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HEMICELLULOSIC POLYMERS FROM THE CELL WALLS OF BEESWING WHEAT BRAN: PART I, POLYMERS SOLUBILISED BY ALKALI AT 2°

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ABSTRACT

Cell-wall material from beeswing wheat bran was sequentially extracted with 0.05M NaOH at 2°, M KOH at 2° and 20°, and 4M KOH at 20° followed by delignification and further extraction with M and 4M KOH at 20°, to leave the α-cellulose residue which contained a significant amount of arabinoxylan. The hemicellulosic polymers solubilised by M KOH at 2°, which represented ~20% of the dry weight of the cell walls, were fractionated by graded precipitation with alcohol prior to anion-exchange chromatography and then subjected to methylation analysis. The major polymers were closely related, highly branched arabinoxylans, slightly branched arabinoxylans, and arabinoxylans in close association with xyloglucans (arabinoxylan–xyloglucan complexes); the arabinoxylans were acidic and were associated with various amounts of phenolics. The various polymers exhibit heterogeneity, and phenolic ester and phenolic ether cross-links play a major role in the architecture of the cell walls.

INTRODUCTION

Despite the importance of wheat bran, a main source of dietary fibre, few detailed studies have been carried out on its major cell-wall polymers[1–5]. Commercial wheat bran contains a range of tissues, including the pericarp with the attached testa, aleurone layer, and some endosperm. The cell walls of wheat bran contain mainly glucuronoarabinoxylans, cellulose, and lignin derived from the lignified outer layers[2,3], and some mixed-linkage β-D-glucans and arabinoxylans from the aleurone layer[3,6]. Alkaline extracts of the cell walls yielded small proportions of ferulic acid and *p*-coumaric acid, which suggested that these acids served to cross-link some of the matrix polymers[2,3]. Firm evidence for an ester link between arabinoxylan and ferulic acid was provided by the isolation of feruloylated arabinoxylan from enzyme digests of cell walls[7,8]. However, there is evidence from studies with other tissues to show that phenolic acids and polyphenolics (including lignin)

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can form alkali-resistant ether linkages with the matrix polysaccharides[9,10].

Previous studies have not dealt in detail with (*a*) the relative amounts of matrix polymers covalently associated with phenolics by ester and/or ether linkages, and (*b*) the heterogeneity of the matrix polymers. This information is crucial for a better understanding of both the biochemistry of cell-wall formation and the biodegradability of fibre from wheat bran by colonic bacteria. In view of this, we have investigated, in some detail, the chemical composition of the cell walls of beeswing wheat bran extracted and fractionated under conditions which cause minimum degradation of the polymers. We now report mainly on the hemicellulosic polymers solubilised by M KOH at 2°. Beeswing bran contains the outer coating of the grain comprising the cuticle, epidermis, and hypodermis. The study was restricted to beeswing bran because of the complexity of the different types of polymers present in the cell walls of various tissues of commercial wheat bran.

EXPERIMENTAL

Plant material. — Wheat grain (cultivar Maris Freeman) was purchased locally. The grains were thoroughly washed, suspended in distilled water for 20 min, and then gently blended in a Waring Blendor (low speed) for 2 min. The mixture was allowed to settle and the beeswing bran, which floated to the surface of the water, was collected. Electron microscopy showed that the beeswing bran was only three cells thick.

Monosaccharide and amino acid analysis. — Neutral sugars were released by a modified Saeman hydrolysis, or by hydrolysis with M H_2SO_4 for 2.5 h at 100°, and analysed[11] as their alditol acetates by g.l.c. Uronic acid was determined colorimetrically by a modification[12] of the method of Blumenkrantz and Asboe-Hansen[13].

Amino acids were released by hydrolysis with 6M HCl and analysed[14] as their heptafluorobutyryl-*n*-propyl derivatives by g.l.c

Phenolic content. — The phenolic content of some of the fractions was determined by the acetyl bromide method[15], which was scaled down for mg amounts of material. U.v. measurements at pH 10, 6.5, and 3 were made with a Pye–Unicam SP800 spectrophotometer[16].

Preparation of cell-wall material from beeswing wheat bran. — Cell-wall material (CWM) was prepared by sequential extraction of the wet ball-milled fresh tissue with aqueous 1% sodium deoxycholate and aqueous 90% methyl sulphoxide to remove cytoplasmic proteins and starch[2]. These two solvents solubilised ~1% of the CWM. Extraction with phenol–acetic acid–water was omitted because the tissue contained only a small proportion of intracellular protein. The CWM was stored as a frozen damp residue at −20°. A freeze-dried portion indicated the yield from 100 g of fresh tissue to be ~39 g (dry weight) of CWM.

Sequential extraction of CWM. — The CWM (10 g, dry weight) was sequentially extracted under argon with the following solvents each containing also 10mM $NaBH_4$: (1) 0.05M NaOH (500 mL), adjusted to pH 10 with 2M HOAc, for 4 h at 2°;

(2) twice with M KOH (2 × 500 mL) for 4 h at 2°, and the extracts were fractionated separately; (3) 500 mL each of M and 4M KOH at 20°. The resulting brown residue was washed thoroughly with distilled water, adjusted to pH 4.5 with HOAc, and then delignified by treatment with sodium chlorite–HOAc at 70° for 2 h under argon. The residue was extracted sequentially with M and 4M KOH at 20° for 2 h, to leave the α-cellulose residue which was washed and freeze-dried.

The 0.05M NaOH-extract was filtered, concentrated under reduced pressure, and then eluted from a column (2 × 32 cm) of Dowex 50W-X8 (H^+) resin (20–50 mesh) with distilled water; the effluent (300 mL) was concentrated and then freeze-dried. The remaining alkaline extracts were acidified to pH 5 with HOAc, dialysed, and concentrated to 100 mL, and an aliquot was freeze-dried. The polymers in the concentrates solubilised by M KOH at 2° were subjected to stepwise precipitation with alcohol.

Graded precipitation with alcohol. — This was effected[5] by increasing the concentration of ethanol in increments of 10%. The mixture was stored at 2° for a minimum of 1 h, and the precipitated polymers were collected by centrifugation and freeze-dried.

Ion-exchange chromatography. — Cell-wall fractions were stirred with distilled water overnight, insoluble material (water-insoluble residue) was removed by centrifugation, and the soluble material was applied to columns (25 × 1 cm) of DEAE-Sephacel (AcO^- form). Elution at 20 mL/h was effected with water (60–80 mL, depending on sample size), followed by a linear gradient of acetate (pH 6.5) 0→0.9M (120–180 mL), then stepwise increases to M (~50 mL) and 5M (~50 mL), and finally 0.2M NaOH (~30 mL). Fractions (2 mL) were collected and monitored for absorption at 280 nm, and aliquots (100 μL) were assayed for total carbohydrate by the phenol–sulphuric acid method[17]. Appropriate fractions were combined, dialysed, and freeze-dried.

The unadsorbed component from one of the fractions, which was rich in glucose, was further fractionated as a borate complex on DEAE-Sepharose, using a borate gradient: 85 mL of 0.05M borate (pH 8) followed by 150 mL of a 0.05→1.0M borate gradient at 20 mL/h. Fractions were monitored for carbohydrate and absorbance at 280 nm, and appropriate fractions were combined, dialysed, freeze-dried, and analysed for constituent sugars.

Partial acid hydrolysis. — The major (acidic) arabinoxylan (30 mg) from the second M KOH-extract was hydrolysed with 0.2M trifluoroacetic acid at 100° for 2 h. The hydrolysate was filtered and the excess of trifluoroacetic acid was removed from the filtrate by repeated evaporation with water under vacuum. The acidic oligosaccharides were trapped on an anion-exchange column and eluted with 2M HOAc. The resulting oligosaccharides were converted into their methylated alditol derivatives and examined by g.l.c.–m.s.[2].

Methylation analysis. — The method of Hakomori was used as described previously[18,19]. The methylated fractions showed negligible i.r. absorption for hydroxyl and were hydrolysed with aqueous 90% formic acid at 100° for 2 h foll-

owed by 0.25M H_2SO_4 at 100° for 12 h. The products were converted into the alditol acetate derivatives and analysed by g.l.c. using OV-225 and ECNSS-M columns[18]. The ECNSS-M column was used to separate the derivatives of (1→2)- and (1→4)-linked xylose from the terminal galactose derivative, which have the same mobilities on OV-225. Attempts to detect uronic acid in some of the polysaccharide fractions were carried out by reduction of the methylated product[2] with $LiAl^2H_4$. G.l.c.–m.s. was performed on a Kratos MS9/50 mass spectrometer, using an OV-225 column. Mass spectra were identified by using the data of Jansson *et al.*[20]; the values for the partially methylated alditol acetates were corrected by using the molar response factors recorded by Sweet *et al.*[21].

RESULTS AND DISCUSSION

Fractionation of CWM. — The CWM was sequentially extracted with 0.05M NaOH at 2°, M KOH twice at 2° and once at 20°, and 4M KOH at 20°. Further extraction of the brown residue with 4M KOH solubilised only small amounts of material, but significant amounts of polymers were solubilised with alkali after delignification. The relative amounts of polymers solubilised and their sugar compositions are given in Table I.

The first extraction was performed with 0.05M NaOH at 2° in order to hydrolyse ester cross-links and solubilise the hemicelluloses with minimum degradation. The small amounts of ferulic and *p*-coumaric acids recovered from the extract indicated that negligible ester cross-links had been broken. Only ~2% of polymeric material was solubilised and carbohydrate accounted for ~4% of this material; the non-carbohydrate component of the polymer was not investigated. The mild alkaline conditions employed have been used to de-esterify pectins with minimum β-

TABLE I

CARBOHYDRATE COMPOSITION OF THE FRACTIONS OBTAINED BY SEQUENTIAL EXTRACTION OF CWM

Fraction	*Yield*	*"Anhydro sugar" (μg)/mg dry wt.*						
	(mg/g)	*Deoxy-hexose*	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Total*
CWM		1.4	306	295	5.0	23.7	335	966.1
0.05M NaOH, 4 h, 2°	18	1.0	13.3	11.6	—	0.9	14.2	41.0
M KOH, 4 h, 2° (A)	102	3.3	355	426	30.8	23.5	57.2	895.8
M KOH, 4 h, 2° (B)	86	1.6	339	367	—	26.4	71.9	805.9
M KOH, 4 h, 20° (C)	67	3.9	472	437	—	36.4	43.3	992.6
4M KOH, 4 h, 20° (D)	79	5.3	342	349	17.2	38.2	101	852.7
0.6% Chlorite, 2 h, 70°	26	Tr[a]	447	448	3.2	38.9	15.1	952.2
M KOH, 2 h, 20°	54	Tr	340	360	3.8	33.8	20.7	758.3
4M KOH, 2 h, 20°	24	4.7	424	405	5.6	33.7	43.5	916.5
α-Cellulose	206	—	154	136	4.7	11.3	692	998

[a]Trace.

eliminative degradation[22]. Further, in preliminary experiments with the cyclohexanediaminetetraacetate-extracted cell walls of onion bulbs, significant amounts of pectic polysaccharides have been solubilised under comparable conditions[23]. Thus, the phenolic-ester cross-links in the cell walls of beeswing bran are more difficult to hydrolyse than methyl-esterified pectins and some of the ester cross-links that exist in the cell-wall matrix of onions.

Therefore, the 0.05M NaOH-insoluble residue was extracted twice with M KOH at 2°, when significant amounts of polymers rich in arabinose and xylose were solubilised. The extracts were not combined, in order to facilitate subsequent fractionation of the heterogeneous mixture of polymers they contained and which are the main concern of this report. The presence of appreciable amounts of ferulic and *p*-coumaric acids and other unidentified phenolics in the extracts showed that phenolic and related ester cross-links had been broken. Further extraction of the residue with M and 4M KOH at 20° solubilised more polymers rich in arabinose and xylose, and some of these were associated with (poly)phenolics. Presumably, the bulk of the polymers solubilised was held in the walls by ester cross-links having various stabilities towards alkali, although some, particularly those solubilised by 4M KOH, may have involved hydrogen bonds also (*cf.* solubilisation of xyloglucans from the cell walls of parenchymatous tissues of dicotyledons)[24,25]. The 4M KOH-insoluble residue had to be delignified before additional amounts of polymers rich in arabinose and xylose could be solubilised, showing that the remaining polymers were highly cross-linked by phenolic ether linkages and/or were encrusted with lignin. The final α-cellulose residue still contained significant amounts of arabinose and xylose, and it was not clear whether the associated arabinoxylans were covalently linked to the cellulose or were physically entangled with the cellulose microfibrils. The amount of uronic acid in the various fractions was $<5\%$ of the total carbohydrate content.

Graded precipitation with alcohol. — Attempts to fractionate the polymers solubilised by M KOH at 2° by anion-exchange chromatography gave recoveries usually of $<30\%$, and the flow rates of the columns decreased because of settlement. The problem was circumvented, to a certain extent, by graded precipitation with ethanol prior to anion-exchange chromatography. Table II shows the yields and carbohydrate compositions of each of the ethanol-precipitated fractions from the first M KOH-extract (A), and some of the fractions from the second extract (B) which were chosen for anion-exchange chromatography.

The total carbohydrate content of the fractions indicated that some of the fractions, particularly A2, A3, A9, and B9, contained significant amounts of non-carbohydrate material. A large proportion of the non-carbohydrate components was shown to be phenolic by the u.v. absorption of aqueous solutions of the polymers at pH 10.5, 6.5, and 3.0. From the ratio of arabinose and xylose in the various fractions, it was clear that highly substituted xylans were generally precipitated less readily with ethanol and it appeared that the amount of phenolics associated with the polysaccharides also influenced their solubility in aqueous alcohol.

TABLE II

CARBOHYDRATE COMPOSITION OF THE POLYMERS PRECIPITATED WITH ETHANOL

		Yield	*"Anhydro sugar" (μg)/mg dry wt.*					
		(%)	*Deoxy-hexose*	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Total*
M *KOH, 2° (A)*									
No EtOH, 18 h, 2°	1	—							
EtOH 0–10%	2	2.8	Tr[a]	146	267	4.6	10.4	42.7	470.7
10–20%	3	3.1	Tr	172	347	3.4	13.6	83.5	619.5
40–50%	4	18.1	Tr	137	569	3.2	13.9	113	836.1
50–60%	5	—							
60–70%	6	5.3	Tr	378	435	2.8	28.1	50.5	894.4
70–80%	7	8.3	1.9	446	436	5.1	28.9	50.9	968.8
80–90%	8	15.8	Tr	466	426	3.3	31.0	33.7	960
90% Supernatant	9	46.6	Tr	197	181	1.9	12.4	23.3	415.6
M *KOH, 2° (B)*									
EtOH 10–20%	3	8.5	Tr	149	308	—	40.9	313	810.9
60–70%	6	9.4	2.2	352	472	5.0	39.7	129	999.9
90% Supernatant	9	61.0	Tr	361	342	5.0	21.5	24.8	754.3

[a]Trace.

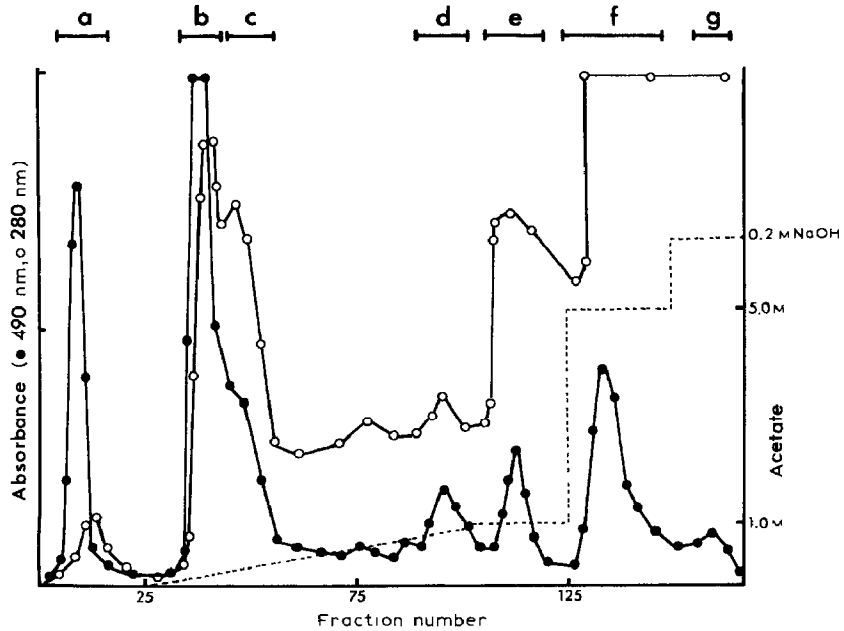

Fig. 1. Fractionation on DEAE-Sephacel of a hemicellulosic polymer (A9) after precipitation with ethanol: —●—, total carbohydrate; —o—, absorbance at 280 nm; ------, solvent gradient. For details, see text.

Thus, the xylans remaining in solution in aqueous 90% ethanol (fractions A9 and B9) were highly substituted and had significant amounts of "covalently" associated phenolics as inferred from the elution profiles of carbohydrate and u.v.-absorbing material of A9 from DEAE-Sephacel (Fig. 1)

Anion-exchange chromatography. — Five fractions (A9, A4, B9, B6, and B3) from the graded precipitation with alcohol were further resolved by anion-exchange chromatography on DEAE-Sephacel. The recoveries from the columns were in the range 58–89%. The carbohydrate elution profiles of these fractions were qualitatively similar, but much less u.v.-absorbing material was associated with the "carbohydrate peaks" from fractions A4, B6, and B3 compared with those from A9 and B9. The carbohydrate and u.v.-absorbance elution profiles of A9 are shown in Fig. 1 (the peak labelling pattern is used for all the other fractions). The carbohydrate compositions of the fractions from A9 and A4 are shown in Table III, and those from B9, B6, and B3 in Table IV.

Fraction A9. — Carbohydrate accounted for only 42% of A9, and the water-insoluble component contained carbohydrate, 11.7%; acetyl bromide-soluble lignin, 64%; and protein, 7.2%. The composition of this residue is comparable to that of fraction *f* (Fig. 1), which was also rich in phenolics and had carbohydrate and protein contents of 24% and 4%, respectively. Each fraction exhibited u.v. spectra at

TABLE III

CARBOHYDRATE COMPOSITION OF THE FRACTIONS FROM *A9* AND *A4* AFTER ANION-EXCHANGE CHROMATOGRAPHY

Fraction	*Yield*	*"Anhydro sugar" (μg)/mg dry wt.*						
	(%)	*Deoxy-hexose*	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Total*
A9								
(Recovery from column, 87%)								
Water-insoluble residue	26.7	6.2	51.9	39.0	1.9	1.9	16.7	117.6
a	9.9	2.6	464	459	3.6	19.1	50.0	998.3
b	27.8	2.7	502	446	—	25.8	20.6	997.1
c	6.5	6.2	276	237	3.7	14.9	22.3	560.1
d	8.0	5.2	294	246	7.8	20.4	39.2	612.6
e	7.8	8.8	298	250	6.0	15.9	31.7	610.4
f	13.1	—	108	87.5	2.1	4.2	29.2	241
A4								
(Recovery from column, 58%)								
Water-insoluble residue	16.0	4.5	125	538	8.2	4.1	174	853.8
a	19.6	1.9	114	465	2.6	38.7	376	998.2
b	9.4	1.7	256	629	3.0	22.8	11.1	923.6
c	8.8	4.0	275	542	7.2	13.7	79.5	921.4
d	Tr							
e	3.7	44.5	326	411	1.1	2.3	4.7	789.6
f	38.6	5.5	132	752	1.7	1.7	14.8	907.7
g	3.9	4.3	135	555	15.6	10.4	47.8	768.1

TABLE IV

CARBOHYDRATE COMPOSITION OF THE FRACTIONS FROM *B9*, *B6*, AND *B3* AFTER ANION-EXCHANGE CHROMATOGRAPHY

Fraction	*Yield*	*"Anhydro sugar" (μg)/mg dry wt.*						
	(%)	*Deoxy-hexose*	*Ara*	*Xyl*	*Man*	*Gal*	*Glc*	*Total*
B9								
(Recovery from column, 89%)								
Water-insoluble residue	16.8	4.3	218	287	6.8	15.5	30.9	562.5
a	6.0	1.4	198	221	15.2	22.8	175	633.4
b	49.8	Tr	513	418	5.7	29.7	27.5	993.9
c	13.4	Tr	343	273	12.1	26.6	58.0	712.7
d	1.5	8.5	378	227	11.5	28.6	68.7	722.3
e	3.8	4.9	431	346	8.2	24.6	60.0	874.7
f	8.3	3.9	240	196	14.2	29.3	42.6	526
g	0.4	11.6	375	83.3	31.3	Tr	240	741.2
B6								
(Recovery from column, 69%)								
a	23.7	4.6	133	351	6.2	51.4	448	994.2
b[a]	58.5	Tr	427	475	1.3	35.0	38.9	977.2
d	3.0	Tr	176	166	3.3	22.8	111	479.1
f	14.8	Tr	105	417	1.9	3.8	22.6	550.3
B3								
(Recovery from column, 62%)								
a	51.4	2.7	62.4	310	4.8	61.2	554	995.1
a1[b]	36.8	11.1	44.0	182	3.5	58.1	543	841.7
a2[b]	10.9	1.1	15.8	112	2.0	53.5	410	594.4
b	33.8	2.7	363	436	1.2	36.3	155	994.2
d	Tr							
e	5.9	Tr	160	175	2.2	10.8	73.3	421.3
f	8.9	Tr	143	660	2.4	4.7	42.3	852.4

[a]Fraction *c* on trailing edge not separated from fraction *b*. [b]After refractionation on DEAE-Sepharose

pH values 10.5 and 3.0 characteristic of ferulic acid. The major component of the water-insoluble residue appeared to be a condensation product of carbohydrate-containing polymeric material rich in phenolics.

The fractions *a–f* were rich in arabinose and xylose, showing that (acidic) arabinoxylans were the major carbohydrate moieties of the constituent polymers, and the ratios of arabinose and xylose ranged from 1.0–1.2. This observation suggested that, amongst other factors, the contents of associated phenolics, determined by measurements of u.v. absorption (relatively low in *a* and *b*, moderately high in *c–e*, and high in *f*) may have influenced the elution pattern of the fractions. Had the original CWM been delignified prior to extraction with alkali, the phenolics associated with the arabinoxylans would have been removed. This probably would have resulted in poor resolution of the arabinoxylans, and some vital information on the

nature of the constituent arabinoxylans and their association with phenolics would have been lost.

Fraction A4. — Carbohydrate accounted for 84% of A4, and fractions *a-f* had carbohydrate contents in the range 77-100%. The ratios of arabinose and xylose of the fractions ranged from 0.18 (*f*) to 0.79 (*e*), showing that the acidic arabinoxylans of A4 were much less branched than those of A9. There was a relatively high content of glucose in the unadsorbed "neutral" fraction (*a*). U.v.-absorption studies showed that each fraction had some associated phenolics.

Fraction B9. — Carbohydrate accounted for 75% of B9. Each of the fractions from the column was rich in arabinose and xylose, and the ratios ranged from 0.9 (*a*) to 1.66 (*d*); the arabinose-xylose ratio of fraction *g* was unusually high (4.5), but the yield was low. Although the carbohydrate composition of the major fraction *b* was comparable with that of the corresponding fraction from A9, the other fractions had carbohydrate compositions and arabinose-xylose ratios different from those of fractions from A9, the most significant differences being shown by the fractions *f*. This finding reflects the heterogeneity of the acidic arabinoxylans (and associated phenolics) and the need to fractionate the M KOH-extracts separately in order to obtain more detailed information about the constituent polymers.

Fractions B6 and B3. — The carbohydrate compositions of the fractions from

TABLE V

AMINO ACID COMPOSITION OF SOME POLYMER FRACTIONS

Amino acid	*Composition (mol %)*					
	A9		*B9*			
	Water-insoluble residue	f	*Water-insoluble residue*	a	b	f
Alanine	18.5	18.4	13.0	7.3	10.6	17.1
Glycine	13.6	16.9	6.1	—	12.5	9.5
Valine	1.3	6.7	13.7	11.5	9.9	11.8
Threonine	5.5	1.2	1.0	1.9	1.0	1.0
Serine	1.6	1.8	0.7	3.5	2.1	1.3
Leucine	8.1	7.0	13.5	19.9	11.2	9.7
Isoleucine	2.3	3.8	6.7	10.5	6.5	5.3
Proline	12.3	12.2	13.6	3.8	8.8	15.2
Hydroxyproline	2.0	0.6	1.4	—	1.7	2.1
Aspartic	11.0	15.7	17.7	22.5	11.8	15.0
Phenylalanine	5.2	5.5	7.9	19.0[a]	9.8	6.5
Glutamic	7.5	5.5	1.7		1.8	2.4
Lysine	2.9	2.0	1.6	—	2.1	1.5
Tyrosine	0.8	0.6	0.8	—	1.0	0.2
Arginine	0.8	0.9	0.4	—	2.6	0.5
Histidine	5.4	1.2	0.1	—	6.8	0.9
Protein (%)[b]	(7.4)	(3.9)	(7.8)	(0.8)	(1.5)	(5.0)

[a]Not resolved from glutamic acid. [b]Based on the sum of the amino acids.

B6 and B3 were comparable and the unadsorbed "neutral" fractions *a* were rich in glucose (*cf.* fraction A4 in Table III). On refractionation on DEAE-Sepharose (borate form), B3a was resolved into two fractions and each was rich in glucose (Table IV). Fractions B6b and B3b were rich in arabinose and xylose, and were comparable with A9b and B9b.

Protein content. — The protein contents of some water-insoluble residues and fractions from the anion columns ranged from 3% to 7%, suggesting the presence of proteoglycans. The amino acid compositions of some of the fractions are shown in Table V. The associated proteins did not contain much hydroxyproline and their compositions are comparable with the hydroxyproline-poor proteoglycans of runner beans. Similar protein contents and amino acid compositions have been found for cell-wall fractions from rye grass[26] and wheat endosperm[27].

Methylation analyses. — Selected fractions from anion-exchange chromatography were subjected to methylation analysis. The fractions gave various amounts of pentitol penta-acetates and hexitol hexa-acetates, even after remethylation of the fractions. The pentitol penta-acetates presumably arose from doubly branched xylosyl and arabinosyl residues; the occurrence of such residues in branched arabinoxylans has been reported[2,3,6,7], but the origin of the hexitol hexa-acetates is not clear. The carbohydrate compositions given in Tables III and IV and the results of methylation analysis of selected polymers (Tables VI and VII) revealed three main groups of fractions, namely, (*1*) highly substituted xylans in which the arabinose–xylose ratios ranged from 0.9 (B9a) to 1.23 (B9b) (Table VI); (*2*) slightly substituted xylans in which the arabinose–xylose ratios ranged from 0.18 (A4f) to 0.25 (B3f) (Table VII); and (*3*) moderately substituted xylans in close association with xyloglucans (Table VII). Although B3b is included in group 1, it contains a significant amount of xyloglucan and thus resembles the polymer complexes of group 3.

In order to identify the uronic acids associated with the polymers A9b, B9b, and A4f, the methylated polymers were reduced with $LiAl^2H_4$ prior to remethylation. Only small proportions of 6,6′-dideuterated terminal glucosyl derivative were detected subsequently. This result could be due to the low contents of Glc*p*A and 4-*O*-Me-Glc*p*A or to incomplete carboxyl-reduction due to steric effects. The latter situation is more probable, as significant amounts of Glc*p*A-(1→2)-Xyl*p* and Glc*p*A-(1→2)-Xyl*p*-(1→4)-Xyl*p* were detected in the partial acid hydrolysates of B9b by g.l.c.–m.s. of the methylated oligosaccharide-alditols. Therefore, it is probable that most, if not all, of the arabinoxylans of the cell walls are glucurono- and 4-*O*-Me-glucurono-arabinoxylans. Hence, below, arabinoxylan connotes acidic arabinoxylan.

In most of the fractions, the number of branch points was in excess of the number of terminal residues. Similar discrepancies have been encountered in studies of xyloglucans from cabbage[28], runner beans[25], and apples[29], and also on methylation analysis of xylosylglucose (isoprimverose)[30], and the possible reasons for the discrepancies have been discussed[28].

Although each of the polymers shown in Table VI contained highly branched

arabinoxylans, some distinctive features could be noted. (*1*) The polymer in A9a is different from that in B9a in having higher levels of terminal arabinosyl and galactosyl groups and lower levels of (1→2,1→4)- and (1→3,1→4)-linked xylosyl residues. (*2*) The polymer in A9f is different from that in B9f in having lower levels of terminal arabinosyl, xylosyl, and galactosyl groups, and higher levels of (1→2,1→4)- and (1→3,1→4)-linked xylosyl and doubly branched xylosyl residues; there were fewer triply branched hexosyl residues in A9f than in B9f. These differences again clearly show the advantage of separate fractionation of the products in the two M KOH-extracts. The polymers in A9a, B9a, and B9b are highly branched arabinoxylans with small amounts of associated phenolics, whereas those in A9e, A9f, and B9f are highly branched arabinoxylans with significant amounts of associated phenolics. Presumably, the associated phenolics are linked to the carbohydrate

TABLE VI

PARTIALLY METHYLATED ALDITOL ACETATES DERIVED FROM THE HIGHLY BRANCHED ARABINOXYLAN FRACTIONS

Alditol	*Relative mol (%)*						
	A9a	*A9e*	*A9f*	*B9a*	*B9b*	*B9f*	*B3b*
2,4-Me_2-Rha	Tr[a]	Tr	Tr	Tr	Tr	Tr	Tr
2,3,5-Me_3-Ara	34.6	29.4	17.6	25.9	25.5	21.9	16.6
3,5-Me_2-Ara	4.4	4.7	3.3	5.6	6.8	3.4	4.0
2,5-Me_2-Ara	6.5	8.5	6.2	7.3	10.1	5.4	5.1
2,3-Me_2-Ara	1.7	2.1	2.1	1.6	1.8	1.1	1.8
2-Me-Ara	—	0.4	1.2	0.8	0.6	—	—
Arabinitol	Tr	4.0	1.1	1.0	0.5	1.0	0.3
2,3,4-Me_3-Xyl	5.5	5.5	2.8	6.5	7.5	4.6	9.6
2,3-Me_2-Xyl	1.4	3.4	3.1	4.6	5.1	3.9	7.3
3,4-Me_2-Xyl	1.8	0.8	0.9	0.8	1.3	0.6	2.1
2-Me-Xyl 3-Me-Xyl	10.0	16.2	26.6	14.9	18.1	17.4	10.3
Xylitol	14.2	14.2	13.6	12.3	15.1	10.2	8.0
2,3,4,6-Me_4-Gal	10.6	3.2	4.3	7.7	4.2	8.9	6.2
2,4,6-Me_3-Gal	—	—	—	—	—	—	0.8
2,3,6-Me_3-Gal	—	—	—	—	—	—	—
2,3,4-Me_3-Gal	—	—	—	0.3	—	—	—
2,3-Me_2-Gal	—	—	—	—	—	—	1.4
2,3,4,6-Me_4-Glc	—	Tr	5.1	0.4	0.2	—	—
2,3,6-Me_3-Glc	Tr	Tr	2.4	3.5	—	—	12.0
2,3,4-Me_3-Glc	1.1	—	—	0.5	—	—	—
2,3-Me_2-Glc	3.1	Tr	—	2.2	0.4	3.4	9.0
2,4-Me_2-Glc	—	—	—	0.2	0.4	0.5	0.1
Me-Hex	—	—	1.1	—	—	—	0.4
Hexitols	4.3	7.3	8.2	3.6	2.3	11.0	4.8

[a]Trace.

TABLE VII

PARTIALLY METHYLATED ALDITOL ACETATES DERIVED FROM THE SLIGHTLY BRANCHED ARABINOXYLANS AND ARABINOXYLAN–XYLOGLUCAN COMPLEXES

Alditol	*Relative mol (%)*					
	Slightly branched arabinoxylans			*Arabinoxylan–xyloglucan complexes*		
	A4f	*B6f*	*B3f*	*A4a*	*B6a*	*B3a*
2,4-Me_2-Rha	—	Tr[a]	Tr	Tr	0.5	Tr
4-Me-Rha	—	—	—	Tr	Tr	—
2,3,5-Me_3-Ara	13.4	9.6	9.7	6.9	10.3	2.5
3,5-Me_2-Ara	0.8	1.1	0.9	0.9	4.6	0.2
2,5-Me_2-Ara	—	0.7	0.7	—	3.2	0.2
2,3-Me_2-Ara	—	—	0.4	—	—	0.3
Arabinitol	—	—	4.6	—	—	—
2,3,4-Me_3-Xyl	1.1	5.4	6.0	7.4	7.5	7.8
2,3-Me_2-Xyl	61.1	53.8	48.1	19.6	13.9	8.2
3,4-Me_2-Xyl	6.4	6.9	5.1	4.7	2.1	2.0
2-Me-Xyl + 3-Me-Xyl	13.3	8.0	8.0	6.9	4.8	9.0
Xylitol	1.8	3.0	4.4	1.4	1.2	1.5
2,3,4,6-Me_4-Gal	0.3	1.0	0.8	6.2	—	3.1
2,3,6-Me_3-Gal	—	—	—	—	1.3	1.2
2,3,4-Me_3-Gal	—	—	—	—	0.2	—
2,3-Me_2-Gal	—	—	1.7	—	—	—
2,3,4,6-Me_4-Glc	—	—	Tr	—	—	0.2
3,4,6-Me_3-Glc	—	—	—	—	—	0.4
2,3,6-Me_3-Glc	—	2.1	0.8	23.0	22.4	34.4
3,6-Me_2-Glc	—	—	—	1.5	1.1	0.3
2,3-Me_2-Glc	—	1.2	1.6	18.5	13.4	25.9
2,6-Me_2-Glc	—	—	—	—	1.7	0.2
Me-Glc	—	—	—	—	3.8	—
Hexitols	1.8	6.4	7.1	2.0	5.4	2.0

[a]Trace.

moieties by alkali-resistant phenolic ether linkages, but the polymers are held within the cell-wall matrix mostly by alkali-labile phenolic ester linkages. The occurrence of alkali-labile and alkali-resistant lignin–carbohydrate linkages has been reported in a number of plant tissues[31–33]. Highly branched acidic arabinoxylans have been isolated from various monocotyledons[27,34–39], but not as a closely related series associated with various amounts of phenolics.

The results of methylation analysis of A4f, B6f, and B3f showed that the main carbohydrate moieties were arabinoxylans which were much less branched than those of the first group. Carbohydrate accounted for 91% of A4f, 55% of B6f, and 85% of B3f, and most of the non-carbohydrate material was shown to be phenolic

by measurement of u.v. absorption. Therefore, it could be inferred that A4f, B6f, and B3f contained slightly branched arabinoxylans associated with various amounts of phenolics. Slightly branched acidic arabinoxylans have been isolated from various monocotyledons[38,40].

Methylation analysis of the polymers in A4a, B6a, and B3a showed that they contained moderately branched arabinoxylans in association with significant amounts of xyloglucans. This composition is in contrast with that of the polymer in B3b (Table VII), which contained a highly branched arabinoxylan in association with a relatively small amount of xyloglucan. The occurrence of xyloglucans in the polymers was inferred from the presence of (1→4)- and (1→4,1→6)-linked glucosyl residues and terminal xylosyl residues. These results suggest that the arabinoxylan–xyloglucan complexes also exhibit heterogeneity. Refractionation of B3a on DEAE-Sepharose (borate form) gave two fractions containing large amounts of glucose and significant amounts of arabinose, galactose, and xylose (Table IV). The failure to resolve the arabinoxylans from the xyloglucans suggested that these polysaccharides may be covalently linked, possibly *via* phenolic cross-links. The occurrence of xyloglucans in monocotyledons has been reported[41,42]. A complex containing arabinoxylan, xyloglucan, and β-D-glucan has been isolated from rice endosperm cell walls[43,44], and the occurrence of small amounts of comparable complexes in the cell walls of runner beans[25], apples[29], immature cabbage leaves[28], and maize[45] has been reported. In most of the above tissues, which were either free of lignin or were only slightly lignified, the xyloglucans in the complexes represented only a small proportion of the "free" xyloglucans[46]. However, in beeswing bran, which contains significant amounts of cell-wall phenolics including lignin, most of the xyloglucans appear to occur as complexes. This is additional evidence that such complexes are native to the cell walls and probably serve to cross-link the matrix polymers.

The above extraction procedures were designed to preserve, as much as possible, the *in vivo* state of the cell-wall polymers. The CWM was used in the hydrated form rather than as a freeze-dried powder. This facilitated the solubilisation of the polymers. Despite this, the final α-cellulose residue contained significant amounts of non-cellulosic polysaccharides, mostly arabinoxylans. Similar observations have been made with α-cellulose residues from beeswing wheat bran[2] and wheat bran[3,4].

Cold, mild alkaline conditions (0.05M NaOH, buffered to pH 10) were not sufficient to disrupt the ester cross-linkages and solubilise significant amounts of hemicellulosic polymers, but significant amounts were solubilised by M KOH at 2°. In view of the low temperature used for extraction, the solubilised polymers would have undergone minimum degradation. A large proportion of the remaining hemicellulosic polymers were solubilised by M and 4M KOH at 20°, but delignification followed by alkaline extraction was required to solubilise the hemicellulosic polymers closely associated or encrusted with lignin.

The hemicellulosic polymers solubilised by M KOH in the cold consisted of (*a*) a range of highly branched arabinoxylans associated with various amounts of

phenolics, possibly by ether linkages, (*b*) less-branched arabinoxylans associated with phenolics, and (*c*) arabinoxylans associated with various amounts of xyloglucans and small amounts of phenolics. Thus, previous reports on the occurrence of arabinoxylan–phenolic complexes in lignified tissues of monocotyledons[38] have been confirmed and extended. Further, the mild conditions used for extraction and fractionation suggest that the heterogeneity exhibited by the various groups of polymers is native to the cell walls and not due to degradation. Recently, using mild extraction and fractionation conditions, apple xyloglucans[47] and onion pectic polysaccharides[23] have been shown to exhibit heterogeneity. As beeswing wheat bran is only three cells thick, it is probable that the heterogeneity reflects the different types of closely related polymers that are deposited during the growth and maturation of the cells. The cross-linking of the various polymers within the cell-wall matrix and the degree of branching of the polymers determine the rate of degradation of fibre from wheat bran by colonic bacteria, and thus has important consequences in the dietary fibre context[48].

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