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A FERULOYLATED ARABINOXYLAN LIBERATED FROM CELL WALLS OF *Digitaria decumbens* (PANGOLA GRASS) BY TREATMENT WITH BOROHYDRIDE

CLIVE W. FORD

Dvision of Tropical Crops and Pastures, C.S.I.R.O., Cunningham Laboratory, St Lucia, Queensland 4067 (Australia)

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ABSTRACT

A water-soluble polysaccharide, liberated from cellulase-degraded stem cell walls of pangola grass (*Digitaria decumbens*) during treatment with borohydride, was purified by gel filtration. The polymer (0.3% of the cell walls) contained no uronic acid, 83% of carbohydrate, 0.2% of esterified ferulic acid, and polyaromatic residues bound by alkali-stable linkages. Acid hydrolysis liberated mainly L-arabinose, D-xylose, and D-galactose, together with a small amount of D-glucose. Methylation analysis established the polysaccharide to be a highly branched arabinoxylan with 60% of the xylose disubstituted, 50% of the arabinose as terminal furanose residues, and most of the galactose as terminal residues. The proportions of xylose branch-points and terminal pentose residues corresponded and, therefore, galactose was postulated to be linked to the polymer complex *via* a non-carbohydrate interface. The polysaccharide may be an example of a cell-wall hemicellulose that is resistant to enzyme-mediated degradation. The possible contribution of ferulic acid as a cross-linking or gel-forming agent is discussed.

INTRODUCTION

Borohydride at pH 9 deacylates[1] acetyl derivatives of glucose, *myo*-inositol, and cellulose under conditions that cleaved 80% of the acetate from cellulase-degraded stem cell walls of a tropical grass. Other chemical linkages in the cell walls were broken to yield borohydride-soluble material some of which had carbohydrate still linked to u.v.-absorbing substances spectroscopically similar to lignin[2]. Identification of the structures of these complexes may further the understanding of the *in situ* binding of cell-wall polysaccharides and help to explain their resistance to biodegradation and the low nutritional value of forage fibre.

Structural studies of the polysaccharide material that had been solubilised during treatment of cellulase-degraded grass cell walls with borohydride are now reported.

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EXPERIMENTAL

Materials. — The isolation of the water-soluble polysaccharide, liberated from cellulase-degraded stem cell walls of pangola grass during treatment with borohydride, has been described[1].

General methods. — Solutions were concentrated at <40° under reduced pressure. Carbohydrate in solution was determined colorimetrically[3] using as reference a 2.5:1 mixture of D-xylose and L-arabinose. The monosaccharide composition of polysaccharides was determined by g.l.c. after hydrolysis with 2M trifluoroacetic acid for 1 h at 120° and conversion of the products into trimethylsilyl ethers[4] or alditol acetates[4]. Phenolic acids were determined by g.l.c. of the trimethylsilyl derivatives[4].

Partially methylated alditol acetates were separated by g.l.c. at 150° or 170° on nickel columns (1.8 m × 2 mm) packed with (*a*) 0.4% of OV-225 on surface-modified Chromosorb[5] or (*b*) 3% of ECNSS on Gas-Chrom Q (100–200 mesh). G.l.c.–m.s. involved a glass column (2.7 m × 6 mm o.d.) packed with 3% OV-225, and a temperature program of 150° for 5 min then to 220° at 10°/min. E.i.-mass spectra were recorded at 70 eV. U.v. spectra were recorded for solutions in water (high-molecular-weight fractions) or aqueous 95% ethanol.

Fractionation of polysaccharides. — *(a) Gel filtration.* Freeze-dried polysaccharide (65 mg, 73% of carbohydrate), isolated[1] from Bio-Gel P-2, was eluted with water at 15–20 mL/h from the following columns (~30 × 2.5 cm) in sequence: Bio-Gel (Bio-Rad) P-4 (200–400 mesh), P-6 (200–400 mesh), P-10 (200–400 mesh), and P-150 (100–200 mesh). The fractions which contained the material in the high-molecular-weight peak from each column were combined and freeze-dried, and a solution in 1 mL of water was applied to the next column. The eluate (2.5-mL fractions) was monitored for carbohydrate[3], absorbance at 280 nm, and changes in the λ_{max} near 285 nm (see Table I).

(b) Treatment with alkali. A solution of the final product (7.1 mg, 79% of carbohydrate) from (*a*) in 0.5M sodium hydroxide (0.5 mL) was kept at 25° under nitrogen for 16 h, then neutralised with 7M acetic acid, applied to a column (32 × 1.6 cm, 100–200 mesh) of Bio-Gel P-150, and eluted with water at 15 mL/h. The eluate was monitored as in (*a*) and the polysaccharide fractions were combined and freeze-dried.

Methylation analysis. — A portion (2 mg) of the product (4.8 mg, 83% of carbohydrate) from (*b*) in a 7-mL vial was dried *in vacuo* over phosphorus pentaoxide. The vial was then sealed, methyl sulphoxide (1 mL) was introduced through a Teflon-coated silicone septum using a syringe, and air was flushed out with nitrogen. Ultrasonication (1 min) aided quick dissolution of the polysaccharide. The tube was cooled in crushed ice and methylsulphinylmethanide[6] (1 mL) was added to the solidified methyl sulphoxide. The mixture was allowed to attain room temperature and then worked-up for g.l.c. as described by Jansson *et al.*[6].

TABLE I

SEQUENTIAL GEL FILTRATION OF POLYSACCHARIDE MATERIAL, SOLUBLE IN NEUTRAL AQUEOUS SOLUTION, EXTRACTED FROM CELL WALLS OF PANGOLA GRASS WITH BOROHYDRIDE

Column	*Polysaccharide applied (mg)*	*Yield of fractions*			*Recovery (%)*	
		I	*II*	*III*	*Solids*	*Carbohydrate*
			(mg)			
P-2[a]	190.6	64.5	96.5	24.8	97	97
P-4	64.5	21.0	19.2	17.5	89	91
P-6	21.0	15.7	6.1		104	87
P-10	15.7	12.0			87	87
P-150	12.0	10.0			83	88

[a]Illustrated in previous work[1].

RESULTS AND DISCUSSION

Polysaccharide material, solubilised during treatment[1] of cell walls of pangola grass with borohydride, was fractionated on several columns of Bio-Gel in sequence, in order to increase the homogeneity with respect to the monosaccharide composition and the co-eluted u.v. absorbance (Table I). Good recoveries of solids and carbohydrate were obtained from each column. The results for Bio-Gel P-4 (Fig. 1) indicated that there was a range of molecular sizes present in the starting material. Of the original polysaccharide, ~33% was eluted as a narrow peak (I) at the exclusion volume, whereas the remainder (II and III) was more polydisperse but still eluted well within the inclusion volume. Fraction I had strong u.v. absorbance (λ_{max} 285 and 319 nm) which matched the profile of the carbohydrate part, whereas the u.v. absorbance (λ_{max} 280 nm) of II and III was weaker and appeared to be independent of the fluctuations in carbohydrate content. The structure of the major u.v.-absorbing components of I clearly differed from those of II and III, the spectra of which were similar to those of unconjugated poly-aromatic substances[2]. Analysis of the u.v. spectra of individual fractions of eluate from the column indicated a steady decrease (288 → 284 nm) in the λ_{max} near 285 nm during elution of I, which suggested heterogeneity. Carbohydrate analysis of I–III also indicated significant differences in monosaccharide composition (Table II). The xylose–arabinose ratios increased from 1.30 in I to 3.79 in III, which, on the assumption that most of the xylose was in the main polysaccharide chains, suggested that the high-molecular-weight fraction I was a highly branched polymer. The degree of branching appeared to decrease as the molecular size of the polysaccharides decreased. Another major difference was the accumulation of most of the galactose in I, which indicated that this monosaccharide was closely associated with the high-molecular-weight complex.

Further gel filtration of I (Table I) on Bio-Gel P-150 gave polysaccharide (0.3% of the cell walls) wih a slightly lower xylose–arabinose ratio and galactose

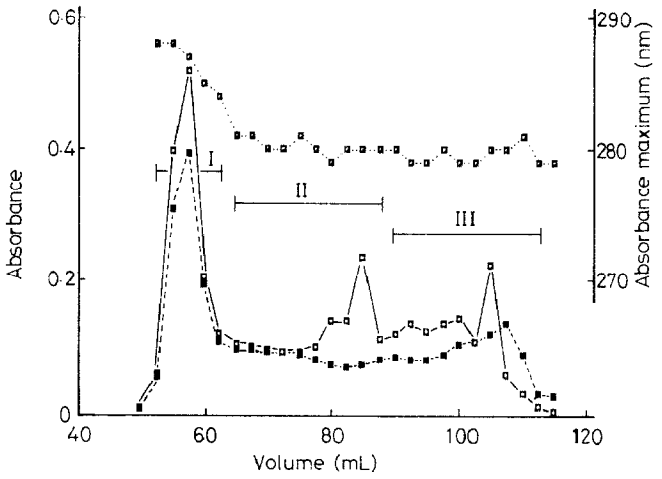

Fig. 1. Fractionation on Bio-Gel P-4 of polysaccharide extracted by borohydride from cellulase-degraded cell walls of pangola grass: ——, carbohydrate; ----, A_{280}; ····, λ_{max}; V_o, 53 mL; V_i, 138 mL.

content (Table II), and a more homogeneous u.v.-absorbing component (λ_{max} 287 and 321 nm) (Fig. 2). Since the u.v.-absorbing material still had an elution profile similar to that of the carbohydrate fraction after chromatography on several different columns, it seemed likely that the polysaccharide and aromatic compounds were linked covalently.

Treatment of the purified polysaccharide with alkali cleaved a major portion of the u.v.-absorbing substances, but did not cause any large change in the molecular-weight-distribution of the carbohydrate (I, Fig. 3) or the monosaccharide composition (Table II). The polysaccharide fraction still had some co-eluting u.v. absorbance (Fig. 3), but the lignin-like u.v. spectrum[2] (λ_{max} 274 nm) was different from that (Fig. 4) of I before treatment with alkali. The weak u.v.-absorbing fraction II (Fig. 3) had a single λ_{max} at 273 nm, whereas fraction III had a u.v. spectrum similar to that of *trans*-ferulic acid (FA). The identity of the phenolic acid

TABLE II

MONOSACCHARIDE COMPOSITION OF POLYSACCHARIDE FRACTIONS FROM BIO-GEL P-4 AND P-150

Column	*Carbohydrate (% of fraction)*	*Ara*	*Xyl*	*Gal*	*Glc*	*Xyl/Ara*
		(Relative molar %)				
P-4, I	75	32.9	42.8	20.7	3.7	1.30
II	67	28.6	59.0	7.0	5.4	2.06
III	66	19.8	75.0		5.2	3.79
P-150, I	79	35.0	44.1	17.5	3.5	1.26
P-150, I(OH)[a]	83	34.4	43.8	18.8	3.1	1.27

[a]After treatment with alkali (Fig. 3).

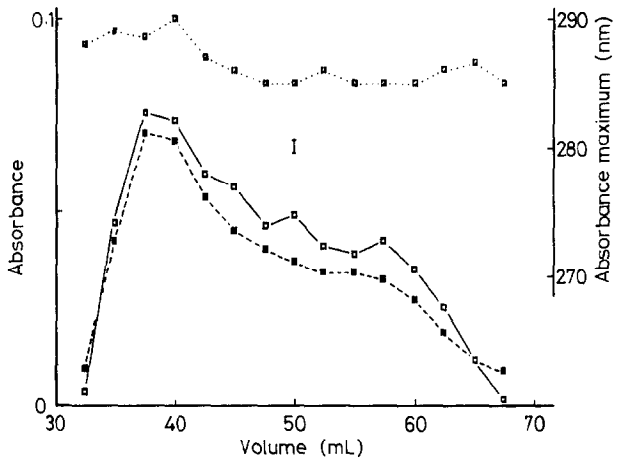

Fig. 2. Refractionation on Bio-Gel P-150 of fraction I from Fig. 1 after elution from columns of Bio-Gel P-6 and P-10 in sequence: ——, carbohydrate; ----, A_{280}; ····, λ_{max}; V_o, 35 mL; V_i, 155 mL.

was confirmed by co-chromatography in g.l.c. and by g.l.c.–m.s., and there was about one FA molecule per 500 sugar molecules.

Methylation analysis indicated the alkali-treated polysaccharide to be a highly branched pentosan (Table III). Of the xylose residues, ~60% were disubstituted with branch-points mainly through the 3-position. Of the arabinose, ~50% was present as terminal furanosyl units, the remainder being 2- and 3-linked furanose residues probably in short side-chains attached to a (1→4)-linked xylose backbone[7].

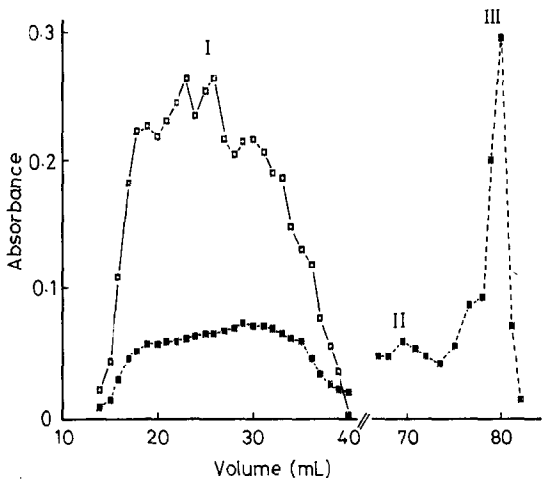

Fig. 3. Fractionation on Bio-Gel P-150 of fraction I in Fig. 2 after treatment with alkali: ——, carbohydrate; ----, A_{280}; V_o, 16 mL; V_i, 70 mL.

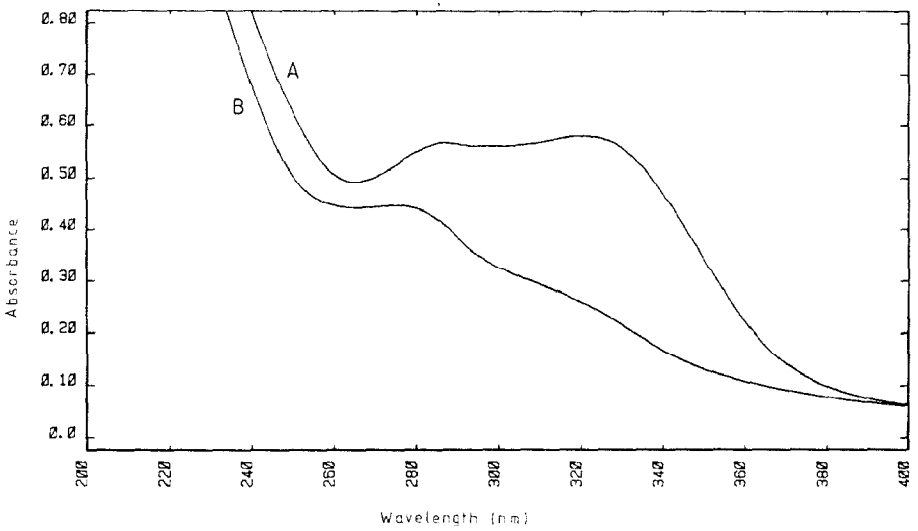

Fig. 4. U.v. spectra of polysaccharide I after fractionation on Bio-Gel P-150: A, before (λ_{max} 287 and 321 nm); B, after (λ_{max} 274 nm) treatment with alkali.

Galactose was present mainly as end-group pyranose residues. Only small amounts (not measured) of 2,4,6-galactose and xylitol penta-acetate were detected. The location of the galactose is difficult to deduce. Since the terminal pentose and disubstituted xylose residues were present in similar amounts, the galactose was not linked to xylose or arabinose. If the components of the complex are covalently linked, then a non-carbohydrate interface of the pentosan and galactose can be visualised. The u.v.-absorbing material, apparently bound by an alkali-stable linkage, may contain hydroxyl groups to which the galactose could be linked.

The FA did not appear to link the polysaccharide and u.v.-absorbing sub-

TABLE III

METHYLATION ANALYSIS OF ALKALI-TREATED POLYSACCHARIDE[a] AFTER FRACTIONATION ON BIO-GEL P-150

Identified component	*Peak area (%)*	*Structure*
2,3,5-Ara[b]	18	Ara-(1→
2,3,4-Xyl	8	Xyl-(1→
3,5-Ara	8	→2)-Ara-(1→
2,5-Ara	10	→3)-Ara-(1→
2,3,4,6-Gal	18	Gal-(1→
2,3-Xyl	11	→4)-Xyl-(1→
2(3)-Xyl[c]	27	→4)-Xyl-(1→ 3(2)

[a]Fig. 3, fraction I. [b]2,3,5-Ara = 1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinitol, *etc.* [c]From m.s. analysis, 2-Xyl and 3-Xyl were present in the ratio of ~3:1.

stances, since no large unidentified u.v.-absorbance was liberated with alkali. It is feasible that the FA was attached to terminal arabinofuranose residues in a manner similar to that reported in pentose trisaccharides produced by enzymic degradation of the cell walls from *Zea*[8] and barley straw[9]. Therefore, the FA could be involved in cross-linkages by esterifying aliphatic carboxyl groups in other cell-wall constituents through HO-4 of the phenolic acid. These aliphatic esters would have been cleaved by borohydride.

The polysaccharide I (Fig. 2) appears to be part of a lignin–carbohydrate complex in which lignin is a minor component. The carbohydrate probably represents "core" material from larger molecules that had been partially degraded during pretreatment of the cell walls with a commercial cellulase[1], a complex mixture of enzymes including xylanase and glycosidase activities. Notable features are the presence of esterified FA, which has been associated more with easily degradable cell-wall tissue[4,10–12], and the appreciable amount of galactose terminal groups which, apparently, are not linked directly to other monosaccharides. The highly branched structure of the pentosan indicated by the methylation analysis suggests that there is considerable heterogeneity with regard to the pattern and frequency of arabinose substitution. Although this may be the first isolation by chemical means of a neutral arabinoxylan containing esterified FA, the enzymic dissociation of a feruloylated glucuronoarabinoxylan from *Zea* cell walls has been described[13]. The latter acidic polymer was solubilised from relatively immature plant tissue, whereas the pangola grass polysaccharide was from mature stem material. Thus, extraction with alkaline borohydride may provide a method for releasing polysaccharide esters of ferulic acid from plant residues that are resistant to enzyme degradation.

Whether the complex is part of the general matrix of the cellulase-degraded cell walls or contributes to material responsible for intercellular binding cannot be specified. However, since pectic substances are present in the intercellular spaces in plant cell walls, it is possible that some of the galactose originated as esterified galacturonic acid, although not necessarily from pectic structures typically associated with dicotyledons. Although the arabinoxylan complex is held in the cell walls by borohydride-labile linkages, the polysaccharide may be an example of grass cell-wall hemicellulose that is resistant to enzymic degradation because of the highly branched structure of the carbohydrate. The quantity and distribution of these branched structures may influence the biodegradability of the cell walls. This resistance could also be enhanced by oxidative gelation involving FA[14], which would form a barrier to penetration by enzymes or micro-organisms. The possible role of ferulic acid in cell walls as a plant growth regulator has also been proposed[15].

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