of xyloglucan gave an extinction of 3.78 at 640nm.

Optical rotations were observed at 20°C in dimethyl sulphoxide solutions. Lignin was determined by the method of Morrison (1972).

Experimental and Results

Qualitative and quantitative analysis of monosaccharide residues

Lignin–carbohydrate complexes (5mg) were hydrolysed with 2M-trifluoroacetic acid at 100°C for 16h (Albersheim *et al.*, 1967) and the derived alditol acetates were determined as described by Sawardeker *et al.* (1965). The identities of the individual monosaccharides were confirmed qualitatively by g.l.c. of the acetate esters of the parent aldoses and the trimethylsilyl ethers of the derived aldonolactones (Morrison & Perry, 1966). The only neutral sugar residues present in the complexes were glucose, xylose, arabinose and galactose; the composition of the complex from stem tissue is shown in Table 1. The complexes from the other tissues gave similar results.

Attempted fractionation of lignin–carbohydrate complexes

(*a*) *With copper (II) acetate.* The complex (100mg) was stirred in water (20ml) overnight, then centrifuged at 50000*g* for 30min to remove water-insoluble material. The supernatant solution was treated with 7% (w/v) copper(II) acetate (10ml), but no precipitation occurred. Ethanol (1vol.) was added and the resultant precipitate was centrifuged off. Further addition of ethanol to the supernatant did not give any further polysaccharide precipitate. The water-insoluble material was dried by solvent exchange from ethanol through acetone to ether (yield 53mg) and the copper complex, after decomposition with 1% HCl in ethanol, was washed free from copper salts with 1% HCl in ethanol before drying by solvent exchange as above (yield 40mg). The proportions of the neutral monosaccharide residues produced on hydrolysis of the copper complex were similar to those of the water-insoluble material (Table 1) and so the fractionation that had taken place was not due to any differences in the constituent neutral monosaccharide residues or the lignin content.

(*b*) *With cetyltrimethylammonium hydroxide.* The complex (100mg) was stirred in water (20ml) overnight, then centrifuged at 50000*g* for 30min to remove insoluble material. The soluble material was treated with an equal volume of 0.1M-cetyltrimethylammonium hydroxide solution. All of the dissolved complex was precipitated and centrifuged off. After the complex was dissociated with 1% HCl in ethanol, washed and dried (yield 37mg) the composition of neutral monosaccharide residues was similar to that of the water-insoluble material. Again fractionation was not achieved on account

Table 1. *Composition of neutral monosaccharide residues (%, w/w, of neutral sugars) and lignin content (% of complex) in fractions obtained from lignin–carbohydrate complexes from stem tissue*

For details see the text.

Fraction	Monosaccharides				Lignin
	Arabinose	Xylose	Galactose	Glucose	
Original complex	12	34	4	50	11.6
Water-insoluble complex	13	35	4	48	11.0
Complex precipitated with copper acetate	11	34	4	51	11.7
Complex precipitated with cetyltrimethylammonium hydroxide	12	34	4	50	11.4
Complex insoluble in 3.7M-$CaCl_2$	15	49	10	25	10.9
I_2-soluble complex	12	20	9	59	10.7
I_2-precipitated complex	6	24	6	63	12.1
Complex eluted with 0.05M-sodium acetate from DEAE-Sephadex column	12	35	4	49	10.6
Complex eluted with 0.5M-sodium acetate from DEAE-Sephadex column	12	34	3	51	11.6

of any differences in the neutral monosaccharide residues or lignin content.

(*c*) *With iodine in* 3.7M-$CaCl_2$. The complex (200mg) was stirred in 3.7M-$CaCl_2$ (20ml) overnight before centrifuging at 50000*g*. The insoluble material was separated, washed and dried (yield 26mg). The soluble material was treated with 3% (w/v) I_2 in 4% (w/v) KI (2ml) (Gaillard, 1961). Precipitated material was centrifuged off and washed with the I_2–$CaCl_2$ mixture. Both the I_2-soluble material and the I_2-insoluble material, suspended in water, were treated with $Na_2S_2O_3$ to reduce I_2 to I^- and the fractionated complexes were recovered by precipitation in 3vol. of ethanol and dried by solvent exchange (yields: I_2-soluble, 32mg; I_2-insoluble, 130mg). The monosaccharide compositions and lignin contents of the three fractions are shown in Table 1. There were differences in the ratio of monosaccharides present but all three fractions had similar lignin contents as shown by quantitative analysis and the u.v. spectra. Fractionation as the I_2 complex is believed to indicate wide variations in the degree of branching of the polysaccharide moiety of the complexes.

Molecular-sieve chromatography

The complexes were dissolved completely in 10% (v/v) dimethyl sulphoxide in water and applied to columns of Sephadex G-50, G-100, G-150 and G-200 [Pharmacia (G.B.) Ltd., London W.5, U.K.] and to columns of Corning controlled-pore glass granules, CPG-10 175Å (17.5nm), 370Å (37nm) and 1250Å (125nm) (BDH Chemicals Ltd., Poole, Dorset, U.K.). The samples were eluted in the void volume of Sephadex G-50, Sephadex G-100 and CPG-10 175Å (17.5nm). The samples were just included inside Sephadex G-150 and CPG-10 370Å (37nm) and well included inside Sephadex G-200 and CPG-10 1250Å (125nm). On all columns except Sephadex G-200 and CPG-10 1250Å (125nm) granules, the samples were eluted as symmetrical peaks containing both lignin and carbohydrate. On the Sephadex G-200 and CPG-10 1250Å (125nm) columns, the peaks were not

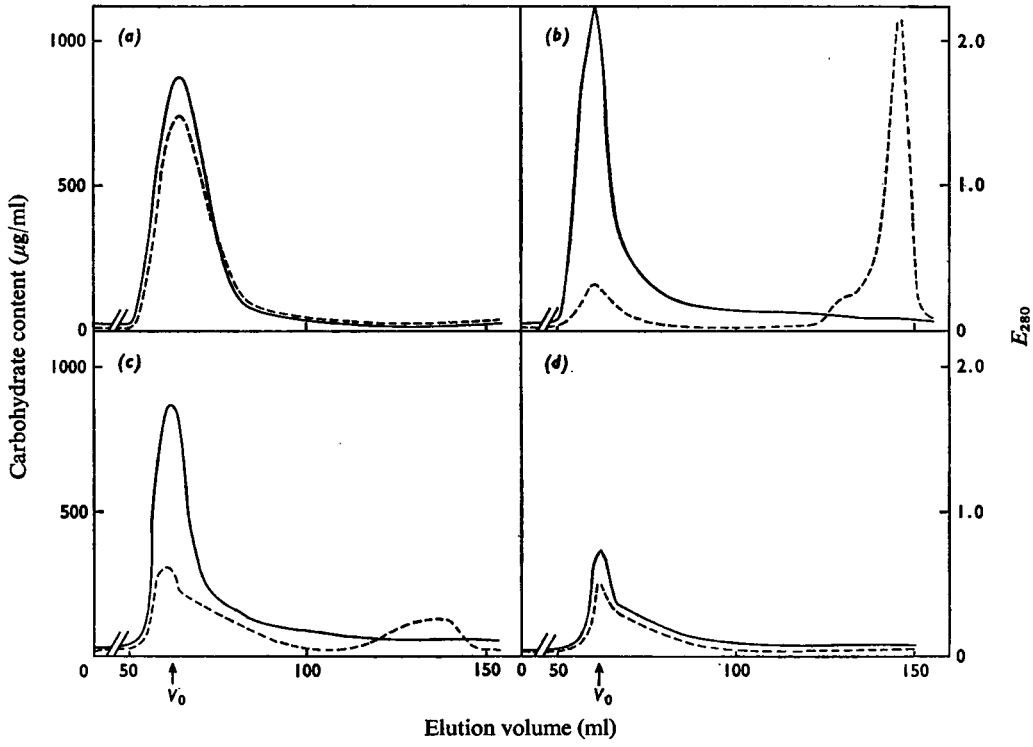

Fig. 1. *Molecular-sieve chromatograms of lignin–carbohydrate complexes from stem tissue on Sephadex G-50*

The column (87cm×1.5cm) was eluted with dimethyl sulphoxide–water (1:9, v/v). (*a*) Untreated complex; (*b*) alkali-hydrolysed complex; (*c*) borohydride-reduced complex; (*d*) lead tetra-acetate-oxidized complex. ——, Carbohydrate content (measured by phenol–H_2SO_4 method); – – – –, lignin content (measured by E_{280}). V_0, void volume.

symmetrical, being skew towards the lower-molecular weight side. Indeed with the leaf sample, two poorly separated peaks were recorded, but even so the lignin and carbohydrate elution profiles coincided.

From these observations the molecular weight of the complexes is estimated at approx. 150000. Also the samples are heterogeneous but cannot be separated satisfactorily by any technique examined. The molecular-sieve chromatogram of a sample on Sephadex G-50 is shown in Fig. 1(*a*).

Ion-exchange chromatography

The complexes were chromatographed on columns of DEAE-Sephadex A-25 [Pharmacia (G.B.) Ltd.] (acetate and phosphate forms). More than 90% of the sample was eluted with 0.025M-NaH_2PO_4 and 0.05M-sodium acetate. A gradient was then applied but the remainder of the complex was not eluted until 0.5M buffer was used. Both fractions had similar composition with respect to lignin content (as estimated by u.v. spectra) and carbohydrate composition (Table 1).

Alkaline hydrolysis of lignin–carbohydrate complexes

The complex from leaf-sheath tissue (8.6mg) was stirred in 0.02% NaOH in dimethyl sulphoxide–water (1:9, v/v; 10ml) for 7 days at 20°C under N_2 and the u.v. spectrum in the region 250–450nm was recorded at various time-intervals (Fig. 2). Alkaline hydrolysis was complete within 4 days. At time zero the major absorbing area (in alkaline solution) was centred around 378nm with two minor areas present centred around 299 and 310nm. As hydrolysis progressed the area around 378nm decreased in intensity and the absorbance maximum moved to shorter wavelengths. Concurrently the absorbing areas at 299 and 310nm increased in intensity until (after 4 days) there were two absorbing areas of approximately equal intensity at 328 and 295nm. On acidification to pH3 the absorbing areas were shifted to shorter wavelengths, namely 309 and 283nm.

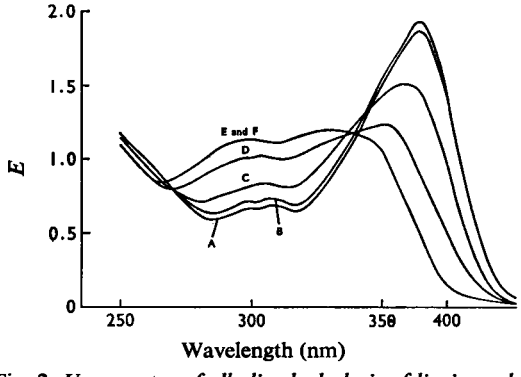

Fig. 2. *U.v. spectra of alkaline hydrolysis of lignin–carbohydrate complex from stem tissue with* 0.016M-*NaOH*

For details see the text. Curve A, 10min; curve B, 60min; curve C, 6h; curve D, 20h; curves E and F, 4 and 7 days.

The acidified sample was extracted with diethyl ether (6×20ml) and the spectra of the water- and ether-soluble fractions were recorded in the range 250–450nm. The water-soluble fraction still contained lignin (as shown by its absorbance at 280nm) whereas the spectrum of the ether-soluble fraction was similar to that of an authentic standard of ferulic acid.

Large-scale alkaline hydrolysis

The stem complex (695mg) was hydrolysed with 0.1M-NaOH in dimethyl sulphoxide–water (1:9, v/v). At this concentration of alkali hydrolysis was complete within 24h. The product was acidified with HCl to pH4.5 and samples were analysed on Sephadex G-50 and CPG-10 370Å (37nm) molecular sieves. The results from the Sephadex G-50 column are shown in Fig. 1(*b*). The total amount of carbohydrate in the high-molecular-weight component was very similar to that in the original material [Fig. 1(*a*)]. However, the lignin content of the complex was greatly decreased, appearing as low-molecular-weight phenolic components.

The remainder of the sample was separated into ether- and water-soluble components by ether extraction. The aqueous solution contained some insoluble material (11mg) that was centrifuged off. This material could not be hydrolysed with 2M-trifluoroacetic acid but gave glucose on hydrolysis with 72% (v/v) H_2SO_4. Cellulose reacts in a similar fashion. The true water-soluble material on treatment with 3vol. of ethanol gave a polysaccharide fraction (524mg) composed of arabinose (15%), xylose (36%), galactose (6%) and glucose (43%).

The ether-soluble fraction (33mg) was analysed for phenolic acids by the g.l.c. method of Hartley (1971). Ferulic acid (25%) and *p*-coumaric acid (3%) were identified and an unidentified fast-moving component was also observed. This compound was tentatively identified as acetic acid (60%) by its chromatographic characteristics at lower column temperatures. From molecular-sieve data there is evidence that units of molecular weights higher than those of the above phenolic acids were released by alkaline hydrolysis and that these units made up the unidentified 12% of the ether-soluble material.

Borohydride reduction of lignin–carbohydrate complexes

The complex from leaf-sheath tissue (8.0mg) was

stirred in 0.1% (w/v) $NaBH_4$ in dimethyl sulphoxide–water (1:9, v/v; 10ml) for 7 days at 20°C. The u.v. spectrum in the range 250–450nm was recorded at various time-intervals; this showed that reduction was complete within 4 days. The original spectrum was identical with that obtained in alkaline solution and the only change observed during the 4 days was a gradual decrease in the absorbance values by about 50%. After the excess of borohydride was oxidized with HCl, the solution was extracted with ether (6×20ml). The ether extract contained a small amount of material absorbing around 280nm. The spectrum of the aqueous solution was similar to that of the original material except that the overall intensity was decreased.

The complex from stem tissue was similarly reduced with $NaBH_4$ and the products were analysed by molecular-sieve chromatography on Sephadex G-50 (see Fig. 1*c*). The high-molecular-weight component contained similar amounts of carbohydrate to both the original material and the alkali extracted complex. The lignin content of this component was less than that in the original complex but considerably greater than that in the alkali-extracted complex. Small amounts of low-molecular-weight phenolic component(s) were also found, but no low-molecular-weight carbohydrate components.

The carbohydrate composition of the high-molecular-weight borohydride-reduced complex was analysed as arabinose (15%), xylose (37%), galactose (7%) and glucose (41%).

Lead tetra-acetate oxidation of lignin–carbohydrate complex

Stem-tissue complex (20mg) was dissolved in a mixture of dimethyl sulphoxide (3.7ml) and acetic acid (1.3ml) and lead tetra-acetate (110mg) was added as described by Zitko & Bishop (1966). After being kept for 24h in the dark, the oxidized complex was precipitated with ethanol (3vol.) and dried. The molecular-sieve chromatogram of a sample of the product on Sephadex G-50 is shown in Fig. 1(*d*). Both the carbohydrate and lignin contents of the high-molecular-weight component were greatly decreased, showing that considerable oxidation had taken place.

Optical rotation of the complexes

The optical rotations of the complexes were all negative and the values were low, namely: leaf-tissue complex, −14.4° (approx. 4.014%); leaf-sheath-tissue complex, −21.5° (approx. 4.116%); stem-tissue complex, −32.7° (approx. 4.000%).

Iodine colour reaction

This reaction, which is positive for xyloglucans (amyloids), was tested on the complex from each tissue and the absorption values obtained were: leaf-tissue complex, 0.200; leaf-sheath-tissue complex, 0.238; stem-tissue complex, 0.215. These values correspond to xyloglucan values of 5.3, 6.3 and 5.7% respectively. The low values suggest that the xylose and glucose residues in the complex were not linked together in the form of xyloglucans.

Methylation of lignin–carbohydrate complexes

The complex (500mg) from each tissue was dissolved in dimethyl sulphoxide (40ml) under N_2. NaH (1g) was added in portions over a 2h period and then methyl iodide (5ml) was added over a further 2h, essentially as described by Hakomori (1964). The addition of reagents was repeated twice on successive days. Chloroform (100ml) was added and the mixture filtered to remove NaI. Dimethyl sulphoxide was removed by repeated extraction with water (10× 100ml), and after drying over anhydrous Na_2SO_4, the chloroform solution was evaporated to dryness. Absorption in the hydroxyl-group region of the i.r. spectrum of all three samples was negligible, showing that methylation was complete. The yields were: leaf, 286mg; leaf sheath, 316mg; stem, 334mg. The methoxyl-group contents were 38.6, 37.8 and 38.0% respectively.

Analysis of the methylated complexes

A portion of each methylated complex was heated with 4% methanolic HCl in a sealed tube at 100°C for 16h. The solution was neutralized with Ag_2CO_3, filtered and evaporated to dryness at 0°C under a stream of N_2. A second portion was hydrolysed with 2M-trifluoroacetic acid for 16h at 100°C; the dried residues were reduced and acetylated. G.l.c. analysis of the derived methyl glycosides and methylated peracetylated glycitols gave the methylated sugars shown below. The major sugars, identified by comparison of their retention times with those of authentic standards, and present in each sample in the following order of abundance were: 2,3,6-tri-*O*-methylglucose, 2,3-di-*O*-methylxylose, 2,3,5-tri-*O*-methylarabinose, 2,3-di-*O*-methylarabinose, 2-*O*-methylxylose, 2,3,4,6-tetra-*O*-methylgalactose, 2,3-di-*O*-methylglucose and 2,3,4-tri-*O*-methylxylose. The possible structure which would yield these methylated derivatives are numerous, but the likely structure would have chains of (1→4)-linked glucose residues and (1→4)-linked chains of xylose residues. The other sugars probably account for side chains on the xylan chains.

Enzymic hydrolysis with cellulase

Samples of the complexes (approx. 5mg), in duplicate, were incubated with a cellulase preparation (2.5mg: a gift from Dr. J. S. D. Bacon) at 37°C for 16h in water (5ml). The samples were reduced with borohydride, and the derived glycitols were analysed by g.l.c. as their acetates. Although small amounts of arabinose (8–11%) and xylose (9–19%) were released, showing that the enzyme preparation contained both arabinohydrolase and xylohydrolase activity, the major sugar released was glucose (79–90%), indicating that β-(1→4)-linked D-glucose residues were features of the complexes.

Discussion

Lignin–carbohydrate complexes have been isolated from many wood sources by use of a number of different solvents (e.g. Björkman, 1957; Pew, 1957; Brownell, 1965; Linnell *et al.*, 1966; Merewether & Samsuzzaman, 1972). These complexes contain up to 25% lignin whereas the carbohydrate components comprise monosaccharide residues that are qualitatively and quantitatively similar to those found in the hemicellulosic fraction of the particular species. The lignin–carbohydrate complexes from grasses are different in that they contain monosaccharide residues similar in ratio to those found in the intact cell wall (Morrison, 1973). The monosaccharide residues present, namely glucose, xylose, arabinose and galactose, were confirmed by formation of three different derivatives and analysis by g.l.c., since Merewether & Samsuzzaman (1972) reported that the lignin–carbohydrate complex from *Eucalyptus obliqua* contained an unknown sugar residue which chromatographed similarly to glucitol hexa-acetate.

Certain covalent bonds in the plant cell wall are known to be extremely labile under either alkaline or acidic conditions (Higuchi *et al.*, 1967; Bolker & Wang, 1969). Lignin–carbohydrate complexes were extracted from ryegrass (Morrison, 1973) with neutral solvents, e.g. dimethyl sulphoxide, dimethyl formamide, 4M-guanidine hydrochloride, which should have no hydrolytic effect on covalent bonds between cell-wall components. In the present paper, attempts were made to fractionate the dimethyl sulphoxide-soluble complexes by precipitation techniques which involve neutral reactions so as to prevent any possible hydrolysis of labile chemical bonds. Copper acetate and cetyltrimethylammonium hydroxide gave no separation, but three distinct fractions were obtained by the I_2-complexing method of Gaillard (1961). Linear β-(1→4)-linked polysaccharides and branched β-(1→4)-linked polysaccharides which only possess a small number of short side chains are precipitated by this reaction, whereas highly branched polysaccharides remain in solution (Gaillard & Bailey, 1966). The lignin content of each fraction was very similar both quantitatively and qualitatively (as determined by u.v. spectroscopy), but the carbohydrate component of each fraction contained different proportions of the constituent monosaccharides. However, the same four monosaccharide residues were found in each fraction implying (by comparison with the results of Gaillard & Bailey, 1966) that fractionation had occurred because of differences in the degree of branching of the carbohydrate portion of the complex. The similarities between the lignin content of each fraction suggested that the chemical linkage(s) between lignin and carbohydrate were similar in each fraction.

Molecular-sieve chromatography indicated that the complexes had molecular weights of approx. 150000 and were not homogeneous. Throughout the molecular-sieve chromatograms, however, carbohydrate and lignin were eluted concurrently, indicating that the complexes of different molecular weights had very similar chemical compositions. Similar results were obtained on ion-exchange chromatography in different buffer solutions: a small proportion of the complex was retarded on the columns and contained relative amounts of carbohydrate and lignin which were almost identical with those of the starting material.

Some of the structural features of the carbohydrate portion of the complexes obtained from the methylation and enzymic analyses are outlined in Fig. 3. These features are similar to many plant-cell-wall polysaccharides (Aspinall, 1959). The most likely structure is either a mixture of xylan- and cellulose-type polymers arranged in some intimate relationship, or a xyloglucan. Xyloglucans have been isolated from seeds and other plant sources (e.g. Kooiman, 1961, 1967; Ramalingham & Timell, 1964; Aspinall *et al.*, 1969; Gould *et al.*, 1971) and there are reports that cellulose fibrils can be made 'soluble' by encapsulation with other cell-wall polysaccharides in both cress (Tyler, 1965) and mustard seeds (Grant *et al.*, 1969). Although the lignin–carbohydrate complexes did not give the characteristic blue stain with I_2 which has been used to identify xyloglucans (Kooiman, 1960), the presence of a xyloglucan cannot be excluded, since the fucoxyloglucan from sycamore callus cells also reacted negatively with I_2 (Aspinall *et al.*, 1969). The complexes had specific optical rotations between −14° and −32°. Aspinall *et al.* (1969) reported the optical rotation of the sycamore fucoxyloglucan to be +27°. The low negative rotations of the complexes are, however, consistent with the idea that the complexes contain mixtures of xylan and cellulose polymers, since xylans have high negative rotations and cellulose derivatives have very low negative values.

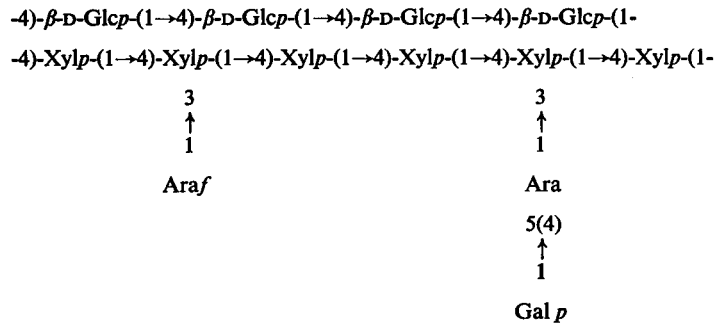

Fig. 3. *Partial structure for carbohydrate component of lignin–carbohydrate complexes from methylation and enzymic analyses*

The ether-soluble compounds obtained from alkaline hydrolysis of the complex contained, among other things, acetic acid, ferulic acid and *p*-coumaric acid. Acetyl groups have long been recognized as constituents of xylans, especially from hard woods (Aspinall, 1959; Hägglund *et al.*, 1956; Timell, 1960), but are lost by hydrolysis during extraction of the hemicelluloses with alkali. Ferulic acid and *p*-coumaric acid have been reported as constituents of milled-wood lignins (Higuchi *et al.*, 1967), ryegrass cell walls (Hartley, 1972, 1973), pineapple tissue (Gortner *et al.*, 1958) and wheat flour glycoprotein (Fausch *et al.*, 1963). They are evidently very widespread in the plant kingdom and may act as cross-linking agents because they possess two functional groups, namely OH and CO_2H.

Chemical treatment of the complexes indicated that the carbohydrate component was resistant to both alkali and $NaBH_4$ but was oxidized by lead tetra-acetate because of the numerous *cis*-diol groups in the structure. The lignin component was partially cleaved from the complex by both alkali and borohydride, but the partially delignified complex obtained after alkaline hydrolysis was very different from that obtained from borohydride reduction. From the u.v. spectra there appeared to be ester bonds that were resistant to reductive cleavage with borohydride.

In conclusion, these results indicate that the lignin–carbohydrate complex extracted with dimethyl sulphoxide from ryegrass contained xylan and cellulose polymers associated with each other. Acetyl and phenolic groups are linked to the complex by ester bonds and there are at least three different lignin linkages, one cleaved on reduction, another cleaved by alkali and a linkage resistant to alkali. From the similarity between the composition of the lignin–carbohydrate complex and the intact cell wall it could be postulated that the complex is a unit of cell-wall structure. Why such a unit can be extracted with dimethyl sulphoxide is not clear, but it could be suggested that the ball-milling technique used to comminute the plant fibres has disrupted hydrogen bonding in the cell wall and allowed the solvent (dimethyl sulphoxide) to penetrate more fully into the particles and dissolve some cell-wall units.

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