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Analysis of wheat arabinoxylans from a large-scale isolation

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Dietary fibre is of interest because of its nutritional activity and its role in the aetiology of some diseases. Arabinoxylans form the major part of wheat dietary fibre and this paper describes the large-scale aqueous and alkaline (0·2 M NaOH) extractions of a wheat milling by-product which yielded approximately 450 g and 1200 g of arabinoxylan-rich materials, respectively. The structures of the arabinoxylans were investigated using acid hydrolysis, methylation analysis and 1- and 2D-NMR experiments. The polysaccharide is confirmed to be a β-(1-4)-xylan substituted with α-L-arabinose (arabinose:xylose ratio of approximately 0·60) substituted at O2 and O3. Methylation analysis and ^{1}H-NMR data indicated that the arabinoxylans from the aqueous extraction were very similar in structure to those isolated by alkaline extraction. Both were similar to arabinoxylans isolated directly from wheat by similar laboratory scale procedures. Detailed ^{1}H-^{1}H-NMR homonuclear and ^{1}H-^{13}C-NMR heteronuclear correlation spectroscopy analysis of a partially depolymerized sample confirmed the structure of the arabinoxylans and allowed an almost complete assignment of resonances in both the ^{1}H- and ^{13}C-NMR spectra. It was concluded that the nutritional activities ascribed to these materials are also likely to occur when they are ingested as components of complete foods.

INTRODUCTION

Non-starch polysaccharides (NSP) are the major components of dietary fibre and are now known to possess important nutritional activities in both man and animals. Health authorities are recommending increases in the dietary intake of fibre which will result in increased protection against degenerative diseases such as diabetes, large bowel cancer and cardiovascular disease (Topping, 1991).

Investigations into the nutritional effects of dietary fibre have mostly involved the feeding of fibre-rich food fractions (e.g. wheat bran) or purified polysaccharides such as guar gum and locust bean gum. However, there have not been many studies of the nutritional activity of purified NSP, commonly found in Western diets and animal feedstuffs (i.e. cereal NSP), owing to the difficulty in isolating and purifying the material in sufficient quantities for feeding trials. Exceptions to this have been the studies of isolated β-glucans from barley and oats in human and animal nutrition (White *et al.*, 1981; Lund *et al.*, 1989; Wood *et al.*, 1990). The arabinoxylans of cereals are more difficult to isolate and purify in large amounts than the β-glucans but this has been done in the case of rye by Ward & Marquardt (1987) who demonstrated that the rye arabinoxylans are anti-nutritive when fed to broiler chickens. More recently it was demonstrated that isolated wheat arabinoxylans also possess anti-nutritive activity when included in broiler chicken diets (Choct

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and Annison, 1990, 1992*a*). In these studies very large amounts (>1000 g) of arabinoxylan-rich material were isolated. However, when this type of approach is adopted it is important to demonstrate that the isolation procedures do not cause major modifications to the structure of the NSP which may affect its nutritional activity. This paper details the structural analysis of arabinoxylans (termed pentosans) isolated from wheat using large-scale procedures. The structure is compared with those of wheat pentosans isolated using more gentle laboratory scale procedures and with data in the literature.

MATERIALS AND METHODS

Large-scale isolation of wheat pentosans

A milling by-product (18·8% arabinoxylans, 16·8% starch and 26·0% protein) was used as the source of wheat pentosans. The materials was incubated in hot water (1 kg/10 L, 80–100°C, 1 h) with a thermostable α-amylase (E.C. 3.2.1.1., Termamyl 120L, Novo A/S, Denmark; 1·5 ml, 360 kU) to degrade the residual starch. The mixture was cooled and incubated with papain (Sigma Chemicals, 2 g, 3·2 kU, 1 h, 65°C) to remove residual protein and centrifuged (1000 *g*). The supernatant was decanted and centrifuged again at high speed (13 000 *g*, 30 min). The clarified supernatant was decanted, adjusted to 80% (v/v) ethanol and allowed to stand at room temperature for several hours. The precipitate was collected, washed with ethanol and air-dried in a forced-air oven (50°C) to give a Water-Extractable Pentosan preparation from milling By-Product (WEPBP).

The insoluble material remaining after the incubation was resuspended in an equal volume of aqueous 0·2 M NaOH containing 1 mg/ml $NaBH_4$ at 80°C for 2 h, neutralized with acetic acid and centrifuged (1000 *g*). The supernatant was decanted, recentrifuged (13 000 *g*, 30 min) and adjusted to 80% v/v ethanol. The precipitate was dried in the same manner as the WEPBP to give an Alkali-Extractable Pentosan preparation from milling By-Product (AEPBP).

From 15 kg of the milling by-product approximately 1200 g of AEPBP and 450 g of WEPBP were isolated.

Laboratory scale isolation of wheat pentosans

Wheat flour (5 g) was extracted with water (100 ml) at room temperature for 1 h with gentle stirring. The slurry was centrifuged (13 000 *g*; 1 h) and the supernatant was collected and adjusted to 80% v/v ethanol. The precipitate was collected by centrifugation (13 000 *g*; 1 h) and resuspended in distilled water and freeze-dried. This Water-Extractable Pentosan preparation from Wheat was termed WEPW.

The pellet remaining from the water extraction was suspended in 0·2 M NaOH (100 ml). The mixture was centrifuged (13 000 *g*; 1 h) and the supernatant was decanted and adjusted to pH 7 by the dropwise addition of glacial acetic acid. Ethanol was added to 80% (v/v) and the resultant precipitate was collected by centrifugation. The pellet was resuspended in water and freeze-dried. The recovered Alkali-Extractable Pentosan preparation from Wheat was termed AEPW.

Column chromatography

Each of the pentosan preparations (150 mg) was dissolved in distilled water and passed through a column (3·5 × 90 cm) of Sephacryl S-400 (Pharmacia, Uppsala, Sweden). The flow rate was 2·4 ml/h. Fractions were collected and those containing carbohydrate as determined by the phenol-sulphuric acid method of Dubois *et al.*, (1956) were pooled and lyophilized. The purified WEPBP, AEPBP, WEPW and AEPW were submitted for analysis.

Apparent molecular weight determination

The apparent molecular weight of each of the preparations was determined by HPLC. Samples were passed through a G5000 P.W. column (Toya Soda Co., Tokyo) using 0·1 M $NaNO_3$ as the mobile phase. A Waters M6000A pump equipped with a Rheodyne 7125 injector and an ERMA model 7510 refractive index detector (ERMA Optical Works Ltd, Tokyo) was used. The flow rate was 0·6 ml/min. The apparent molecular weights were estimated by comparison with the retention time of pullulan standards ($5{\cdot}3 \times 10^3$–$7{\cdot}8 \times 10^5$ Da). The term 'apparent molecular weight' is used, because the standards consist of (1-4)-α-glucans which have different conformation to the (1-4)-β-xylans of the samples and therefore probably chromatograph differently (Rees *et al.*, 1982).

Sugar analysis

The monosaccharide composition of the pentosan preparations was determined by gas chromatography (GC) of the alditol acetate derivatives of sugars following the hydrolysis procedures of Olsen *et al.*, (1988) and the derivatization procedures described by Blakeney *et al.*, (1983). The instrument used was a Varian 3400 gas chromatograph equipped with a Varian Series 8000 autosampler, a flame ionization detector and a capillary column (DB 1701, 15 m, J&W Scientific). During analyses the column was held at 200°C for 1 min, then raised by 5°C/min to 220°C for 4 min. Duplicate samples were hydrolysed and the derivatized products were each injected twice.

The levels of polymeric arabinoxylans were calculated assuming a xylan backbone with arabinose side chains using the following relationship:

Arabinoxylan = pentose × 0·88.

Methylation analysis

Freeze-dried polysaccharide (5 mg) was further dried *in vacuo* under an infra-red lamp for 2–3 h. The sample was dissolved in dimethylsulphoxide (1 ml), the vial was flushed with N_2 and sealed. When all the polysaccharide was dissolved dimethyl sulphanyl (DIMSYL) anion (0·5 ml), prepared as described by Harris *et al.* (1984), was added and the mixture was stirred for 1 h. To ensure that an excess of DIMSYL anion remained an aliquot (0·1 ml) of the mixture was put onto a few crystals of triphenylmethane (excess base being indicated by the development of a red colour). The reaction mixture was cooled to 10°C, methyl iodide (0·25 ml) was added and the mixture was held at room temperature for 3 h. Excess methyl iodide was removed with a stream of N_2 gas at room temperature.

The methylated polysaccharide was recovered by adding water (2 ml) and transferring the sample to a larger tube and extracting with chloroform (4 ml). The chloroform was washed four times with water (3 ml) and dried with 0·5 g of anhydrous magnesium sulphate. The chloroform layer was transferred into a clean tube and dried. To ensure complete methylation, recovered polysaccharide was treated with methyl iodide (5 ml) and silver oxide (0·5 g) as described by Purdie & Irvine (1903). The mixture was stirred at 40°C overnight. The methyl iodide was decanted and evaporated from a clean tube. The methylated polysaccharide was then hydrolysed and converted to the partially methylated alditol acetates (PMAA) using the same procedures as described for the sugar analysis of polysaccharides.

The PMAA were analysed and quantified using GC. The instrument used was a Hewlett Packard 8980 Series II gas chromatograph equipped with an FID detector and a capillary column (BPX70, 25 m, Scientific Glass Engineering Inc., Melbourne, Australia). The oven temperature was held at 180°C for 1 min and then raised at 3°/min to 200° and held for 2 min.

Samples from WEPW and AEPBP were also analysed by GC–MS using a Hewlett Packard 8980 gas chromatograph (capillary column BPX70, 25 m, Scientific Glass Engineering Inc., Melbourne, Australia) coupled to a Finnigan 3200 mass spectrometer operating in the electron impact mode. The identity of each of the PMAA was determined by comparison of the mass spectra obtained with those of known standards reported by Jansson *et al.* (1976). By comparison of GC retention times, the methylation analysis products of WEPBP and AEPW were also identified and quantified.

Partial depolymerization of AEPBP

AEPBP was depolymerized using a β-xylanase enzyme (MegaZyme Aust. Pty Ltd, Sydney, Australia). Preliminary experiments (data not presented) were conducted to ascertain appropriate conditions of incubation to cause a significant drop in the viscosity of a solution of AEPBP. The depolymerized AEPBP analysed by NMR was a sub-sample of material which was prepared by adding β-xylanase (25 μl, 4000 U/ml) to a solution of AEPBP (1·5% w/v, 1000 ml) being held at 50°C. After 2 min the temperature of the solution was raised rapidly to boiling point to deactivate the enzyme. The depolymerized material was recovered by drying the solution quickly at 80°C as a thin film over stainless-steel trays. The residue was ground to a fine powder.

^{1}H-NMR analysis

All spectra were acquired at 360 K on a Bruker AM500 nuclear magnetic spectrophotometer operating at 500 MHz in the pulsed Fourier transform mode with quadrature detection. Before Fourier transformations, resolution enhancement was achieved by the Lorentz–Gaussian multiplication and Gaussian broadening factor with a typical value of 0·4–0·45. The HOD peak was suppressed by preset automation of saturation decoupling. Data were acquired over 80–100 scans. Chemical shifts are expressed relative to an external acetone standard ($-CH_3$; 2·2 ppm).

^{13}C-NMR analysis of partially depolymerized AEPBP

The proton decoupled ^{13}C-NMR spectrum of AEPBP was acquired at 360 K with the spectrometer operating at 125 MHz. The number of scans was 80 000. Before Fourier transformation, signal-to-noise enhancement was achieved by exponential multiplication. Chemical shifts were expressed relative to an external standard (dioxane, 67·8 ppm).

^{1}H-^{1}H-NMR and ^{1}H-^{13}C-NMR correlation spectoscopy (COSY)

The ^{1}H-^{1}H-NMR COSY spectrum was acquired as described by Aue *et al.* (1976) and Nagayama *et al.* (1980).

The ^{1}H-^{13}C-NMr COSY spectrum was acquired at 360 K as described by Bax (1983) and modified by Rutar (1984) (4 K carbon acquisition; 1063·8 Hz carbon sweep-width; 1050 Hz proton sweep-width; 256 scans pre t_1; 178 experiments zero-filled to 512 points, with 1 s relaxation delay; processed with an exponential window function in the $f2$ direction; line broadening factor 5 Hz, and a shifted sine bell squared for $f1$, shifted by $\pi/4$).

RESULTS

The pentosans from the large-scale isolations (WEPBP and AEPBP) had a high degree of polymerization with apparent molecular weights of 500 000 Da and 758 000 Da (Table 1). The alkali-extracted polymer isolated directly from wheat (AEPW) had a similar apparent molecular weight (500 000 Da). The WEPW pentosans were more polydispersed containing material up to an apparent molecular weight of 758 000 Da). The depolymerized AEPBP was also highly polydispersed with molecules ranging from 5300 to 758 000 Da.

The sugar composition of each of the fractions is shown in Table 2. The materials from the large-scale isolation (WEPBP and AEPBP) were predominantly pentosan (84·9 and 86·8% dry wt, respectively). A small amount of glucose was also present in these materials. WEPW and AEPW had lower levels of pentosans (41·7 and 46·7% dry wt., respectively). WEPW had a relatively high level of glucose (14·6% dry wt) and some galactose (4% dry wt). The arabinose to xylose ratio was similar in WEPBP, AEPBP and AEPW (approximately 0·60) but WEPW had a relatively higher level of arabinose (arabinose:xylose = 0·80).

Methylation analysis indicated that the pentose sugars of the four preparations had similar linkages (Table 3). The arabinose residues existed exclusively in the furanose conformation and almost all were present as non-reducing terminal units (a small amount of 3,5-*O*-methylated arabinose was detected). Except for non-reducing terminal units, all the xylose residues were linked through O4. In AEPBP, AEPW and WEPW approximately 50% of the xylose residues carried further substitutents with approximately half of these being monosubstituted (at O3) and the other half di-substituted (at O2 and O3). Less arabinofuranosyl residues were detected in the methylation products of WEPBP and this was reflected in the lower level of xylose branch point residues being present. A small amount of 2,3,6-tri-*O*-methylglucitol was detected amongst the methylation products of WEPW indicating the presence of 4-linked glucan.

Table 1. Apparent molecular weight of arabinoxylans in pentosan fractions

Fraction	Molecular weight (Da)
WEPBP[a]	500 000
AEPBP	758 000
WEPW	Polydispersed <758 000
AEPW	500 000
AEPBP (depolarized)	Polydispersed 5 300–758 000

[a]Fraction identity as described in text.

Table 2. Sugar composition (% dry wt) of wheat pentosan preparations

Sugar	Large-scale isolation		Laboratory isolation	
	WEPBP	AEPBP	WEPW	AEPW
Arabinose	35·9	36·4	20·7	20·2
Xylose	60·6	62·1	26·0	32·4
Mannose				
Galactose			4·0	1·0
Glucose	2·5	4·7	14·6	7·2
Arabinoxylan[a]	84·9	86·8	41·7	46·7
Arabinose:xylose	0·59	0·59	0·80	0·62

[a]Calculated level of polymeric arabinoxylans.

The anomeric regions of ^{1}H-NMR spectra of the pentosan preparations are shown in Fig. 1. Comparison with data in the literature allowed assignments of the major peaks to be made. The ^{1}H-NMR spectra were very similar being characterized by three, equally intense, well resolved resonances in the α-anomeric region (5·31-2, 5·21-2, 5·14 ppm) which were assigned to α-L-arabinose. WEPW also had a poorly resolved complex peak in this region of the spectrum. The β-anomeric region of the spectrum contained a large complex resonance (4·35–4·50 ppm) which was assigned to xylose. Each of the assignments and the integrals are presented in Table 4.

The proton-decoupled ^{13}C-NMR spectrum of a partially depolymerized sample of AEPBP is shown in Fig. 2. Most of the major resonances were assigned (Table 5) by reference to data in the literature. The assignments were confirmed after inspection of the 2D ^{1}H-^{1}H-NMR COSY (Fig. 3) and ^{1}H-^{13}C-NMR COSY spectra (Fig. 4).

DISCUSSION

The nutritive activity of dietary fibre polysaccharides is likely to be mediated by their degree of polymerization and by their chemical structure, both of which directly influence their physical properties. The pentosans (AEPW and AEPBP) isolated by the large-scale procedure retained a high degree of polymerization (Table 1) and the values obtained for their apparent molecular weights are in the range of those previously observed by Hoffman *et al.*, (1991*a*,*b*) who isolated wheat arabinoxylan fractions from 25 to 1000 kDa. The large-scale procedure yielded pentosan preparations which were much more enriched in arabinoxylans than those isolated in the laboratory scale procedures. This is because starch and protein are enzymically removed during the large-scale isolation. Despite extensive purification, the calculated arabinoxylan content of these materials is 84–87% (Table 2). The hydrolysis conditions used were optimized for the recovery of soluble polysaccharides, but small losses

Table 3. Methylation analysis products and their proportions from wheat pentosan preparations

Methylation products	Deduced structural unit	(mol% arabinoxylan)[a]			
		Large-scale isolation		Laboratory isolation	
		WEPBP	AEPBP	WEPW	AEPW
Pentaacetyl xyl[b]	-2,3,4-xyl(*p*)-[c]	17·9[d]	15·4	15·6	14·7
2-*O*-Me-xyl[e]	-3,4-xyl(*p*)-	14·7	13·1	14·8	16·1
2,3-*O*-Me-xyl[e]	-4-xyl(*p*)-	44·7	33·5	29·3	36·3
2,3,4-*O*-Me-xyl	xyl(*p*)-	2·8	7·6	6·4	5·9
3,5-*O*-Me-ara	-2-ara(*f*)	0·9	4·2	3·1	2·7
2,3,5-*O*-Me-ara	ara(*f*)-	19·0	26·3	30·8	24·3
2,3,6-*O*-Me-glc	-1,4-glc(*p*)-			(2·51)[f]	
	arabinose[g]	19·9	30·4	33·9	27·0
	xylose[g]	80·1	69·6	66·1	73·0

[a] Arabinose and xylose methylated products are expressed as mol percent of summed pentose residues.
[b] 1,2,3,4,5-penta-*O*-acetylxylitol.
[c] Xylose residue, pyranose form linked through positions 1, 2, 3 and 4.
[d] Calculated from the FID response factors described by Sweet *et al.* (1975).
[e] 1,3,4,5-tetra-*O*-acetyl, 2-*O*-methylxylitol; 1,4,5-tri-*O*-acetyl, 2,3-di-*O*-methylxylitol etc.
[f] Expressed as mol percent of total methylated products.
[g] Total mol per cent of arabinose and xylose residues in arabinoxylan.

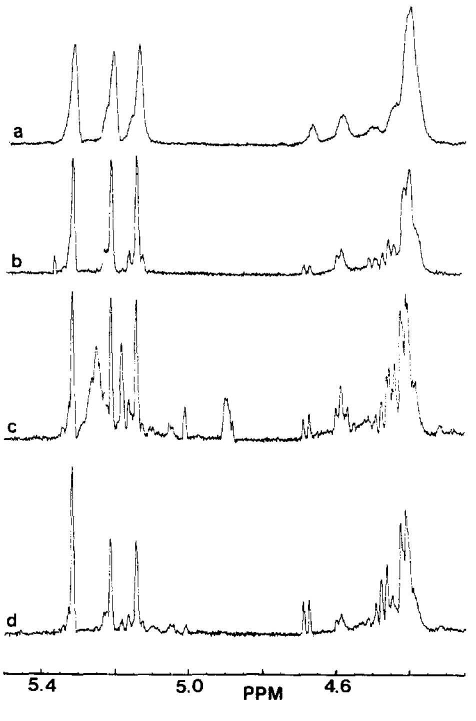

Fig. 1. ^{1}H-NMR spectra (anomeric region) of the wheat pentosan fractions. (a) WEPBP; (b) AEPBP; (c) WEPW; (d) AEPW. Samples codes as described in text.

may have occurred. It is also likely that small amounts of uronic acids were present in the material, as has been reported by Brillouet & Joseleau (1987) but these would not have been detected using the alditol acetate derivation procedure. The laboratory isolation procedure was designed to extract the wheat pentosans as gently as possible to avoid degradation of the polymer and it consisted essentially of cold water and then cold-alkali extractions which will dissolve some protein and starch. Starch is the likely source of the high level of glucose in WEPW and AEPW. The galactose found in WEPW and AEPW is probably from an arabinogalactan which is also present in wheat at low levels (Mares & Stone, 1973; Iszydorczyk *et al.*, 1990).

The difference between the arabinose:xylose ratio of WEPBP and WEPW (0·59 and 0·80) suggests that some arabinose residues may be lost in the large-scale procedure. The glycosidic linkages of arabinofuranosyl residues are very labile (Brillouet *et al.*, 1982), but this difference in the ratios represents a relatively small loss of arabinose (i.e. arabinose content drops from 44% to 37% of the arabinoxylan). The arabinose:xylose ratios of the WEPBP and AEPBP are similar to those reported previously for wheat arabinoxylans (Brillouet & Mercier, 1981; Henry, 1987; Gruppen *et al.* 1989; Annison, 1990). The closeness of the arabinose:xylose ratios suggests that the structure of the arabinoxylan in each isolate is very similar and this is confirmed by the results of the methylation analyses (Table 3). The levels of the methylation products of each of the pentosan preparations were similar and the structure of the arabinoxylan is confirmed to be a (1-4)-linked xylan main chain with side chains consisting mostly of one arabinofuranosyl residue. An anomaly in the results of the methylation analyses is that the number of non-reducing terminal residues detected is less than the number of branch points. Theoretically, the amount of

Table 4. Chemical shifts (ppm) of the anomeric resonances in the ^{1}H-NMR spectra of wheat pentosan preparations. The intensities of resonances relative to the resonance assigned to xylose are shown in parentheses

Assignments	Chemical shift (ppm)			
	Large-scale isolation		Laboratory isolation	
	WEPBP	AEPBP	WEPW	AEPW
α-ara-3X[a]	5·32[b] (0·24)	5·31 (0·24)	5·32 (0·16)	5·31 (0·23)
α-ara-3(A2)X[c]	5·22 (0·25)	5·21 (0·24)	5·21 (0·14)	5·21 (0·17)
α-ara-2(A3)X	5·14 (0·29)	5·14 (0·28)	5·14 (0·17)	5·14 (0·28)
α-glc			5·22-5·27 (0·47)	
β-xyl	4·35-4·45 (1)	4·35-4·45 (1)	4·35-4·45 (1)	4·35-4·50 (1)

[a] α-L-arabinose linked to O3 of β-D-xylose.
[b] Chemical shift (ppm) relative to acetone (-CH_3, 2·2 ppm).
[c] α-L-arabinose linked to O3 of β-D-xylose which is also substituted with arabinose at O2.

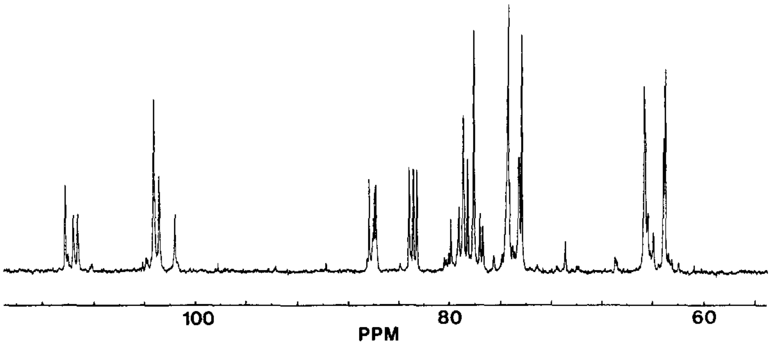

Fig. 2. ^{13}C-NMR spectrum (broadband decoupled) of partially depolymerized AEPBP.

Table 5. Resonances in the ^{13}C-NMR spectrum of partially depolymerized wheat pentosan preparation AEPBP

ppm	Assignment	ppm	Assignment	ppm	Assignment
110·5	C1[1];*ara*-2(A3)X[a]	83·5	C2;*ara*	75·7	C3;*xyl*
109·9	C1[1];*ara*-3(A2)X	83·2			
109·6	C1[1];*ara*-3X	82·9		74·9	C2;*xyl*
				74·7	C2;(A-3)*xyl*
103·6	C1[2];(A-2,3)*xyl*	80·2	C3;(A-3)*xyl*		
103·2	C1[2];(A-3)*xyl*			65·0	C5;*xyl*
101·9	C1[2];*xyl*	79·6	C2;(A-2)*xyl*	64·7	C5;term-xyl
		79·3	C3;*ara*	64·2	
86·7	C4;*ara*	78·9	C3;*ara*		
86·3		78·4	C4;*xyl*	63·5	C5;*ara*
86·2		78·0	unassigned	63·3	
		77·7	unassigned		

[a] C1 of α-L-arabinose attached to C3 of β-D-xylose also substituted at C2 with arabinose.
[1] Assigned from cross-peaks in the ^{1}H-^{13}C-NMR COSY spectrum (Fig. 4).
[2] Assigned from Ebringerova *et al.* (1990).

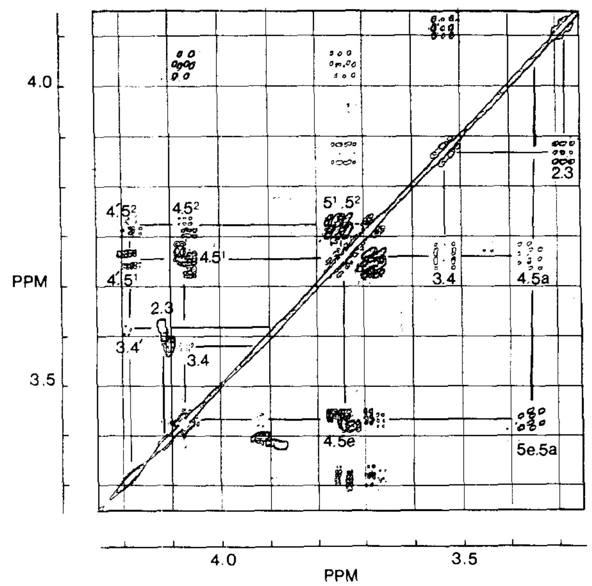

Fig. 3. ^{1}H-^{1}H-NMR COSY spectrum of partially depolymerized AEPBP. Cross peaks assigned to the arabinose protons are labelled above the diagonal, those assigned to xylose are labelled below the diagonal. The equatorial and axial protons of xylose at position 5 are labelled 5e and 5a, respectively. The two protons of arabinose at position 5 are labelled 5^1 and 5^2, respectively. Two resonances of H4 were identified (4·06 and4·19 ppm) and these have been labelled 4 and 4' respectively.

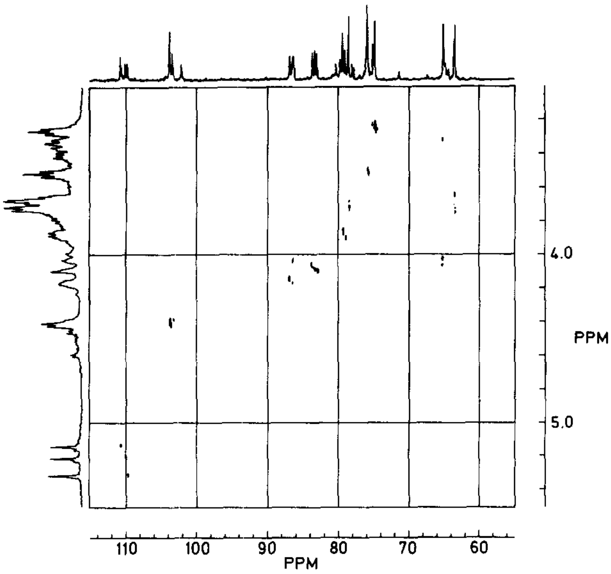

Fig. 4. ^{1}H-^{13}C-NMR COSY spectrum of partially depolymerized AEPBP.

2,3,5-tri-*O*-methylarabinitol present should be equivalent to two times the amount of 1,2,3,4,5-penta-*O*-acetylxylitol (this derivative indicates a double side-chain substitution on O2 and O3) plus the amount of 2-*O*-methylxylitol (this derivative indicates a single side-chain substitution on O3). The shortfall in the amount of detected non-reducing terminal arabinose is probably due to losses in the work up. The 2,3,5-tri-*O*-methylarabinitol derivative is very volatile as indicated by its low retention time on GC analysis. It is unlikely that under-methylation is the cause as very little 3,5-di-*O*-methylarabinitol and none of the other products expected from under-methylation were detected. Hoffman *et al.* (1991*b*) detected 3,5-di-*O*-methylyarabinitol in the methylation products of arabinoxylans isolated from wheat and interpreted it as indicating the presence of two arabinofuranosyl residues; one a non-reducing terminal residue and the other linked through O2. The presence of extended side-chains has also been suggested by other workers (Brillouet & Mercier, 1981; Brillouet *et al.*, 1982). It is of interest that in many studies of arabinoxylans, methylation analysis data are inconsistent in the ratio of non-reducing terminal arabinose units to in-chain xylose branch points (Brillouet *et al.*, 1982; Brillouet & Joseleau, 1987; Dupont & Selvendran, 1987; Ebringerova *et al.*, 1990; Herath *et al.*, 1990; Hoffman *et al.*, 1991*a*,*b*). Other workers have been more successful at obtaining consistent results (Bengtsson & Aman, 1990; Aman & Bengtsson, 1991). It is possible that the apparent excess number of branch points is due to the presence of uronic acid substituted onto the main chain which would not be detected in the analyses used in this study (Dupont & Selvendran, 1987) but while this may account for some of the excess it seems unlikely, as wheat arabinoxylans contain only a small amount of uronic acid (Brillouet & Joseleau, 1987). If one assumes that each of the substituted positions on the xylose residues (i.e. O2 and O3) carried an arabinose residue then the arabinose:xylose ratio which can subsequently be calculated agrees very closely with the arabinose:xylose ratio calculated from the sugar analysis data (Table 2). This strongly suggests that the problem with the analyses is the recovery of the volatile methylated arabinose derivative.

^{1}H-NMR analyses of the pentosan preparations confirmed that the polysaccharide components all have a similar structure (Fig. 1, Table 4). The assignments were made by reference to data in the literature (Smith & Hartley, 1983; Gubler *et al.*, 1985; Bengtsson & Aman, 1990; Aman & Bengtsson, 1991). The α-L-arabinofuranosyl units exist in three distinct environments as indicated by three intense resonances (5·31-2, 5·21-2 and 5·14 ppm), which is consistent with a mono- and di-substitution pattern. The ratio of integrals of the resonances indicates that approximately one third of the α-L-arabinofuranosyl residues were present as a mono-substituent and two thirds as di-substituents on the xylose, which is consistent with the methylation data. The resonances from the β-D-xylose are less resolved, indicating that the pattern of residues carrying arabinose is highly complex or random. The large extra peak in the α-anomeric region of the spectrum of WEPW is probably α-D-glucose from starch as suggested by the sugar analysis and methylation analysis data.

A very well-resolved ^{13}C-NMR spectrum of a partially depolymerized portion of AEPBP was obtained (Fig. 2, Table 5). The depolymerization was achieved using a β-xylanase and this may account for the excellent resolution. Partial depolymerization of polysaccharides is often required to reduce the viscosity of sample solutions in order to obtain good NMR spectra. In the case of arabinoxylans, enzymic depolymerization is preferred over mild acid hydrolysis as the arabinofuranosyl residues are very acid labile. The depolymerization procedure was carried out on a large portion of AEPBP as part of large-scale preparation of depolymerized pentosan material used in nutritional studies (Choct & Annison, 1992*b*).

Almost all the major resonances of the ^{13}C-NMR spectrum were assigned by reference to data in the literature and subsequent analysis of the sample by 2D-NMR. The structure was consistent with a main-chain xylan being substituted at the O2 and O3 with arabinofuranose. The arabinofuranose C1, C2 and C4 resonances were split into three signals, which clearly reflect the three chemical environments of this residue. This was also seen in the anomeric resonance of the xylose residues. The very low field resonance (86·1-7 ppm) of C4 of arabinose confirms the furanose conformation of this residue. The substitution of O3 of xylose causes a downfield shift of the C3 resonance from 75·7 to 80·2 ppm which was clearly shown in the ^{1}H-^{13}C COSY spectrum (Fig. 4). A similar downfield shift of the C2 resonance occurs when xylose is substituted at the O2 position in model compounds (Bradbury & Jenkins, 1984). Thus the C2 of xylose carrying arabinose at O2 can be assigned to 79·6 ppm.

Both the ^{1}H-^{1}H-NMR COSY and ^{1}H-^{13}C-NMR COSY spectra were very well resolved (Figs 3 and 4). By comparing data within each of them it was possible to assign most of the peaks in the 1D spectra. The values recorded for the xylose residues are very similar to those reported by Herath *et al.* (1990).

In the ^{1}H-^{1}H-NMR COSY spectrum it was possible to trace the couplings around the ring protons of both the arabinose and xylose residues although the H3–H4 cross-peak of arabinose was weak and difficult to identify. This is unexpected as the J_{H3-H4} for methyl α-L-arabinofuranoside is 5·5 Hz which is larger than the other couplings ($J_{H1,H2}$, $J_{H2,H3}$, $J_{H4,H5}$ are 1·8, 3·2 and 3·4 Hz, respectively; Angyal, 1979). Cross-peaks in the ^{1}H-^{13}C-NMR spectrum indicated that the H4 proton has two main chemical shifts (4·06 and 4·19 ppm). The reason for this is unclear but it is not due to some of the arabinose being present in the pyranose as the ^{13}C-NMR and methylation data indicate that all the arabinose residues are in the furanose form.

The complex pattern of substitution of the arabinose on the xylan backbone of the polysaccharide results in complex ^{1}H-NMR and ^{13}C-NMR spectra even when partially depolymerized samples are analysed. The combination of 1D and 2D experiments has made possible chemical shift assignments of all the carbons (Table 5) and protons (Table 6) in the major structural features of the polysaccharide. To the best of the authors' knowledge this is the first time such a complete report of NMR chemical shift data has been reported for arabinoxylans.

Table 6. ^{1}H-NMR chemical shift assignments of partially depolymerized pentosan preparation AEPBP

	Chemical shift (ppm)	
Proton	β-xylopyranosyl	α-arabinofuranosyl
H1	4·35–4·50^a	5·31, 5·21, 5·14
H2	3·27–3·30	4·12
H3	3·50–3·55	3·89–3·95
H4	3·75	4·06, 4·19
H5	4·07(eq)^b	3·68^c
	3·35(ax)^b	3·75^d

^{a}A range of values indicates a complex multiplet of resonances.
^{b}Equatorial and axial protons of xylose; assigned from Smith & Hartley (1983) and Herath *et al.* (1990).
^{c,d}Resonances of H5^2 and H5^1 arabinofuranosyl protons as labelled in Fig. 4.

It is clear from the above study that it is possible to isolate large amounts of highly enriched wheat pentosan preparations in a manner which does not greatly alter the chemical structure of the major arabinoxylan constituent. It is therefore likely that the nutritional activities which have been ascribed to these preparations (Choct & Annison, 1990; 1992*a*) will also be present when the pentosans are consumed as part of complete foods.

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