Physicochemical and Functional Properties of Rye Nonstarch Polysaccharides. V. Variability in the Structure of Water-Soluble Arabinoxylans

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ABSTRACT

Subsequent increases in the degree of ammonium sulfate saturation of a rye water extract allowed the precipitation of at least three distinct arabinoxylan fractions that differed markedly in molecular weight distribution and fine structure. A first (main) fraction, precipitated between 25 and 50% saturation, had an arabinose-to-xylose ratio (A/X) of 0.5 with virtually all branched xylose residues substituted at O-3 with arabinose (as shown by 500 MHz ^{1}H-nuclear magnetic resonance [NMR]). This fraction contained ferulic acid (0.04–0.07%), had a lower molecular weight than the other fractions, and gelled oxidatively with the hydrogen peroxide-peroxidase system.

A second (small) fraction had an A/X of 1.4 and precipitated between 75 and 100% ammonium sulfate saturation. It did not gel. All branched xylose residues were substituted at both O-2 and O-3 with arabinose. Structures of one, two, and more consecutive, disubstituted xylose residues in the xylan chain could be recognized in the ^{1}H-NMR spectra.

Fractions precipitating between 50 and 75% ammonium sulfate saturation were not mixtures of these two arabinoxylan fractions because the ^{1}H-NMR spectra showed differences in the ratio of isolated to paired disubstituted xylose residues.

Arabinoxylans (also often referred to as pentosans) occur as a minor carbohydrate fraction in cereal grains. However, because of their water-binding capacity and viscosity-enhancing properties, they have important functional properties. They influence dough and breadmaking (Meuser and Suckow 1986, Kühn and Gross 1989, Delcour et al 1991, Maat et al 1992). Arabinoxylans gel oxidatively when hydrogen peroxide and peroxidase are added. This is caused by the formation of covalent linkages through oxidative coupling of ferulic acid residues esterified to the arabinoxylan (Neukom and Markwalder 1978), but it is unknown whether the gelation process takes place during breadmaking (Delcour et al 1991). To establish the molecular basis of functionality, it is necessary to gain more insight in the structural diversity of the arabinoxylans.

The structure of wheat (*Triticum aestivum* L.) arabinoxylans has been the subject of a significant number of investigations since Perlin (1951a) identified a water-soluble polysaccharide in wheat flour containing both L-arabinose and D-xylose residues. Perlin (1951b) found that this pentosan consists of a main chain of 1→4-linked β-D-xylopyranose, containing branches of terminal L-arabinofuranose linked at O-2 or O-3 of the xylose residues. The branches occur most frequently on isolated xylose residues or on two consecutive xylosyl units (Ewald and Perlin 1959). Goldschmid and Perlin (1963) degraded such arabinoxylan with an endoxylanase and concluded that branching deviates substantially from a uniform type of arrangement. They suggested a structure with highly branched regions (in which isolated and paired branches are separated by single xylosyl units) and more open regions.

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Hoffmann et al (1991a) demonstrated a wide diversity in wheat flour water-soluble arabinoxylans. The components differed in molecular weight, ferulic acid content, arabinose-to-xylose ratio, and in the ratio of D-xylose moieties, which are di- (O-2,3), mono- (O-3), or unsubstituted with L-arabinose. The structure of oligosaccharides, generated by endo-1,4-β-D-xylanase digestion, was established (Hoffmann et al 1991b, Hoffmann et al 1992a). Each branching type yielded typical ^{1}H-nuclear magnetic resonance (NMR) spectra. They found all four combinations of O-3-mono- and O-2,3-disubstituted β-D-Xylp residues (with single α-Araf residues attached) occurring in internal -β-D-Xylp-(1→4)-β-D-Xylp- backbone elements, but they were not present in equal quantities. A wheat water-soluble arabinoxylan preparation, analyzed by ^{1}H-NMR (Hoffmann et al 1992b), contained isolated and/or paired monosubstituted xylose residues, isolated disubstituted residues, and clusters of two disubstituted xylose residues. Few structure elements containing a monosubstituted xylose residue next to a disubstituted residue were present.

It is generally accepted that the structural features of the water-soluble arabinoxylans from rye (*Secale cereale* L.) are quite similar to those of wheat arabinoxylans. Rye contains more water-soluble arabinoxylans than wheat (Meuser and Suckow 1986, Girhammar and Nair 1992a). Aspinall and Ross (1963) investigated the structure of rye-flour arabinoxylans and found a ratio of 7.5:2.2:1 for isolated branched xyloses, pairs of branched xyloses, and sequences of three consecutive substituted xylose residues. They concluded that the arabinose side chains are attached randomly along the main xylan chain.

More recently, for the major fraction of rye water-soluble arabinoxylans, Bengtsson and Aman (1990) found that 50% of the xylose residues were substituted at O-3 and 2% at O-2 and O-3 by terminal arabinose. Periodate oxidation and degradation studies revealed that, in this fraction, the branch points were predominantly isolated residues (36%) or small blocks of two residues (62%), and they were not randomly distributed (Aman and Bengtsson 1991). Digestion by an endoxylanase preparation (of a fraction in which 43% of the xylose residues were monosubstituted and 7% were disubstituted with arabinose) resulted in oligosaccharides and a remaining polymeric fraction that was enriched with disubstituted xylose residues. In this fraction, 60–70% of the xylose residues were substituted at both O-2 and O-3 with arabinose units. These results indicated that the mono- and disubstituted xylose residues were present in different polymers (arabinoxylan I and II, respectively) or different regions of the same polymer (Bengtsson et al 1992a). On average, 46% of the xylose residues in arabinoxylan I and 57% of those in arabinoxylan II were branched, and the degree of substitution in respective polymers was similar for a wide range of samples. The ratio of contents of arabinoxylan I compared to that of arabinoxylan II was different for the different rye varieties grown in different countries and ranged from 1.1 to 2.8 (Bengtsson et al 1992b).

Therefore, to date, it has not been determined whether the structures designated as arabinoxylan I and arabinoxylan II are present in different polymers or in different regions of the same polymer. Although Bengtsson and Aman (1990) isolated an almost pure arabinoxylan I, an intact pure arabinoxylan II has never been found. In this article, we describe the separation of the two polymers by ammonium sulfate fractionation of a water extract, and we document the structural variability in the water-soluble arabinoxylans from rye whole meal. A graded ammonium sulfate fractionation was used recently by Izydorczyk and Biliaderis (1992) to isolate groups of wheat arabinoxylans differing in chemical structure, ferulic acid content, intrinsic viscosity, gelling capacity, and molecular weight.

MATERIALS AND METHODS

Isolation of Arabinoxylan Fractions

Rye (Danko, harvested in 1991 in Belgium) was milled on a DDD President mill. Whole grain meal was heated at 130°C for 90 min to inactivate enzymes. Dry meal (1,000 g) was extracted with 4.0 L of water at room temperature for 90 min by end-over-end rotation. After centrifugation (30 min, 10,000 × *g*, 20°C), the supernatant was treated with 2.0 ml of α-amylase (Type XII-A from *Bacillus licheniformis*, Sigma Chemical, St. Louis, MO) for 1 hr at 85°C. After centrifugation, the supernatant was filtered with Celite (10 g/l). Ammonium sulfate was added to 25% saturation; the solution was kept overnight at room temperature to equilibrate and was centrifuged (30 min, 10,000 × *g*, 20°C). The residue was discarded. The supernatant was further saturated to 50%, kept overnight, and centrifuged as above. In the next step, the solution was saturated to 75% and, subsequently, in the last step, to 100%, with intermediate steps as outlined above. The residues precipitated at 50, 75, and 100% (fractions AX50, AX75, and AX100, respectively) were dialyzed against distilled water at 4°C for two days. The protein content of the resulting solutions was measured according to Lowry et al (1951), and a quantity of a 5% suspension of Wyoming montmorillonite, containing ten times as much clay (w/w) as protein to be removed, was added. The pH was adjusted to 3.0, and the solution mixed for 30 min and centrifuged (30 min, 10,000 × *g*, 20°C). The supernatant was neutralized, and four volumes of ethanol were added to precipitate the arabinoxylans. The precipitate was filtered and dried by washings with ethanol and acetone.

AX50 was further fractionated by dissolving 500 mg in 250 ml of water, adding ammonium sulfate to 47.5% saturation, centrifuging, dialyzing the supernatant (AX50S) and residue (AX50R), and adding ethanol to precipitate both arabinoxylan fractions.

A similar fractionation of Danko rye harvested in 1990 resulted in fractions precipitating at different degrees of saturation with ammonium sulfate: 25% (AX25B), 50% (AX50B), 65% (AX65B), 100% (AX100B). (AX25B not studied.)

AX100B was further fractionated into a fraction precipitating at 75% ammonium sulfate saturation (AX100BR) and a soluble fraction (AX100BS).

Analytical Methods

Polysaccharides were hydrolyzed in 12*M* H_2SO_4 for 1 hr at room temperature followed by 3 hr at 100°C in 1*M* H_2SO_4. Monosaccharides were measured as their alditol acetates (Supelco SP-2330 capillary column) according to the Englyst method (1984). Losses due to degradation during hydrolysis were countered by giving standards the same treatment as samples. Inositol was used as an internal standard. Analyses were performed in duplicate. The standard deviation for the arabinose-to-xylose (A/X) ratio was, on average, 0.016. The average standard deviation for percent total carbohydrates was 1.4. A representative chromatogram is shown in Figure 1.

Ferulic acid was measured by high-performance liquid chromatography (HPLC, Alltech ROSIL C18 column, eluent water/acetic acid/methanol 95:5:30, monitoring absorbance at 280 nm) after treatment of the sample with 0.5*M* KOH (flushed with nitrogen) for 24 hr at room temperature. After acidification of the solution, ferulic acid was extracted with ethyl acetate. *p*-Hydroxybenzoic acid was used as an internal standard as it was by Gruppen et al (1989).

Proteins were measured according to the method of Lowry et al (1951), with bovine serum albumin as the standard.

Relative viscosities of arabinoxylan solutions (2 mg/ml) were measured at 30.0°C in an Ostwald II viscometer before and after the addition of horseradish peroxidase (POD) and H_2O_2 as described previously (Vinkx et al 1991).

Size-exclusion chromatography of dissolved fractions was performed on a Sephacryl S500 HR column (fractionation range for dextran is 4.10^4 to 2.10^7 da) with 0.3% NaCl as the eluent as described previously (Vinkx et al 1992). The fractionation was monitored by reading the absorbance at 280 nm and by analyzing eluted fractions for total carbohydrates according to Dubois et al (1956).

^{1}H-NMR spectra were recorded on a Bruker 500 MHz apparatus at 67°C at the Bijvoet Center, Utrecht University, The Netherlands. This equipment is the same as that used for recording Hoffmann's NMR spectra (Hoffmann et al 1992b). Pulse repetition time was 0.01 sec. The number of scans varied from 100 to 500. Acetone was used as an internal standard. Samples were prepared by dissolving in D_2O (99%), lyophilizing, and renewed dissolving in D_2O, so that OH-signals were removed from the spectrum.

A ^{1}H-decoupled ^{13}C-NMR spectrum was recorded on a Bruker AM-300 apparatus (75 MHz) at 67°C for a concentration of ~1.5% of the sample in D_2O. Pulse repetition time was 2 sec, and the number of scans was ~20,000. Dioxane was used as an external standard (67.4 ppm). Peak assignments were made on the basis of the data by Hoffmann et al (1992b) and Bengtsson and Aman (1990).

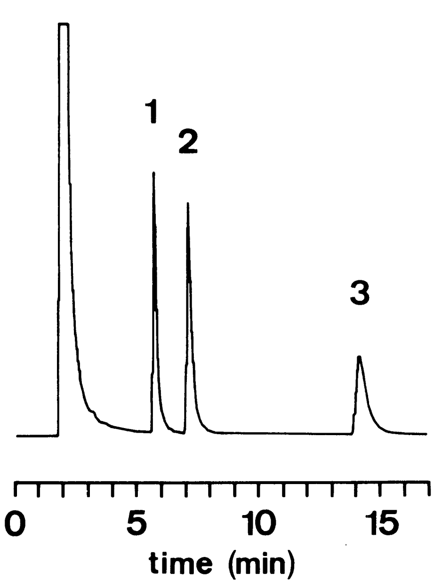

Fig. 1. Gas chromatogram of alditol acetates of arabinoxylan fraction AX65B (Supelco SP-2330 capillary column, 220°C). **1,** arabinose; **2,** xylose; **3,** inositol (internal standard).

TABLE I
Protein Content, Monosaccharide Composition and Fine Structure, Ferulic Acid Content, and Viscosities of Arabinoxylan Fractions Precipitated at Different Degrees of Ammonium Sulfate Saturation

	Rye Danko 1991					Rye Danko 1990		
	AX50	AX75	AX100	AX50R		AX50B	AX65B	AX100BS
Protein (%)	0.0	1.1	1.7	ND[a]	Protein (%)	0.1	0.9	ND
Carbohydrate (%)	90	82	70	92	Carbohydrate (%)	90	86	89
A/X[b]	0.55	1.09	1.42	0.50	A/X	0.52	0.81	1.34
xyl_{di}/xyl_{subst}[c]	0.07	0.79	1.00	0.02	xyl_{di}/xyl_{subst}[c]	0.04	0.50	1.00
Ferulic acid (%)	0.07	0.02	0.03	0.08	Ferulic acid (%)	0.04	0.05	Trace
Relative viscosity	3.2	8.3	2.2	ND	Relative viscosity	3.0	ND	4.2
Relative viscosity after gelation	6.0	13.0	2.2	ND	Relative viscosity after gelation	5.2	ND	4.4

[a] ND = not determined.
[b] A/X = ratio of arabinose to xylose as determined by gas chromatography.
[c] xyl_{di}/xyl_{subst} = ratio of disubstituted xylose residues to the sum of disubstituted and monosubstituted xylose residues, as determined from integrating anomeric arabinose peaks in the ^{1}H-nuclear magnetic resonance spectra.

RESULTS AND DISCUSSION

Yields of Fractions

The yields of AX50, AX75, and AX100 were 0.28, 0.17, and 0.04%, respectively, of dry meal. The yield of AX50S was 60% and the yield of AX50R was 17% of AX50. The yields of AX50B, AX65B, and AX100B were 0.43, 0.06, and 0.35%, respectively, of dry meal. The yields of AX100BR and AX100BS were 49 and 24% respectively, of AX100B. Yields were relatively low (less than 1% compared to ~2% water-soluble arabinoxylans reported by Bengtsson et al 1992b) due to the clay treatment in the purification procedure (Vinkx and Delcour 1992). Differences in arabinoxylan yields of rye samples from different harvest years were observed in the present study. Since no replicate fractionations were performed, it is difficult to estimate the significance of this finding, although it was reported earlier (Saastamoinen et al 1989) that the pentosan content of rye depends on harvest year (effects of environmental conditions [weather] and growing time).

Analysis of the Arabinoxylans

All the samples were virtually pure arabinoxylans (Table I and Fig. 1); they had a very low protein content, and the only monosaccharides observed were arabinose and xylose (only a trace of glucose was present in AX100).

The fractions had a ferulic acid content (trace amount to 0.08%) in accord with previous results on rye arabinoxylans (Vinkx et al 1991), but it was low compared to literature data on wheat water-soluble arabinoxylans. Hoffmann et al (1991a) listed contents of up to 0.39% ferulic acid.

Size-exclusion chromatography (Figs. 2 and 3) showed that the fractions AX50, AX50R, AX75, AX50B, and AX65 contained one broad carbohydrate peak coinciding with a peak of absorbance at 280 nm, which was due to ferulic acid (Vinkx et al 1992). Differences in ferulic acid content for the different fractions were clearly confirmed. AX100 and AX100BS contain more protein and less ferulic acid: the 280-nm absorbance peaks and the carbohydrate peaks did not coincide.

These two fractionations demonstrated that variation in molecular weight distribution occurs between samples of the same variety from different harvest years (Figs. 2 and 3).

Relative viscosities of arabinoxylan solutions (Table I) were related to molecular weight distribution, as was also reported by Girhammar and Nair (1992b).

Much like Izydorczyk and Biliaderis (1992), we found a fraction (AX100) that contained ferulic acid but possessed no gelling capacity (Table I). It is likely that the combination of a minimum molecular weight and ferulic acid content (and arabinoxylan concentration) is necessary for gelation, but also the stiffness of the arabinoxylan chain (increasing with amount of substituted xyloses) might play a role (Izydorczyk and Biliaderis 1992). However, more work is clearly needed to understand the relative impact of ferulic acid content, molecular weight, and chain stiffness on gelling capacity.

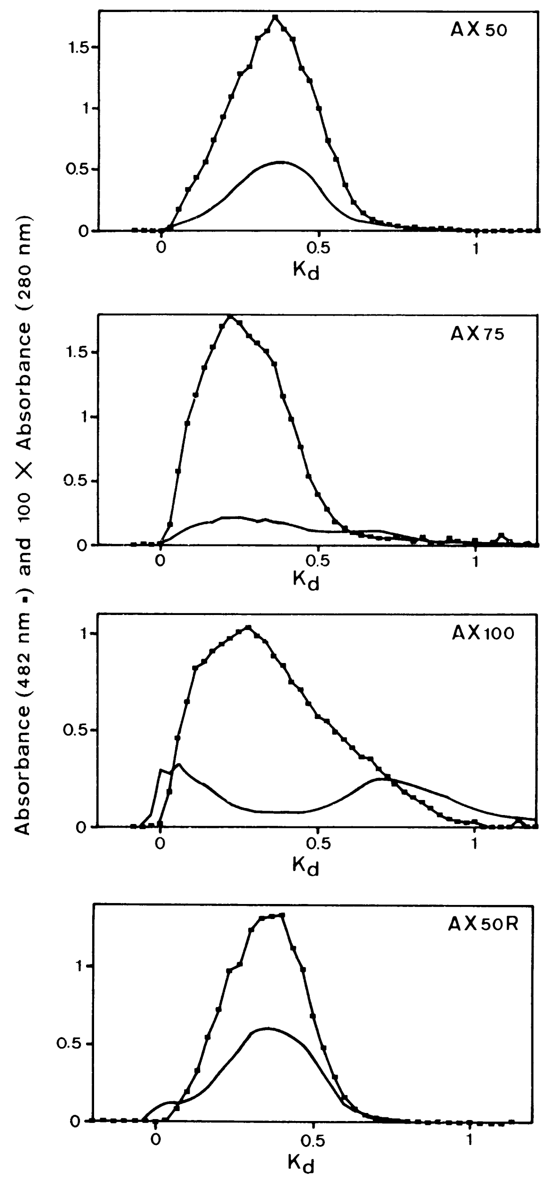

Fig. 2. Size-exclusion chromatography of AX50, AX75, AX100, and AX50R as monitored for total carbohydrates (• •) and absorption at 280 nm (—).

Ammonium Sulfate Fractionation

The major rye water-soluble arabinoxylan fraction (AX50 and AX50B, 50–60% of total isolated arabinoxylan) was characterized by an A/X ratio of 0.50–0.55. It gels on addition of H_2O_2/POD and has a relatively high ferulic acid content and a relatively low molecular weight (Table I and Figs. 2 and 3). This fraction had a very low disubstituted xylose residue content, which is in agreement with the findings of Bengtsson and Aman (1990). This could be concluded from the ^{1}H-NMR spectra (Figs. 4 and 5, Table II) as well as from the data of Bengtsson and Aman (1990) and Hoffmann et al (1992b). Thus, the anomeric proton of an arabinose of a monosubstituted xylose results in a peak with a chemical shift of δ5.40 ppm. Peaks with chemical shifts at δ5.21–δ5.31 ppm demonstrate the presence of xylose O-2,3 disubstituted with arabinose. A small peak at δ5.42 ppm can be attributed to a monosubstituted xylose adjacent to a disubstituted xylose. Limitations imposed by the ^{1}H-NMR technique left it unclear whether monosubstituted xylose residues in the xylan chain were isolated or in clusters.

With increasing ammonium sulfate saturation, rye water-soluble arabinoxylan fractions precipitated with increasing A/X ratio (0.50–1.42), increasing ratio of disubstituted to total branched xyloses (from almost zero to 1), decreasing ferulic acid content (0.08% to trace amounts), and decreasing gelling capacities. The tendencies were established beyond any reasonable doubt for A/X ratio and branching pattern only. These observations are in agreement with recent reports for wheat water-soluble arabinoxylans (Izydorczyk and Biliaderis 1992). However, ranges for A/X ratios (0.58–0.88 for wheat) and amounts of disubstituted xylose residues (^{1}H-NMR spectra) were were much larger for the rye fractions described in this article than they were for the wheat arabinoxylans described by Izydorczyk and Biliaderis (1992). In our study, the fraction that precipitated at the lowest ammonium sulfate saturation had the lowest molecular weight, in contrast to what was found for wheat arabinoxylans by the cited authors.

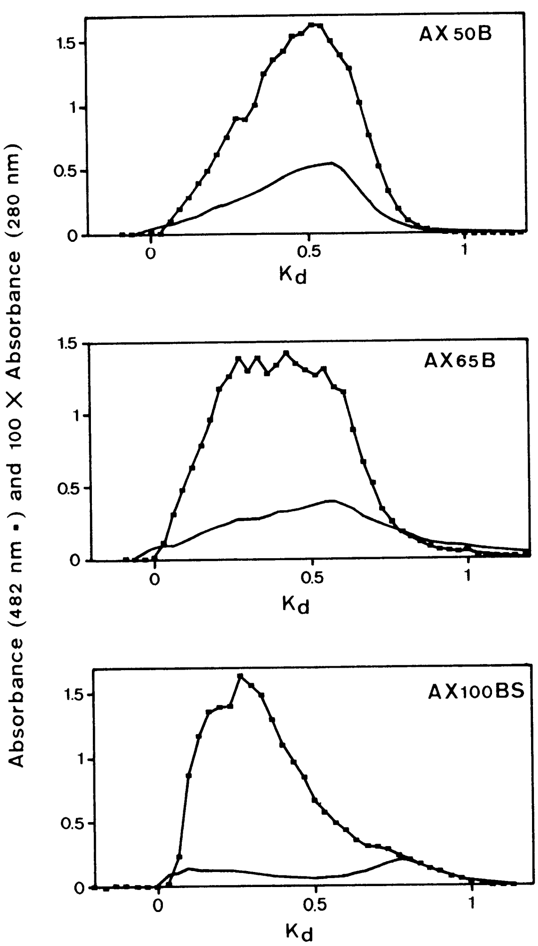

Fig. 3. Size-exclusion chromatography of AX50B, AX65B, and AX100BS as monitored for total carbohydrates (· ·) and absorption at 280 nm (—).

Distribution of Disubstituted Xylose Residues

A close examination of the ^{1}H-NMR spectra (Figs. 4–10) revealed differences in the way disubstituted xyloses (xyl_{di}) were present in the arabinoxylan. Thus, the peaks of the anomeric protons of the arabinose moieties in such xyl_{di} (δ5.21–δ5.31 ppm) indicated a difference in the number of consecutive xyl_{di} residues in the xylan chain. Indeed, it is clear from the work by Hoffmann et al (1992b) that an isolated xyl_{di} residue is characterized by a peak at δ5.222 ppm, as well as by one at δ5.287 ppm. Two neighboring xyl_{di} result in peaks at δ5.222, δ5.243, δ5.298, and δ5.308 ppm. With 500 MHz ^{1}H-NMR spectrometry, sufficient resolution could be obtained to distinguish, especially in the AX100BS spectrum (Fig. 10), two more peaks for rye water-soluble arabinoxylans. These peaks very probably arose from sequences of three or more consecutive xyl_{di} residues. Based upon literature data (Hoffmann et al 1992b) and relative peak areas, tentative assignments were made (Table II). For AX100BS, it could be calculated from monosaccharide analysis (1.34 A/X) and relative integrated peak areas in the ^{1}H-NMR spectrum that the xyl_{di} occurrences were ~23% isolated, 26% paired, and 51% in sequences with an average of 3.5 consecutive disubstituted xyloses. As a consequence, almost all unsubstituted xylose residues would occur isolated in this fraction. Further evidence for this conclusion came from the ^{13}C-NMR spectrum.

When considering relative peak heights in the spectra of the different fractions, we could conclude that the fractions with the lower proportion of xyl_{di} residues contained more isolated xyl_{di} (or xyl_{di} adjacent to xyl_{mono}), whereas the fraction with the highest

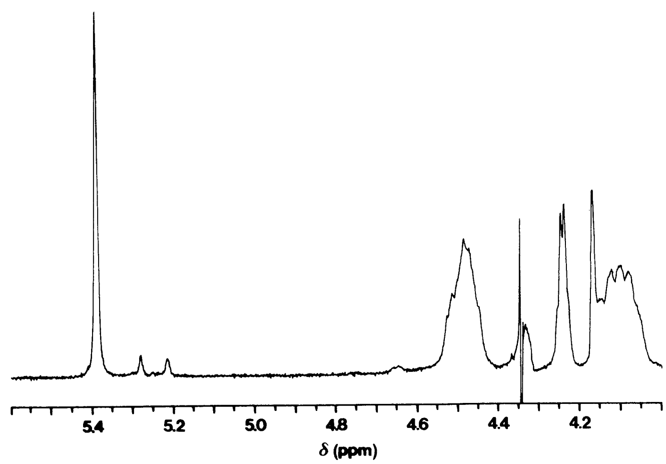

Fig. 4. ^{1}H-nuclear magnetic resonance spectrum of arabinoxylan fraction AX50 (D_2O, 67°C). See Table II for peak assignments.

TABLE II
^{1}H-Nuclear Magnetic Resonance Chemical Shifts (δ, ppm) of Anomeric Protons of Arabinose in Rye and Wheat[a] Water-Soluble Arabinoxylans

	Rye		Wheat	
Elements[b]	Ara^2 [c]	Ara^3 [d]	Ara^2	Ara^3
a	. . .	5.39	. . .	5.396
b	5.21	5.28	5.222	5.287
c I	5.21	5.30	5.222	5.308
II	5.24	5.29	5.243	5.298
d I	5.21*[e]	5.30*	. . .	. . .
II	5.23*	5.31*	. . .	. . .
III	5.24*	5.30*	. . .	. . .

[a] Hoffman et al (1992b).
[b] Element *a* = monosubstituted xylose (isolated or paired); *b* = isolated disubstituted xylose; *c* = paired disubstituted xyloses (I and II); *d* = sequence of three (I, II, and III) or more (I: first, II: all middle, and III: terminal) consecutive disubstituted xylose residues.
[c] Ara^2 = arabinose substituted at O-2 of xylose.
[d] Ara^3 = arabinose substituted at O-3 of xylose.
[e] * = tentative assignment.

proportion of xyl_{di} residues had a low content of isolated xyl_{di} residues. Earlier reports that sequences with more than two consecutive substituted xylose residues are very rare (Aspinall and Ross 1963, Aman and Bengtsson 1991) need not be in disagreement with our results because the highly substituted fraction was only recovered in low yields. This is supported by findings in the anomeric regions of xylose residues (δ4.4–δ4.7 ppm) and the nonanomeric regions in the spectra (δ3.3–δ4.3 ppm), although resolution and, accordingly, interpretation was much more difficult here. The peak at δ4.4–d4.5 ppm was assigned (Hoffmann et al 1992b) to unsubstituted xylose (δ4.46 ppm refers to an unsubstituted xylose adjacent to a substituted xylose, δ4.48 ppm to a unsubstituted xylose following another unsubstitued xylose). Hoffmann et al (1992b) further reported peaks at δ4.53 ppm for a monosubstituted xylose, at δ4.66 ppm for an isolated xyl_{di}, and at δ4.65 and δ4.59 ppm for paired xyl_{di} residues. However, the peak at δ4.53 ppm in the AX100 spectrum (Fig. 8) could not be ascribed to a monosubstituted xylose residue because of the arabinose anomeric signals. It is not unlikely that it originates from one of the xylose units of a sequence of more than two xyl_{di}. It is clear that AX100 contained a relatively low amount of unsubstituted xyloses, which were mostly adjacent to a substituted xylose. Hoffmann et al (1992b) further reported that the peaks of the H-2 of arabinose appear at δ4.16 ppm for arabinose attached at O-2 of xylose and at δ4.17 ppm for arabinose attached at O-3 of xylose. These peaks were easily recognized in the spectra and confirmed previous conclusions.

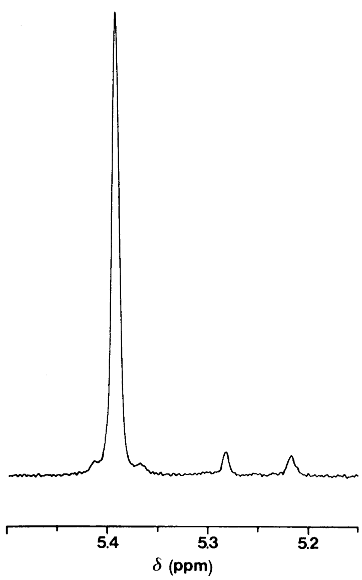

Fig. 5. [1]H-nuclear magnetic resonance spectrum of arabinoxylan fraction AX50B (D_2O, 67°C). See Table II for peak assignments.

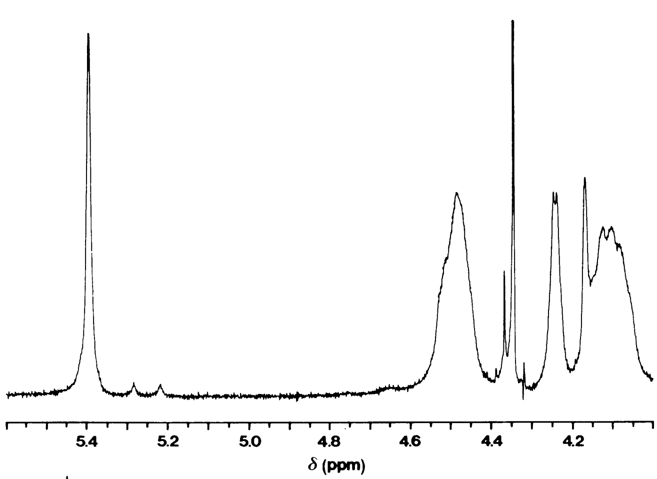

Fig. 6. [1]H-nuclear magnetic resonance spectrum of arabinoxylan fraction AX50R (D_2O, 67°C). See Table II for peak assignments.

Some smaller unidentified peaks (δ5.43 and δ5.37 ppm) were present in the spectra of AX75, AX100, AX50B, AX65B, and AX100BS. One possibility is that they indicated short araban branches, which have been suggested by Ebringerova et al (1990) in rye bran arabinoxylans.

^{13}C NMR Spectrum of a Disubstituted Arabinoxylan

The ^{13}C NMR spectrum of the AX100BS fraction is shown

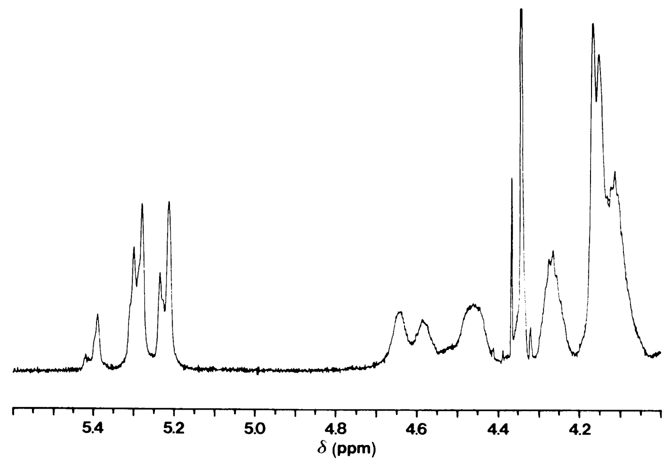

Fig. 7. [1]H-nuclear magnetic resonance spectrum of arabinoxylan fraction AX75 (D_2O, 67°C). See Table II for peak assignments.

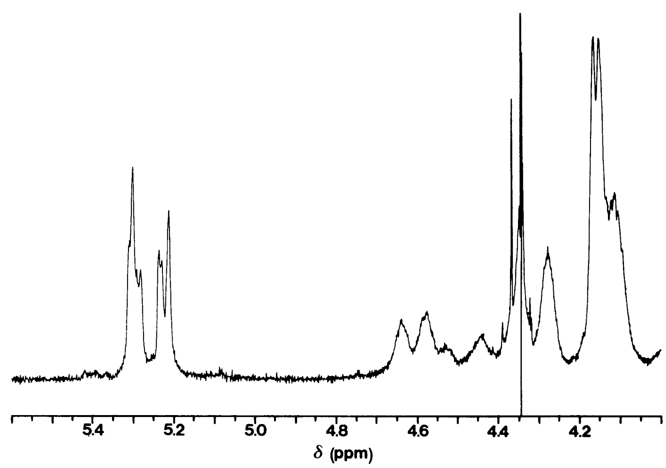

Fig. 8. [1]H-nuclear magnetic resonance spectrum of arabinoxylan fraction AX100 (D_2O, 67°C). See Table II for peak assignments.

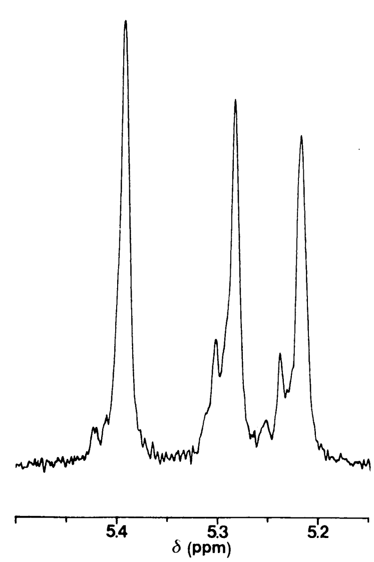

Fig. 9. [1]H-nuclear magnetic resonance spectrum of arabinoxylan fraction AX65B (D_2O, 67°C). See Table II for peak assignments.

in Figure 11. Peaks were assigned according to literature data of wheat water-soluble arabinoxylans (Hoffmann et al 1992b): peaks at δ109.5, δ82.3, δ77.7, δ85.0, and δ62.2 ppm were assigned to C-1, C-2, C-3, C-4 and C-5 of an arabinose attached to O-2 of a xyl_{di}. Peaks at δ108.8, δ82.0, δ78.1, δ85.3, and δ62.2 ppm were assigned to C-1, C-2, C-3, C-4, and C-5 of an arabinose linked to O-3 of a xyl_{di}. A small peak at δ109.1 ppm, probably from an anomeric carbon of arabinose, could not be assigned. The anomeric region of the xylose monomers (δ100–δ103 ppm) showed a major peak at δ100.9 ppm assigned to xyl_{di}, a smaller peak at δ102.0 ppm assigned to an unsubstituted xylose adjacent to a substituted xylose (xyl_{adj}), and only a very small peak at δ102.5 ppm, indicating trace amounts of monosubstituted xylose (xyl_{mono}) residues or unsubstituted xyloses adjacent to another unsubstituted xylose (xyl). The C-2 atom signals of xylose at ~δ74 ppm confirmed this observation: a major peak at δ74.7 ppm of xyl_{di} and a small peak at δ73.8 ppm of a xyl_{adj}. No other xylose C-2 peaks were observed, indicating that virtually all unsubstituted xylose residues were isolated. This is in agreement with ^{1}H-NMR spectra and monosaccharide analysis. The peak at δ74.7 ppm was also attributed to C-3 of a xyl_{adj}. Other C-3 and C-4 peaks of xylose were more difficult to recognize in the area ~δ78 ppm. The C-5 peaks of xylose (δ63.3, δ63.5, and δ63.7 ppm) could not be fully interpreted on the basis of the literature data. Hoffmann et al (1992b) listed δ63.21 ppm for xyl_{di}, δ63.85 ppm for xyl_{adj} and xyl, and δ63.59 ppm for xyl_{mono}; Bengtsson and Aman (1990) listed δ63.9 ppm for unsubstituted xyloses and δ63.7 ppm for xyl_{mono}.

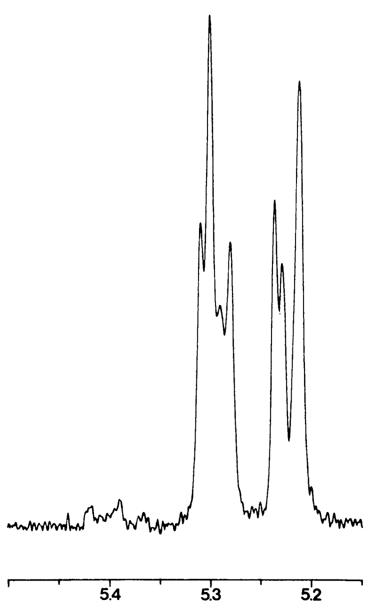

Fig. 10. ^{1}H-nuclear magnetic resonance spectrum of arabinoxylan fraction AX100BS (D_2O, 67°C). See Table II for peak assignments.

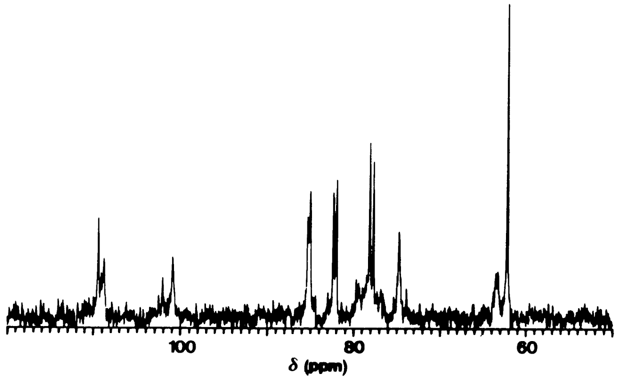

Fig. 11. ^{13}C-nuclear magnetic resonance spectrum of arabinoxylan fraction AX100BS (D_2O, 67°C).

Variability in Arabinoxylan Structure

Monosubstituted xylose residues adjacent to disubstituted xylose residues were present only in very small amounts, as was also reported for wheat arabinoxylans (Hoffmann et al 1992b). The separation of one arabinoxylan fraction containing only un- and disubstituted xylose residues (which has not, to the best of our knowledge, been reported earlier) and of a second one, with virtually all branched residues monosubstituted, lends support to the hypothesis (Bengtsson et al 1992a,b) that two separate polymers exist, even though intensive efforts aimed at the total purification of the (still hypothesized) monosubstituted polymer were not successful. AX50R has 1% disubstituted xyloses (Fig. 6). Furthermore, the ^{1}H-NMR data (Figs. 4–10) clearly show that a range of structures exists. Indeed, spectra of intermediate fractions (AX65B and AX75) cannot be visualized as being the sum of spectra of the polymer with virtually only un- and monosubstituted xyloses and that of the pure polymer containing only un- and disubstituted xylose residues (when considering the ratio of isolated and paired disubstituted xylose moieties). Hence, the variability in rye arabinoxylan structures might be a continuum varying between the two extremes found in this work.

CONCLUSIONS

A range of polymer structures exists in the group of the arabinoxylans present in the rye grain. This is in contrast to previous suggestions of two classes. The water-soluble arabinoxylans differ in molecular weight, arabinose-to-xylose ratio, ferulic acid content, ratio of di- to monosubstituted xylose, and ratio of disubstituted xyloses that are isolated, paired, or present in longer sequences in the xylan chain. The differences in chemical fine structure established in the present work for rye arabinoxylans are much larger than those described previously for wheat arabinoxylans. Almost pure monosubstituted arabinoxylan (~54%) and pure disubstituted arabinoxylan (~9%) could be recovered. Ammonium sulfate fractionation can be a good tool for obtaining more homogeneous arabinoxylan preparations, which should allow investigations of the molecular basis of functionality. The present study shows that the different groups of arabinoxylans have different viscosity-enhancing properties and gelling capacities.

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