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Carbohydrate Polymers 42 (2000) 3–11

Carbohydrate Polymers

www.elsevier.com/locate/carbpol

Structural features of (1 → 3),(1 → 4)-β-D-glucan and arabinoxylan fractions isolated from rye bran

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Accepted 13 August 1999

Abstract

A water unextractable fraction from rye bran was isolated by means of a sequential extraction. Gel filtration revealed a homogeneous β-glucan and at least two different arabinoxylans. ^{1}H and ^{13}C NMR spectroscopy were used to identify the structural units in both polysaccharides. The isolated arabinoxylan contained a relatively high amount of unsubstituted xylose residues (56.7%). The molar ratio of cellotriosyl to cellotetraosyl units and the ratio of (1 → 4) to (1 → 3)-linkages in β-glucan were determined using high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) and ^{1}H NMR, respectively. The molar ratio of cellotriosyl to cellotetraosyl was found to be 1.94. From this, the ratio of (1 → 4) to (1 → 3)-linkages was calculated to be 2.34, as compared to 2.31 obtained by ^{1}H NMR, showing that the results from both methods are in close agreement.

Keywords: Mixed-linkage (1 → 3),(1 → 4)-β-D-glucan; Arabinoxylan; Rye; Structure

1. Introduction

Mixed-linkage (1 → 3),(1 → 4)-β-D-glucan (hereafter referred to as β-glucan) and arabinoxylan, together with other polysaccharides, glycoproteins and phenolics are important components in the gel-like matrix of the endosperm cell wall of cereals and contribute to cell wall characteristics such as water-holding capacity, porosity and plasticity (Fincher & Stone, 1981). The general occurrence of β-glucan in plant structures and the difficulty in removing it from the cell wall indicates that it acts as a structural component (Darvill, McNeil, Albersheim & Delmer, 1980). On the other hand, other authors have stated that β-glucan also might serve as an energy source (Loescher & Nevins, 1972, 1973; Nevins, Huber, Yamamoto & Loescher, 1977).

β-Glucan belongs to a family of polymers which are heterogeneous with respect to molecular size and fine structure, varying with tissue, stage of maturity and source (Bacic, Harris & Stone, 1988). The highest levels of β-glucan are found in grains of barley (3.0–6.9%, Åman & Graham, 1987) and oats (2.2–4.2%, Åman & Graham, 1987). The content in rye grain varies generally from 1–2% for different varieties from different countries (Saastamoinen, Plaami & Kumpulainen, 1989). Henry (1987) found 2.5% for whole rye grain and 1.7% for rye starchy endosperm, whereas Nilsson, Saulnier, Andersson and Åman (1996) reported 3.4% of the rye bran as being β-glucan. The amount of arabinoxylan in rye bran was much higher, up to 37% (Nilsson et al., 1996).

β-Glucan appears to be an unbranched polysaccharide composed of (1 → 4)-linked glucopyranosyl (Glc*p*) residues (~70%) substituted at position 3 or 4, hereafter referred to as 3G4 and 4G4, respectively and (1 → 3)-linked-Glc*p* residues (~30%) substituted at position 4, hereafter referred to as 4G3 (Aspinall & Carpenter, 1984; Fleming & Manners, 1966; Luchsinger, Chen & Richards, 1965; Parrish, Perlin & Reese, 1960; Perlin & Suzuki, 1962). About 90% (w/w) of the β-glucan in barley flour consists of cellotriosyl and cellotetraosyl units linked by single (1 → 3)-linkages (Woodward, Fincher & Stone, 1983) and this structural feature has been shown to be valid for other cereals as well (Wood, Weisz & Blackwell, 1994). The (1 → 3)- and (1 → 4)-linkages are distributed neither statistically randomly nor in a strictly repeating sequence (Staudte, Woodward, Fincher & Stone, 1983). The introduction of (1 → 3)-linkages results in "kinks" within the molecule giving rise to an irregular shape which prevents extensive intermolecular association and makes the β-glucan partially water-soluble (Clarke & Stone, 1963). Longer sequences of (1 → 4)-linkages have been identified, those for rye being mainly cellopentaosyl, cellohexaosyl and cellononaosyl (Wood et al., 1994). These longer

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PII: S0144-8617(99)00129-0

sequences are believed to be responsible for the partial water-insolubility of the β-glucan, because they provide surfaces that are capable of forming junction zones (Woodward et al., 1983).

Different sources of β-glucan have been subjected to extensive structural analysis, mainly with respect to the arrangement of the linkages (Staudte et al., 1983; Wood et al., 1994). Barley and rye have a consistently higher ratio of cellotriosyl to cellotetraosyl units, 2.8–3.3 and 3.0–3.2, respectively, than oats (2.1–2.4) (Wood et al., 1994).

A previous study on a bran fraction from rye (Nilsson et al., 1996) revealed the presence of a β-glucan which was water extractable after $Ba(OH)_2$ extraction. $Ba(OH)_2$ preferentially extracts arabinoxylan from the water unextractable material (Gruppen, Hamer & Voragen, 1991). The β-glucan may be released from the cell wall as a result of arabinoxylan removal by breakage of ester-linkages and hydrogen bonds. In the cell walls of the starchy endosperm of barley, a ferulic acid esterase from *Aspergillus niger* can disrupt ferulic acid-arabinoxylan ester bridges. It has been suggested that the arabinoxylan is stripped off directly from the cell wall and subsequently brings the β-glucan, which is physically associated with the arabinoxylan, into solution (Moore, Bamforth, Kroon, Bartolomé & Williamson, 1996). Another effect might be chemical degradation of the polymers by alkaline treatment.

The water extractable arabinoxylan in rye has been shown to contain a backbone of (1 → 4)-linked-β-D-xylopyranosyl (Xyl*p*) residues with sidechains of terminal α-L-arabinofuranosyl (Ara*f*) residues essentially linked to C3 or to C2 and C3, but also to some extent to C2 (Åman & Bengtsson, 1991; Bengtsson, Åman & Andersson, 1991; Vinkx, Delcour, Verbruggen & Gruppen, 1995; Vinkx, Reynaert, Grobet & Delcour, 1993).

On the whole, little information is available about the structure of water unextractable β-glucan from rye bran. In this study we have isolated, and structurally elucidated, a β-glucan together with an arabinoxylan, which were water extractable after $Ba(OH)_2$ extraction.

2. Experimental

2.1. Polysaccharide preparation

Rye bran was obtained from a Swedish commercial mill (AB Nord Mills, Malmö) and ground in a Retsch laboratory mill using a 0.5 mm screen. Samples (40 g) were extracted by ethanol and after enzymatic starch degradation with water (Nilsson et al., 1996). The unextractable material was further extracted with $Ba(OH)_2$ followed by water as described by Nilsson et al. (1996). The latter water extractable material was freeze dried and denoted BN.

An aliquot of the freeze dried sample (135 mg) was dispersed in 60.5 ml 0.05 M NaOH and extracted with magnetic stirring for 16 h at room temperature followed by centrifugation (900g, 15 min). The supernatant was analyzed for sugar residue composition by the Uppsala procedure (Theander, Åman, Westerlund, Andersson & Pettersson, 1995), omitting the swelling step in 72% H_2SO_4. The supernatant was concentrated in an Amicon concentrator (Grace Company, Beverly, MA, USA) and both the concentrate and permeate were analyzed for sugar residues and protein (ninhydrine test; Sigma). The pellet of the 0.05 M NaOH extraction was dispersed in 20 ml 0.1 M NaOH for 16 h. Thereafter, the sample was centrifuged (900 g, 15 min) and the supernatant decanted. The sugar composition of the pellet was determined.

The supernatant of the 0.1 M NaOH extraction was applied (10 injections of 1 ml; 5.6 mg/ml) on a Sephacryl S-500 column (86.5 × 2.6 cm) (Amersham Pharmacia Biotech, Uppsala, Sweden) and eluted with 0.05 M NaOH containing 0.01% NaN_3. This separation was performed using a FPLC system (Amersham Pharmacia Biotech, Uppsala, Sweden) equipped with a differential refractometer (R401, Waters Associates, Milford, MA, USA). Fractions of 3.5 ml were collected. All separations were performed at room temperature with a flow rate of 1 ml/min.

Sugar residues in each collected fraction were analyzed and the fractions were pooled on the basis of the sugar profile (fraction 1–10 [F1], 11–45 [F2] and 46–80 [F3]), neutralized to pH 7 with 0.05 M HCl, dialyzed against deionized water for 3 days and freeze dried.

2.2. Size exclusion chromatography—low angle laser light scattering

Determination of the average molecular weight ($\bar{M}_w$) on F2 and F3 was performed by size exclusion chromatography low angle laser light scattering (SEC-LALLS). The system was equipped with a TSP pump (Spectra System P 1500, Spectra Physics, Fremont, CA, USA), a temperature controlled autoinjector (Shimadzu SCL 10A, Shimadzu Corporation, Kyoto, Japan), RI detector (Shodex RI SE-61, Showa Denko KK, Tokyo, Japan) and a light scattering detector (Chromatix KMX-6, LDC Analytical, USA). Serially connected TSK G6000 PWXL, G5000 PWXL and G4000 PWXL columns were used. The solvent was 0.05 M Na_2SO_4 + 0.01 M EDTA, pH 6.0. All fractionations were performed at room temperature with a flow rate of 0.5 ml/min. Calculations were based on a d*n*/d*c* of 0.150 (Vårum, Smidsrød & Brant, 1992).

2.3. NMR spectroscopy

NMR spectroscopy was performed on a Bruker DRX-400 spectrometer (Bruker Spectrospin Canada, Milton, Ont., Canada) operating at 80°C. The samples (F1: 3 mg; F2: 12.7 mg; F3: 17.5 mg) were dissolved in 0.7 ml D_2O and acetone-d_6 (δ_H 2.225; δ_C 31.00) was used as internal reference. Heteronuclear multiple bond correlation (HMBC) used a delay for evolution of long range coupling ($^3J_{C,H} = 15.38$ Hz), while heteronuclear single quantum

Table 1
Content of sugar residues in the BN fraction (% of dry weight), the relative sugar composition of the 0.05 M and the 0.1 M NaOH extracts (rel. %) and within parentheses the proportion of solubilized sugar residues in the two extracts, respectively (% of the sugar residue in BN)

Sugar residue	BN	0.05 M NaOH extract	0.1 M NaOH extract
Arabinose	7.7	23.3 (58.4)	5.3 (40.3)
Xylose	11.2	24.6 (42.3)	10.7 (56.3)
Mannose	0.7	2.7 (76.2)	0.2 (21.5)
Galactose	1.8	8.4 (87.8)	0.3 (11.2)
Glucose	57.9	41.0 (13.7)	83.5 (85.1)
Total	79.3	100	100

coherence-distortionless enhancement by polarization transfer (HSQC-DEPT) used the echo-antiecho sequence and a CNST2 of 145 Hz (Willker, Leibfritz, Kerssebaum & Bermel, 1993).

The anomeric signals in ^{1}H NMR spectra of β-glucan were integrated by lorentzian curve fitting in MATLAB (The Mathworks Inc., Natick, MA, USA). The signals at 4.55 ppm originating from 3G4 and 4G4 were assumed to consist of two overlapped doublets with the same coupling constant as the signal of the anomeric 4G3 at 4.75 ppm. The partly overlapped doublet from xylose at 4.49 ppm was accounted for when present.

2.4. Determination of oligosaccharides after lichenase degradation of the β-glucan

Samples of β-glucan (5 mg) were incubated with lichenase (1U/ml, E.C. 3.2.1.73, Megazyme, Bray, Ireland) for 20 h at 40°C in 1 ml 20 mM sodium phosphate buffer at pH 6.5 (McCleary & Glennie-Holmes, 1985; Wood, Weisz & Blackwell, 1991; Wood et al., 1994). The enzyme was inactivated by treatment for 10 min in a boiling water bath and diluted 2 times. Samples (100 μl) were analyzed by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) on a Dionex DX500 instrument (Sunnyvale, CA, USA), equipped with a Carbopac PA1 column (4 × 250 mm) which was eluted at 1 ml/min with solvent A: 0.15 M NaOH and solvent B: 0.15 M NaOH + 0.5 M NaAc using the following gradient: T = 0 min, A = 90%, B = 10%; linear gradient; T = 30 min, A = 0%, B = 100%. The electrode pulse potentials and durations were as follows: E1 = 0.4 V, 0.5 s; E2 = 0.9 V, 0.8 s; E3 = −0.3 V, 0.49 s. The signals were integrated during 0.2 s (0.3 to 0.5 s) (Saulnier, Gévaudan & Thibault, 1994).

Maltotriose, maltotetraose, maltopentaose, maltohexaose, maltoheptaose, laminaribiose (Sigma, St. Louis, MO, USA) and cellobiose (Kebo Lab, Stockholm, Sweden) were used as standards. A lichenase hydrolysate (10 mg) was fractionated on a Bio-gel P-2 column (94 × 1.6 cm, Bio-Rad Laboratories, Hercules, CA, USA) with 0.02 M sodium phosphate buffer pH 6.5 as an eluent at a flow rate of 0.1 ml/min and 1.5 ml fractions were collected. The fraction with the highest concentration of each oligosaccharide (tri-hexasaccharide) was analyzed by HPAEC-PAD and also for sugar residues. The peak area of the oligosaccharide from HPAEC-PAD divided by its weight concentration gave the response factor.

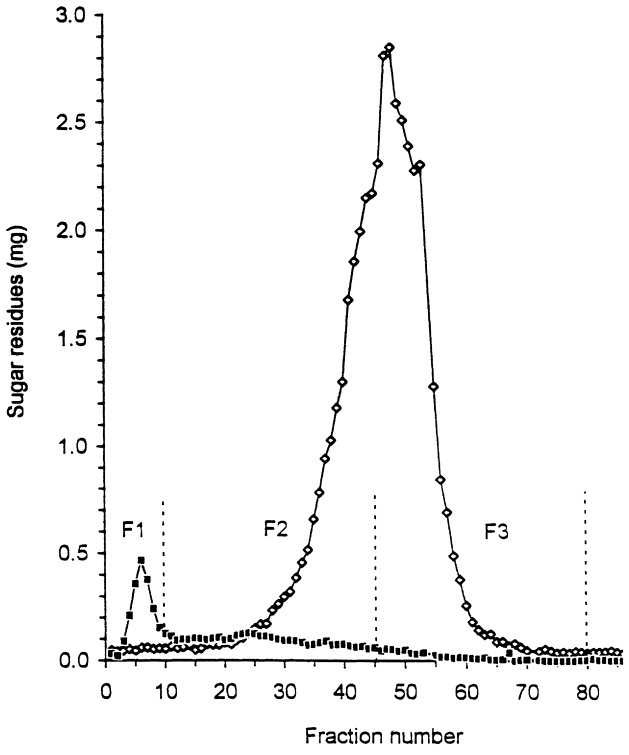

Fig. 1. Fractionation of the 0.1 M NaOH extract of BN on Sephacryl S-500. Three fractions F1-F3 were collected. The distribution of sugar residues of β-glucan (◇) and arabinoxylan (■) is also shown.

3. Results and discussion

3.1. Isolation and purification of β-glucan

A fraction BN extractable with water after sequential extraction with ethanol, water and alkali was obtained from rye bran (Nilsson et al., 1996). BN, which constituted 11.5% of the bran, contained a polysaccharide mixture with arabinoxylan (18.9%) and β-glucan (57.9%) (Table 1). These results were similar to those previously obtained by Nilsson et al. (1996). The possibility of extracting these polysaccharides with water from the pre-extracted bran fraction gave rise to the expectation that water could be used as a solvent for further separation. However, the freeze dried polysaccharide fraction was very difficult to dissolve in water. Therefore, 0.05 M NaOH was used as the extractant and the supernatant, obtained after centrifugation, contained peptides or proteins, detected by ninhydrine, which could be removed by ultrafiltration. The peptides or proteins in the permeate were of lower molecular weight than the filter cut off (10 kD) and tests to render them visible

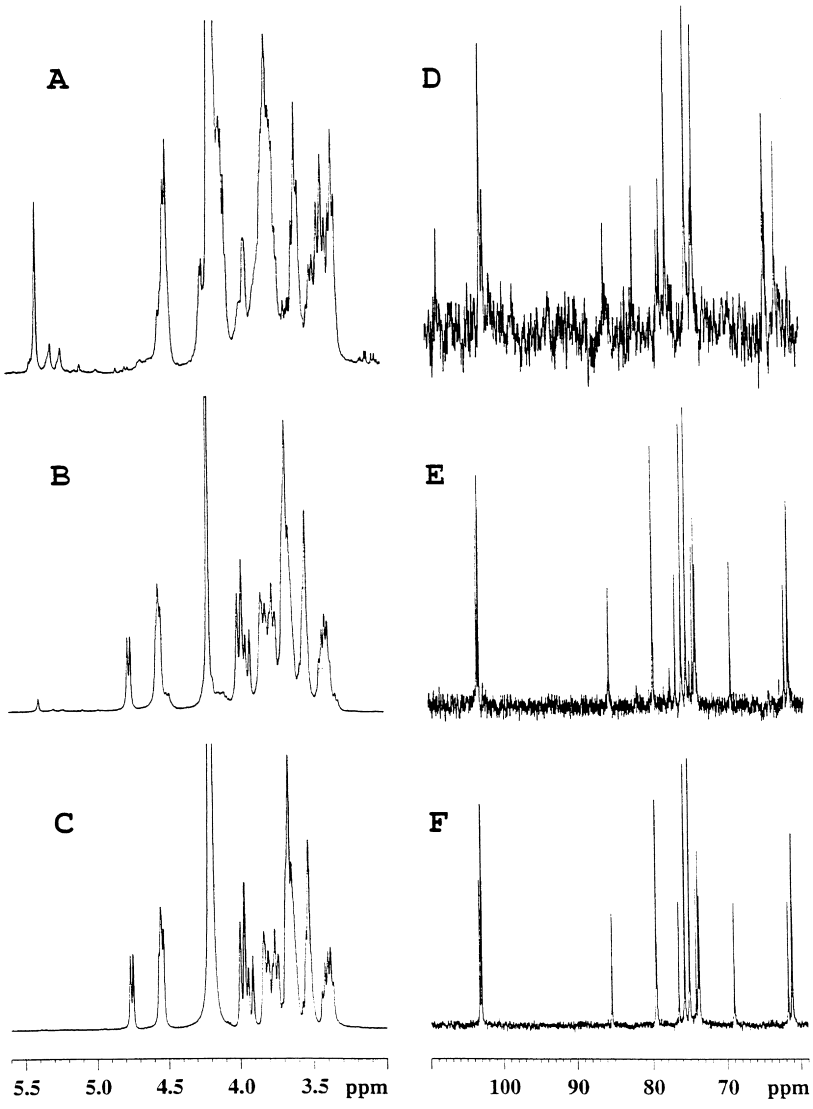

Fig. 2. ^{1}H-spectra of F1 (A), F2 (B) and F3 (C) to the left and ^{13}C-spectra of F1 (D), F2 (E) and F3 (F) to the right.

with SDS-PAGE failed. The ninhydrine test could not detect any peptides or proteins in the concentrate containing the dissolved polysaccharides. 0.05 M NaOH solubilized most of the mannose (76%) and galactose (87%) residues and about half of the arabinoxylan. In comparison, only 14% of the glucose residues were solubilized, while 85% were solubilized in 0.1 M NaOH.

The arabinoxylan with an Ara/Xyl ratio of 0.95 was highly branched and apparently had a higher susceptibility to solubilisation in 0.05 M NaOH than the notably less branched arabinoxylan with Ara/Xyl ratio 0.50 in the 0.1 M NaOH extract. About 7% of the sugar residues in BN remained insoluble even after the extraction with 0.1 M NaOH.

Differences in solubility of β-glucan might arise from changes in flexibility of the linear chain (Buchala & Wilkie, 1971). The low solubility in water might be due to intermolecular associations, whereas in alkali, these associations are avoided. Thus, in this study, the β-glucan was purified with a sequential extraction procedure. 0.05 M NaOH removed most of the mannose and galactose residues and about half of the arabinoxylan, while 0.1 M NaOH solubilized a partially purified β-glucan together with a more sparsely branched arabinoxylan.

3.2. Size-exclusion chromatography

The 0.1 M NaOH extract (Table 1) was applied on a Sephacryl S-500 column and eluted with 0.05 M NaOH. This concentration was used, because 0.1 M might degrade the column material. If water was used, only 12% of the applied material was recovered. The use of 0.05 M NaOH increased the yield to 100%. Three fractions (F1, F2 and F3) were collected and the distribution of the sugar residues over the column was 5.3% in F1, 44.4% in F2 and 50.3% in F3 (Fig. 1). F1 contained a high molecular weight

Table 2
Chemical shifts in ppm (measured at 400 MHz in D_2O at 80°C; internal acetone, δ_H 2.225) and within parentheses $J_{H,H}$ (Hz) of the anomeric protons in F1and F3. The shifts are listed in descending order and the identified sugar residues, mainly from arabinoxylan and β-glucan, are given

Sugar residue	F1	F3
→ 4)-α-Glc*p*(1 → 4)	5.433 (4.16)	–
α-Ara*f* (1 → 3)[a]	5.394	–
α-Ara*f* (1 → 2)[b]	5.288	–
α-Ara*f* (1 → 3)[c]	5.218	–
→ 4)-β-Glc*p*(1 → 3)	4.756 (7.90)	4.757
→ 2,3,4)-β-Xyl*p*(1 → 4)[d]	4.660	–
→ β-Glc*p*[e]	–	4.656
→ 3)-β-Glc*p*(1 → 4)	–	4.555
→ 4)-β-Glc*p*(1 → 4)	–	4.543
→ 4)-β-Xyl*p*(1 → 4)[f]	4.494 (7.09)	–

[a] Terminal arabinofuranose linked to O-3 of monosubstituted Xyl*p*.
[b] Terminal arabinofuranose linked to O-2 of a disubstituted Xyl*p*.
[c] Terminal arabinofuranose linked to O-3 of a disubstituted Xyl*p*.
[d] A disubstituted Xyl*p*.
[e] A reducing end.
[f] An unsubstituted Xyl*p*.

arabinoxylan with an Ara/Xyl ratio of 0.52 and the one in F2, with lower molecular weight, had a similar ratio (0.49). β-Glucan was mainly recovered in F2 and F3, which contained 88 and 98% of β-glucan as total sugar residues, respectively.

3.3. NMR spectroscopy

Fractions F1, F2 and F3 were analyzed by ^1H and ^{13}C NMR spectroscopy. F1 (Fig. 2a and d) contained essentially arabinoxylan of high molecular weight, F2 (Fig. 2b and e) mainly β-glucan contaminated with an arabinoxylan and F3 (Fig. 2c and f) a very pure β-glucan. The anomeric ^1H and ^{13}C signals were assigned to the different sugar residues (Tables 2 and 3).

Ara*f* (1 → 3) on the monosubstituted Xyl*p*, Ara*f* (1 → 2) and Ara*f* (1 → 3) on the disubstituted Xyl*p* were clearly distinguishable in the ^1H-spectrum of F1 (Fig. 2a, Table 2). The chemical shifts were in close agreement with the published data from Gruppen, Hoffmann, Kormelink, Voragen, Kamerling and Vliegenthart (1992); Hoffmann, Geijtenbeek, Kamerling & Vliegenthart (1992) even though these data refer to low molecular weight oligosaccharides.

The doublet downfield of the Ara*f* (1 → 3) signal at 5.433 ppm ($J_{H,H} = 4.2$ Hz) is not a signal derived from arabinose, because DQF-COSY (spectrum not shown) show crosspeaks to the H-2 signals between 3.5–3.6 ppm whereas the H-2 of arabinose would be between 4.1 and 4.3. This anomeric signal is probably derived from traces of starch. It should be noted that a monosubstituted Xyl*p* residue adjacent to a disubstituted Xyl*p* residue as suggested by Cleemput, Roels, Van Oort, Grobet & Delcour (1993) was probably not present, because the shoulder downfield of the signal of the Ara*f* (1 → 3) on the monosubstituted Xyl*p* was absent.

The amount of mono- di- and unsubstituted Xyl*p* can be calculated from the integrals of the Ara*f* signals and the sugar residue concentration of each fraction. The amount of monosubstituted (mXyl = 34.6%) exceeded the amount of disubstituted (dXyl = 8.7%) and the amount of unsubstituted xylose (uXyl) was 56.7%.

The absence of notable shoulders in the disubstituted signals in the ^1H-spectrum (Fig. 2a) indicated that two disubstituted Xyl*p* residues adjacent to each other were not very prevalent. This, and the low amount of disubstituted Xyl*p* residues, indicates that they were not present as blocks.

The relatively high signals of the unsubstituted *Xylp* residue in the ^{13}C-spectrum (Fig. 2d) indicate that longer sequences of unsubstituted Xyl*p* residues might be present. The spectrum was almost identical to the spectrum reported by Bengtsson and Åman (1990). However, some of their assignments were corrected after 2D-NMR experiments conducted by Nilsson, Andersson, Andersson, Autio and Åman (1999). Due to the low amounts of the disubstituted Xyl*p* residues, these signals were not visible with ^{13}C NMR (Table 3), while the monosubstituted and unsubstituted signals could be assigned easily by comparison to published

Table 3
Chemical shifts in ppm (measured at 100 MHz in D_2O at 80°C; internal acetone, δ_C 31.00) of the ^{13}C resonances of the sugar residues from arabinoxylan (F1) and β-glucan (F3)

Sugar residue	C1	C2	C3	C4	C5	C6
α-Ara*f* (1 → 3)[a]	108.377	81.673	78.040	85.500	62.282	
→ 3,4)-β-Xyl*p*-(1 → 4)[b]	101.999	73.700	78.366	74.488	63.530	
→ 4)-β-Xyl*p*-(1 → 4)[c]	102.414	73.501	74.554	77.225	63.802	
→ 4)-β-Glc*p*(1 → 3)	103.310	74.158	79.029	75.529	75.693	61.070[d]
→ 3)-β-Glc*p*(1 → 4)	103.024	73.695	85.343	68.998	76.484	61.625
→ 4)-β-Glc*p*(1 → 4)	103.066	73.866	79.029[e]	75.529[e]	75.693	61.070[d]

[a] Terminal arabinofuranose linked to O-3 of monosubstituted Xyl*p*.
[b] The monosubstituted Xyl*p*.
[c] An unsubstituted Xyl*p*.
[d] Another signal was observed at 61.112 ppm. Whether this signal was derived from → 4)-β-Glc*p*(1 → 3) or → 4)-β-Glc*p*(1 → 4) could not be determined.
[e] A signal at 79.420 ppm was observed, which could be derived from another → 4)-β-Glc*p*(1 → 4) with a different chemical environment.

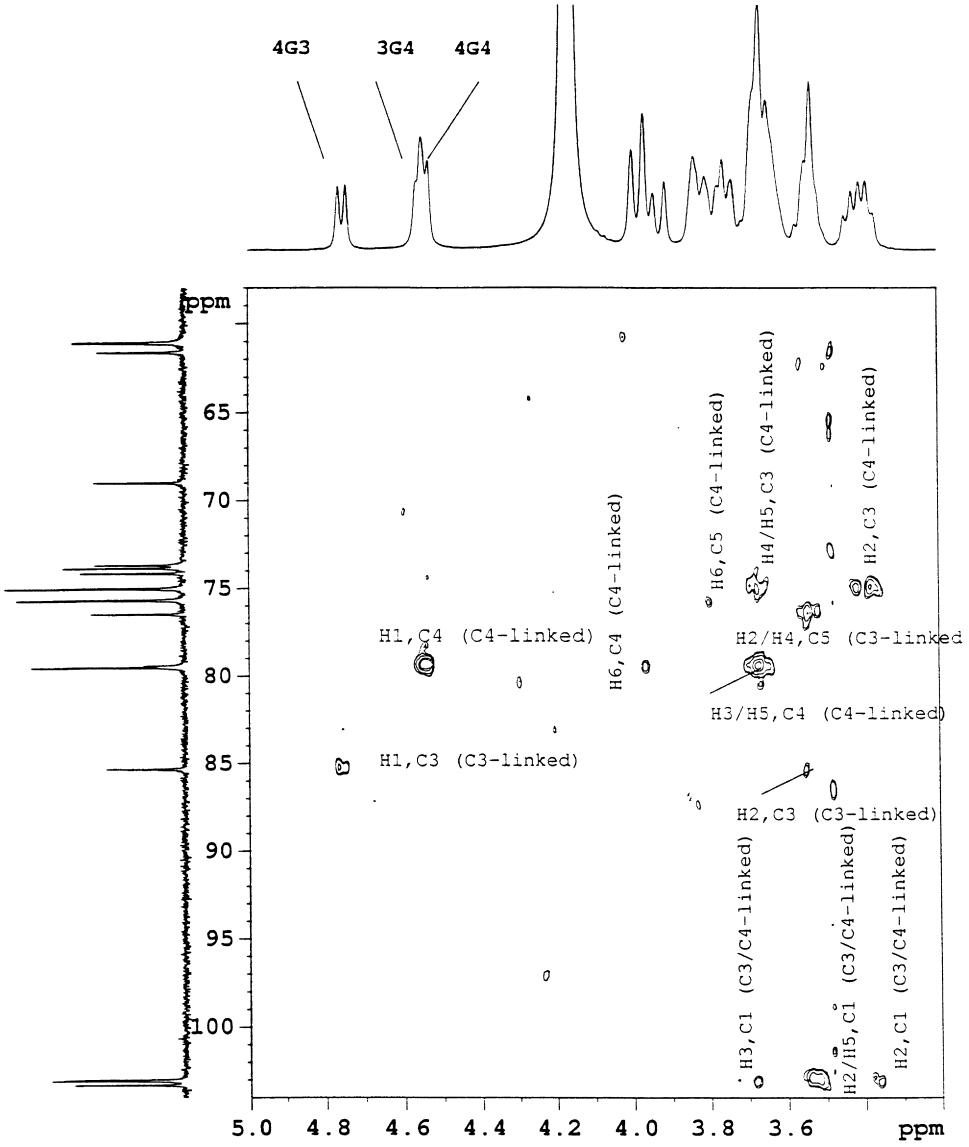

Fig. 3. HMBC of isolated β-glucan. Long-range coupling $^{2}J_{C,H}$ and $^{3}J_{C,H}$ is shown as crosspeaks and denoted as such. C–H coupling in anomeric region is indecisive whether Glc*p* residue is C3 or C4 substituted.

data (Bengtsson & Åman, 1990; Hoffmann, Roza, Maat, Kamerling & Vliegenthart, 1991). Besides the presence of the arabinoxylan reported by Nilsson et al. (1996), a structurally different arabinoxylan, low in substitution, appears to be present in the BN fraction of rye bran. F3 (Fig. 2c and f) showed a pure β-glucan spectrum with 16 different ^{13}C-signals (Table 3). Barley β-glucan with 15 ^{13}C-signals (Bock, Duus, Norman & Pedersen, 1991) and oat β-glucan with 18 ^{13}C-signals (Dais & Perlin, 1982) have previously been reported. These authors however, do not report the molecular size of their investigated β-glucans. Therefore differences in resolution are difficult to explain. However, the use of DMSO-d_6 at elevated temperatures instead of D_2O is known to give better resolution (Dais & Perlin, 1982). Still, it is difficult to compare two β-glucans from different sources even though the spectra are probably very similar.

The C1 of the residue 4G3 is mainly influenced from the proton on C3 from the adjacent residue and therefore has its chemical shift more downfield than the C1 from the other two residues 4G4 and 3G4. The relatively sharp singlets of the 3-O-substituted residues might support the evidence for the single occurrence of the (1 → 3)-linkage (Dais & Perlin, 1982; Parrish et al., 1960). The purity of F3 allowed us to obtain rather legible 2D-NMR spectra. To our knowledge, no 2D-spectra on rye β-glucan have been published before. HMBC (Fig. 3) shows the long-range coupling $^{2}J_{C,H}$ and $^{3}J_{C,H}$ and most of the crosspeaks could be assigned. However, due to limited resolution in the COSY spectrum, mainly for H3 and H4, it was not possible to assign all crosspeaks. On the other hand, the signals obtained by HSQC-DEPT (Fig. 4), which shows the direct coupling $^{1}J_{C,H}$ could all be assigned. DEPT sequence was used to

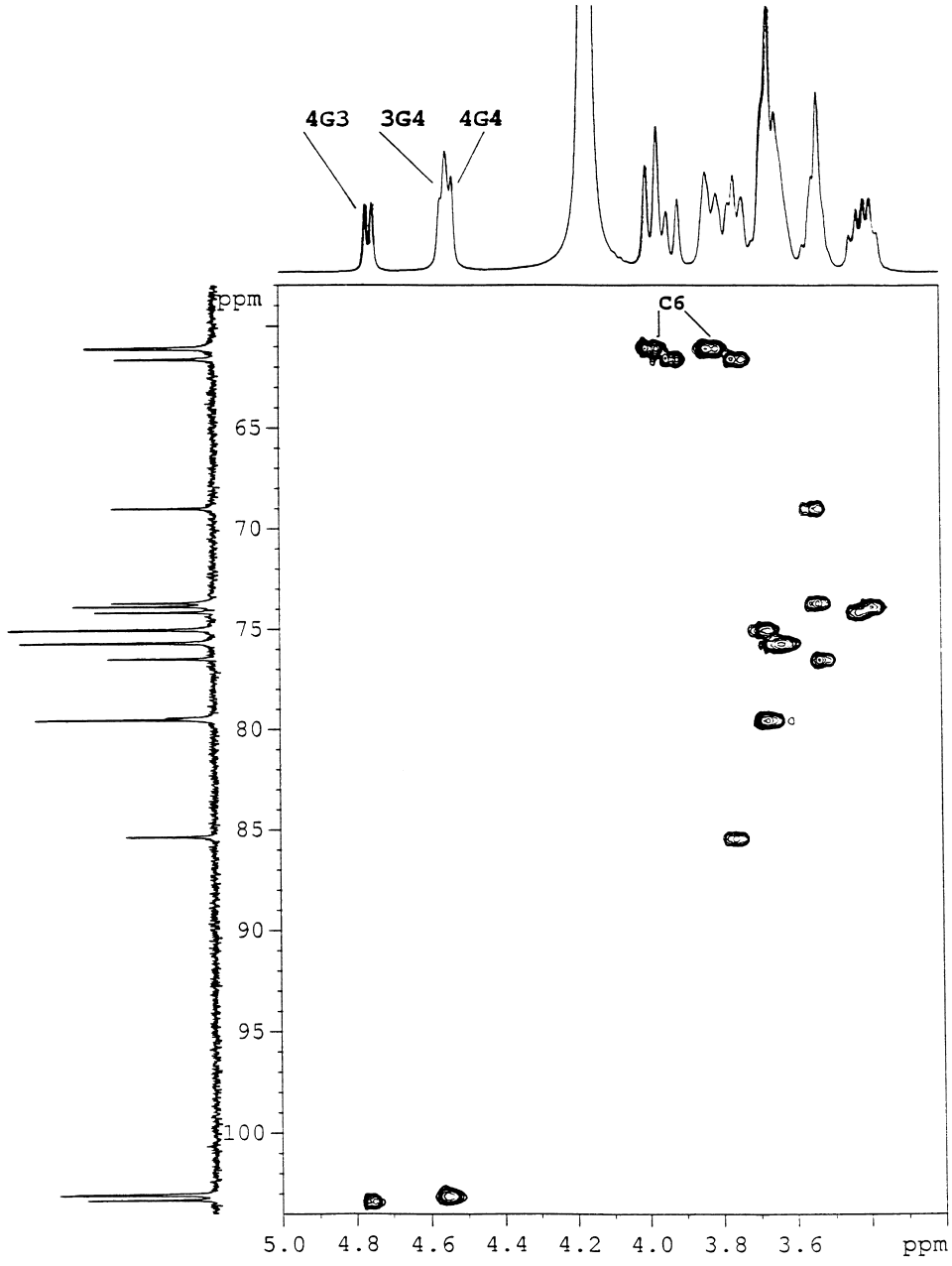

Fig. 4. HSQC-DEPT of isolated β-glucan. Direct coupling $^{1}J_{C,H}$ is shown as crosspeaks. $-CH_2$ signals are denoted as C6.

differentiate between negative $-CH_2$ and positive $-CH/-CH_3$, respectively, as indicated in the figure. COSY and TOCSY were used to assign the ^{13}C-signals.

The ratio of $(1\rightarrow4)$ to $(1\rightarrow3)$-linkages could be determined by integration of the anomeric signals of 3G4, 4G4 and 4G3 in the NMR spectra. For F3 the ratio was found to be 2.31. A complication in calculating the ratio for F2 was the interference of the xylose doublet with the 3G4 and 4G4 signals. Integrals were therefore calculated from curve fitted theoretical peaks in order to minimize this interference and the ratio was found to be 2.26. This showed that the ratio was similar in these two, β-glucan containing, fractions. However, the calculated area for 4G3 was about 6% lower than the area for 3G4, both in F2 and F3. These areas should be similar, because the amounts of 4G3 and 3G4 are theoretically equal. The anomeric signals in F3 were more narrow than in F2, indicating a slower relaxation rate, probably because of lower molecular weight as confirmed by SEC-LALLS. The $\bar{M}_w$ for F2 was 64,000 with a $\bar{M}_w/\bar{M}_n$ of 1.59 and the $\bar{M}_w$ for F3 was 21,000. The $\bar{M}_n$ showed a large variation between replicates of F3, due to unreliable data in the lower molecular weight ranges.

The proportion of $(1\rightarrow4)$-linkages in cereal β-glucan exceeds that of $(1\rightarrow3)$-linkages by at least a factor of two (Parrish et al., 1960). If the $(1\rightarrow3)$-linked cellotriosyl and cellotetraosyl units are present in equal amounts, the ratio is

2.5 and the presence of longer sequences of consecutive (1 → 4)-linkages will increase this ratio. The ratio of (1 → 4) to (1 → 3)-linkages in barley and oats, estimated from the anomeric ^{13}C-signals, was 2.3 (Dais & Perlin, 1982). It should be noted that these samples concern total β-glucan from the grain while the highly purified samples in the present investigation were isolated from rye bran.

3.4. Determination of cellotriosyl and cellotetraosyl units

Purified β-glucan was hydrolyzed with lichenase and a tri- and tetrasaccharide, corresponding to cellotriosyl and cellotetraosyl units in the intact β-glucan, were isolated after fractionation on Biogel P-2 and analyzed on HPAEC-PAD. Response factors were calculated relative to cellobiose. The trisaccharide had a higher relative response factor (0.66) than the tetrasaccharide (0.45), showing that the HPAEC-PAD was more sensitive for the trisaccharide. Decreasing response factors with increasing degree of polymerisation have previously also been shown for fructo- and malto-oligosaccharides (Koch, Andersson & Åman, 1998; Timmermans, van Leeuwen, Tournois, de Wit & Vliegenthart, 1994).

Chromatograms from HPAEC-PAD show two main peaks being the trisaccharide and tetrasaccharide. Other oligosaccharides of higher DP (5–9) were also found in small amounts, but these could not be quantified. The weight ratio of cellotriosyl to cellotetraosyl was 1.75 for F2 and 1.47 for F3. The corresponding molar ratios were 2.31 and 1.94, respectively. Wood et al. (1991, 1994) did not obtain any difference between response factors and reported molar ratios of 2.73 and 3.1 depending on variety, or expressed as weight ratios 2.07 and 2.35, respectively. When our response factors are applied to these values, the molar ratios become 1.86 and 2.11. Assuming that the whole β-glucan molecule consists of cellotriosyl and cellotetraosyl units, it is possible to determine the ratio of (1 → 4) to (1 → 3)-linkages, which in our case would be 2.34. This assumption applied to the data from Wood et al. (1991,1994) gives ratios of 2.35 and 2.32. This clearly indicates that results from ^{1}H NMR and HPAEC-PAD are comparable for determination of the ratio of cellotriosyl to cellotetraosyl and the ratio of (1 → 4) to (1 → 3)-linkages.

4. Conclusions

A homogeneous, low molecular weight ($\bar{M}_w$ 21,000) β-glucan and a high molecular weight arabinoxylan were isolated and characterized from a water-unextractable fraction of rye bran. Besides these, another arabinoxylan with a high degree of substitution was detected in a 0.05 M NaOH extract while another arabinoxylan and the major part of β-glucan remained insoluble. The isolated arabinoxylan had a higher proportion of unsubstituted Xyl*p* residues than previously reported from rye bran. The β-glucan showed strong similarities to β-glucans from oats and barley, although the ratio of cellotriosyl to cellotetraosyl units as determined with HPAEC-PAD was lower. The ratio of (1 → 4) to (1 → 3)-linkages was 2.34, when the assumption was made that the whole β-glucan molecule consists of cellotriosyl and cellotetraosyl units. This value was very similar to that obtained from ^{1}H NMR, showing that these methods were comparable when our response factors were used. It should be noted that the structure of the studied β-glucan does not necessarily represent the structure of the total β-glucan in rye since it constitutes a fraction with specific extractability properties.

Acknowledgements

The authors wish to express their gratitude for the skillful technical assistance of Ann-Sissel Ulset of the Norwegian Biopolymer Laboratory, NTNU, Trondheim, Norway for running the SEC-LALLS experiments and Rolf Andersson of the Department of Chemistry at the SLU, Uppsala, Sweden for his kind help and fruitful discussions on the interpretation of the NMR spectra.

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