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# Periodate Oxidation and Degradation Studies on the Major Water-soluble Arabinoxylan in Rye Grain

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*ABSTRACT*

*The major water-soluble arabinoxylan from rye grain has previously been shown to contain a main chain of 4-linked β-D-xylopyranosyl residues in which, on average, every second is substituted at position 3 with terminal α-L-arabinofuranosyl residues. Periodate oxidation, reduction and fragmentation by mild acid hydrolysis produced a series of glycerol xylosides containing 4-linked xylopyranosyl residues linked at the reducing end to position 2 of glycerol. It was shown that a one-step periodate oxidation was incomplete due to the formation of relatively stable hemiacetal linkages. A sequential oxidation and reduction procedure was used to bring about complete oxidation of arabinose and unbranched xylose residues in the intact polysaccharide. Quantitative analysis of the products liberated by mild acid hydrolysis revealed the presence of glycerol xylosides with one, two or three xylose residues in the molar ratio of 1·00:0·86:0·02. The xylose residues must have originated from branched residues in the main chain of the arabinoxylan. The units or small blocks of two residues are therefore distributed mainly as isolated branched residues and not randomly as previously reported.*

## INTRODUCTION

Rye grain contains a high proportion of water-soluble polysaccharides among which arabinoxylans predominate (Podrazký, 1964). The major fraction contains a main chain of 4-linked β-D-xylopyranosyl residues in which, on average, every second unit is substituted at position 3 with a terminal α-L-arabinofuranosyl residue (Aspinall & Sturgeon, 1957; Bengtsson & Åman, 1990). A small proportion of the xylose units are



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doubly substituted at positions 2 and 3, also by terminal α-L-arabinofuranosyl residues.

Xylopyranosyl residues branched at position 3 are stable to periodate oxidation. Reduction of rye flour arabinoxylan after a single treatment with periodate, followed by mild acid hydrolysis afforded glycerol xylosides with one (**1**), two (**2**) or three (**3**) xylopyranosyl residues (see Fig. 1) in the molar ratio of 7·5:2·2:1 (Aspinall & Ross, 1963). No higher homologues were identified and it was concluded that the arabinofuranosyl side chains were attached to isolated and, less frequently, to two and three, but not more, continuous xylopyranosyl residues. The molar ratio in which the three glycosides were formed was considered to be consistent with a fairly random distribution of side chains along the polysaccharide chain.

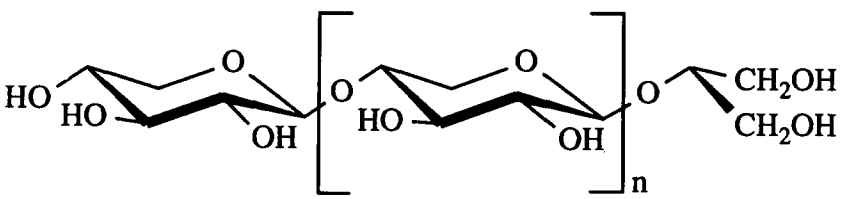


**Fig. 1.** Glycerolxylosides (**1–8**) isolated after periodate oxidation, reduction and mild acid hydrolysis of the water-soluble arabinoxylan. **1–8** correspond to $n = 0–7$.

Later research, however, showed that during oxidation of 4-linked β-D-xylan the second-order rate coefficient rapidly decreased, and became constant at about 2% of the initial value after consumption of 0·6 mole of periodate per anhydropentose unit (Painter & Larsen, 1970; Ishak & Painter, 1971). It was concluded that deviation from the initial rate-constant was caused by the formation of relatively stable hemiacetal rings between aldehyde groups in oxidised residues and hydroxyl groups in adjacent, but not yet oxidised, residues. These hemiacetal linkages are cleaved by reduction with sodium borohydride, and the protected but formerly vulnerable diol groups are re-exposed for oxidation. For xylans it was reported that the oxidation–reduction sequence has to be repeated twice before full oxidation is achieved.

In the present paper the major fraction of water-soluble arabinoxylans in rye grain was oxidised with periodate and reduced in sequence and thereafter fragmented by mild acid hydrolysis. Glycerol xylosides were obtained, identified and quantified and the results were interpreted as evidence for the distribution of side chains in the polysaccharide.

## MATERIALS AND METHODS

### General methods

Concentrations were carried out in a vacuum centrifuge or by rotary evaporation at a temperature below 40°C. All analyses were carried out in duplicate.

Isolated samples (1 mg) were hydrolysed in 1 M trifluoroacetic acid (121°C, 90 min). The formed sugars were reduced, acetylated and analysed by GLC on a CP-Sil 88 capillary column (Theander & Westerlund, 1986).

Isolated samples (1 mg) were dried in vacuum, methylated by a modified Hakamori method, hydrolysed with trifluoroacetic acid, reduced and acetylated (Harris *et al.*, 1984). The identities of the *O*-acetylated and *O*-methylated alditols were established by their GLC-retention times on a DB-1 capillary column (Oakley *et al.*, 1985) and by their e.i. mass spectra (Björndal *et al.*, 1970) using a Finnegan 4021 mass spectrometer at 70 eV and an Incos 2000 data system.

$^{1}$H-NMR spectra (400 MHz) were recorded at 85°C on a Varian VXR-400 instrument. About 2000 pulses were collected, pulse repetition time was 3·68 s and r.f. pulse angle 45°. The dried carbohydrate-containing fractions (0·5 mg) were dissolved in $D_2O$ (0·7 ml) and sodium 3-trimethylsilyltetradeuteriopropionate was used as reference. The shoulders seen in Figs 3 and 5 are artefacts due to the NMR-technique used. $^{13}$C-NMR spectra (101 MHz) were run at room temperature on the same instrument. About 50 000 pulses were collected, pulse repetition time was 1·5 s and r.f. pulse angle 45°. The dried carbohydrate-containing fraction (1 mg) was dissolved in $D_2O$ (0·7 ml) and sodium 3-trimethylsilyltetradeuteriopropionate used as reference.

### Isolation of arabinoxylan

The main water-soluble arabinoxylan in rye grain (cv. Kungs II) was isolated as described previously (Bengtsson & Åman, 1990). The method includes reflux in 90% aqueous ethanol, extraction of water-soluble components from the residue at 40°C and isolation of crude arabinoxylans by precipitations with ammonium sulfate and 67% aqueous ethanol. The crude arabinoxylan was fractionated on DEAE-cellulose and the main fraction, eluted with water and constituting about 0·3% of dry grain, contained arabinose and xylose residues in a ratio of about 1 : 2·1, together with only traces of other components.

**Periodate oxidation**

Arabinoxylan (50 mg) was dissolved in 50 ml 0·02 M sodium metaperiodate (Chaplin & Kennedy, 1986). The reaction flask was covered with aluminium foil and left in a refrigerator at 4°C for 18 h. At the end of this time, excess of periodate was destroyed by the addition of 2 ml ethylene glycol. The reaction mixture was dialysed against distilled water for 48 h and thereafter concentrated to about 10 ml. An aliquot of the oxidised material was withdrawn for sugar and methylation analysis. The rest of the material was reduced with sodium borohydride (5 mg) and the mixture left for 24 h at room temperature. The sample was then dialysed against distilled water for 24 h and freeze-dried.

In another experiment arabinoxylan (50 mg) was dissolved in 50 ml 0·025 M sodium metaperiodate and the covered reaction flask left at room temperature for 70 h (Painter & Larsen, 1970). At the end of this time excess periodate was destroyed by the addition of 2 ml ethylene glycol. The reaction mixture was dialysed against distilled water for 48 h, concentrated to about 10 ml, reduced with sodium borohydride (5 mg), dialysed for 24 h against distilled water and concentrated to dryness. The oxidation and reduction procedures were repeated twice, but the last time the oxidation time was only 24 h. After the last oxidation an aliquot of the unreduced material was removed for sugar and methylation analysis.

The oxidised and reduced material from the two experiments was dissolved in 1 ml 0·02 M HCl and partially hydrolysed in a boiling water-bath for 20 min.

**Fractionation on Biogel P-2**

The hydrolysate was applied on a Biogel P-2 column (2 × 90 cm) and eluted with water (0·25 ml $min^{-1}$) in a Pharmacia FPLC system. The refractive index was registered continuously and fractions of 2 ml were collected. The concentration of xylose residues in the fractions was determined by the phenol–sulphuric acid assay (Dubois *et al.*, 1956).

## RESULTS AND DISCUSSION

Water-soluble arabinoxylan from rye grain was oxidised with 0·02 M sodium metaperiodate in one step. During this treatment all arabinose residues in the polysaccharide were degraded since xylose was the only identified component in the sugar analysis. Methylation analysis of the

oxidised product led to the recovery of almost all of the periodate-resistant xylose residues as unmethylated xylose; only traces of terminal xylose residues were methylated. Since 2-*O*-methyl-D-xylose, originating from branched residues, was the only component expected in the analysis if all unbranched residues were oxidised, the result clearly demonstrated the formation of hemiacetal linkages between the aldehyde groups of oxidised residues and hydroxyl groups in stable or not yet oxidised residues (Painter & Larsen, 1970; Ishak & Painter, 1971). The oxidised material was reduced, partially hydrolysed and fractionated on a Biogel P-2 column. Seven fractions (I–VII) were isolated (Fig. 2) of which I and II were identified as mixtures of low-molecular-weight components and not further studied. Sugar analysis of fractions III–VII showed that all contained xylose and glycerol residues. The $^{1}$H-NMR spectrum of fraction III (Fig. 3) revealed a doublet at 4·53 ppm, identified as the anomeric proton of the xylose residue in **1** (see Fig. 1). Resonances of the protons at C-1 and C-3 of the glycerol residue of **1** were identified as multiplets between 3·5 and 3·8 ppm and of the proton at C-2 as a quintet at 3·87 ppm. The $^{13}$C-NMR spectrum of **1** showed eight resonances of which those at 103·2, 73·5, 76·2, 69·8 and 65·6 ppm were identified as C-1–C-5 of the xylopyranosyl residue and those at 61·3, 81·0 and 61·8 ppm as C-1–C-3 of the glycerol residue (Reicher *et al.*, 1989). The identification of the two primary carbons of the glycerol residue may, however, be reversed. The $^{1}$H-NMR spectrum of fraction IV revealed doublets at 4·46 and 4·56 ppm in equal amounts

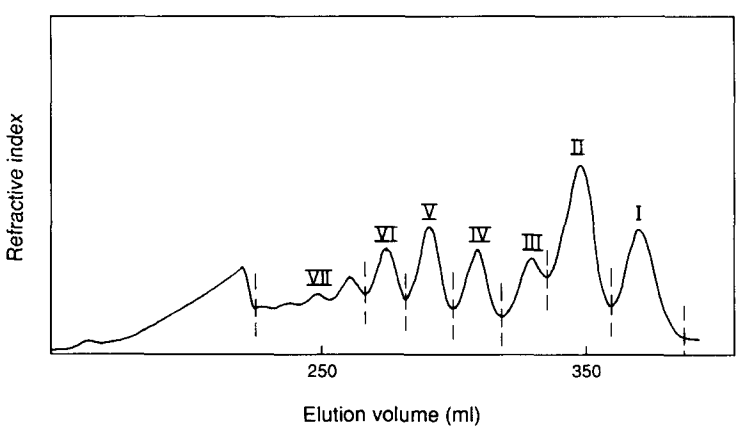


**Fig. 2.** Gel-permeation chromatography on Biogel P-2 of glycerolxylosides isolated after periodate oxidation, reduction and mild acid hydrolysis of the water-soluble arabinoxylan. The fractionation was followed continuously by refractive index and seven fractions (I–VII) were collected.

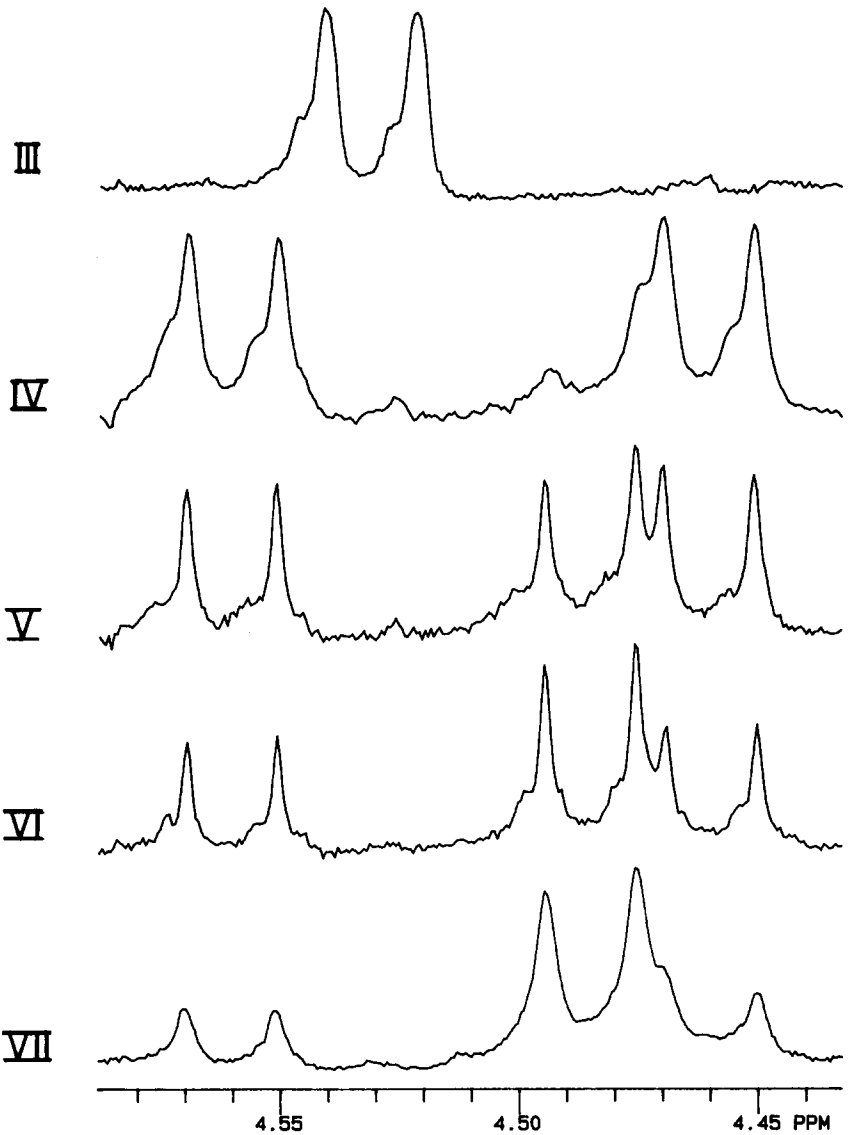


**Fig. 3.** $^1$H-NMR spectra of the anomeric region of isolated fractions (III–VII) of glycerolxylosides (see Fig. 2) obtained on gel filtration.

(Fig. 3). The first doublet was identified as the anomeric proton of the non-reducing end and the second as the other anomeric proton of **2** (Bengtsson *et al.*, 1991). In fraction V, a doublet at 4·49 ppm of similar intensity to the other anomeric protons in the spectrum was seen. This signal was identified as the anomeric proton of the central xylose

residue of **3**. In fraction VI the intensity of the signal at 4·49 ppm was twice that of the other anomeric protons, showing that the anomeric protons of the two interjacent xylose residues of **4** had the same chemical shift. In fraction VII, which was identified as a mixture of **5**–**8**, the doublet at 4·49 ppm was, as expected, even more intense compared to the other anomeric protons of the spectrum. From these results it is evident that information on the chain length of the glycerol xylosides can easily be calculated from the intensity of the anomeric protons in the $^1$H-NMR spectra.

Sugar analysis of the in-sequence oxidised arabinoxylan showed that xylose was the only sugar residue present and methylation analysis gave 2-*O*-methyl-D-xylose as the main fragment with less than 10% of unmethylated xylose. The results clearly demonstrate that the hemiacetal linkages formed between aldehyde groups in oxidised residues and hydroxyl groups in intact residues were reduced to a small proportion by the sequential oxidation and reduction procedure (Painter & Larsen, 1970). The low content of 2,3,4-linked xylose residues may originate from under-methylation or the small proportion of doubly branched xylose residues present in the polymer (Bengtsson & Åman, 1990).

The in-sequence oxidised and reduced arabinoxylan was fragmented by mild acid hydrolysis and fractionated on the Biogel P-2 column. Refractive index detected five peaks which were individually pooled and concentrated to dryness (fractions VIII–XII). Sugar and $^1$H-NMR analysis revealed that fraction VIII contained small amounts of free xylose together with other components and fraction IX no xylose residues. Fraction X–XII (Fig. 4), on the other hand, all contained xylose and glycerol residues and were identified, as discussed previously, as **1**–**3**.

The phenol–sulphuric acid colorimetric assay was used to determine the xylose content in the fractions (Fig. 4) and quantification of the peaks revealed that the glycerol xyloside (**1**) and the two higher homologues (**2** and **3**) were present in a molar ratio of 1·0; 0·86; 0·02.

$^1$H-NMR analysis of the in-sequence oxidised, reduced and partially hydrolysed material showed three main doublets of similar intensity in the anomeric region (Fig. 5). By comparison with the chemical shifts in Fig. 3, the signals were identified as resonances from the anomeric protons of **1** (4·53 ppm) and **2** (4·56 ppm and 4·46 ppm). Since the anomeric protons of non-terminal xylopyranose residues (4·49 ppm) were present in only minor amounts, higher homologues of **1** could only be present in very small amounts. The molar ratios of **1**–**3**, as calculated from integration of the anomeric resonances, were in excellent

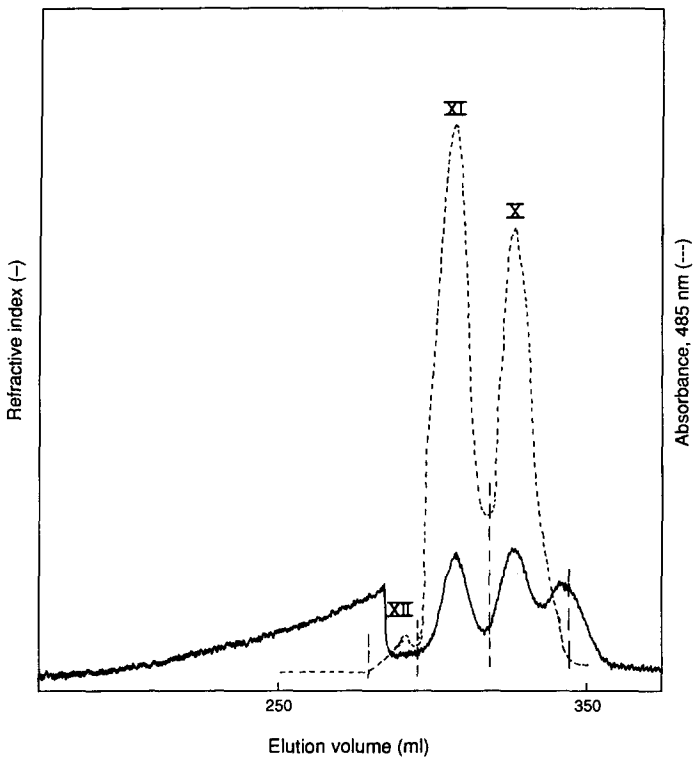


**Fig. 4.** Gel filtration of in-sequence oxidised and reduced and thereafter weak-acid-hydrolysed arabinoxylan on a Biogel P-2 column. Fractions VIII and IX are not seen since they were removed by a pre-fractionation. The fractionation was followed continuously by refractive index (——) and collected fractions (2 ml), were analysed for carbohydrates by the phenol–sulphuric acid method (– – –).

agreement with those obtained by the colorimetric quantification of the individual components.

In a previous study, the three glycerol xylosides (**1**–**3**) were also identified after periodate oxidation, reduction and partial acid hydrolysis of water-soluble rye arabinoxylan (Aspinall & Ross, 1963). The molecular ratio of the formed glycerol xylosides differed greatly, however, from that obtained in the present study. This was expected since a one-step oxidation procedure was used. The results from the present study show that the branched xylose residues are present predominantly as isolated units (36%) or small blocks of two residues (62%) and not randomly as previously reported.

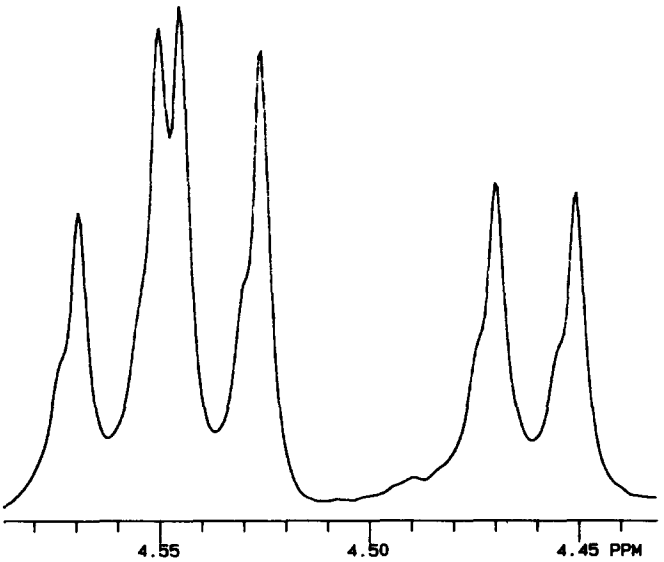


**Fig. 5.** $^1$H-NMR spectra of the anomeric region of the mixture of glycerolxylosides (see Fig. 2) obtained from water-soluble arabinoxylan after a sequential oxidation and reduction procedure followed by mild acid hydrolysis.

## ACKNOWLEDGEMENTS

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