Carbohydrate Research, 163 (1987) 73–79
Elsevier Science Publishers B.V., Amsterdam - Printed in The Netherlands

STRUCTURAL FEATURES OF A RYE-BRAN ARABINOXYLAN WITH A LOW DEGREE OF BRANCHING

ZDENKA HROMÁDKOVÁ, ANNA EBRINGEROVÁ, EVA PETRÁKOVÁ
Institute of Chemistry, Slovak Academy of Sciences, 842 38 Bratislava (Czechoslovakia)
AND JAN SCHRAML
Institute of Chemical Process Fundamentals, Czechoslovak Academy of Sciences, 165 02 Prague (Czechoslovakia)

(Received June 26th, 1986; accepted for publication, December 24th, 1986)

ABSTRACT

Two fractions (AX-I and AX-II) of an L-arabino-D-xylan with a low degree of branching were isolated from rye bran by different extraction procedures with and without a chlorite-delignification step. The yields and compositions of AX-I and AX-II were similar, but the d.p. of AX-II was ~100 units higher. AX-I was shown by partial hydrolysis, methylation analysis, and ^{13}C-n.m.r. spectroscopy to contain a backbone of (1→4)-linked β-D-xylopyranosyl residues substituted with single α-L-arabinofuranosyl groups at position 3 of every sixth or seventh D-xylosyl residue.

INTRODUCTION

Rye bran is important as a source of cereal "dietary fibre"[1–4]. It contains a large proportion of cell-wall material which is rich in hemicelluloses[5]. The influence of pentosans on the milling behaviour of grain[6], the quality of flour[7], and the rheological properties of doughs from rye and other cereal flours has been reported[8,9]. A severe effect of rye grain in feeding chicks on nutrient adsorption in general, and fat in particular, has been attributed to the hemicelluloses present[10]. It is therefore important to obtain more information about the structure and properties of rye-bran hemicelluloses. In comparison to rye-endosperm hemicelluloses, which were investigated by Aspinall *et al.*[11,12], there are few data about the non-starchy polysaccharides of rye bran. Theander and Åman[13] isolated water-soluble and water-insoluble polysaccharides from rye bran and studied their chemical properties. The fractionation and analysis of rye-bran hemicelluloses has been presented[14] and we now report on the structural features of an arabinoxylan with a low degree of branching isolated from the native and chlorite-delignified bran.

RESULTS AND DISCUSSION

Arabinoxylan AX-I. — The arabinoxylan (AX-I) with a low degree of bran-

0008-6215/87/$ 03.50

ching was isolated by extraction of the chlorite holocellulose prepared from de-lipidated, de-starched, and de-pectinated rye bran with aqueous 4.5% sodium hydroxide and precipitation from the alkaline extract by acidification[14]. After repeated precipitation of the polysaccharide from a solution in aqueous 4.5% sodium hydroxide by acidification to pH 5 followed by dialysis, a polysaccharide which appeared homogeneous in free-boundary electrophoresis was obtained. The polysaccharide was water-insoluble and contained D-xylose and L-arabinose in the molar ratio 7.1:1 together with small proportions of D-glucose and uronic acid (Table I). The uronic acid was mainly 4-*O*-methyl-D-glucuronic acid, detected by p.c. (solvent *B*) mainly as 2-*O*-(4-*O*-methyl-α-D-glucopyranosyluronic acid)-D-xylose. The viscosity $[\eta]$ of AX-I in methyl sulphoxide was 2.65 dL/g which, according to the equation[15] $[\eta] = 5.9 \times 10^{-3} \times \text{d.p.}_w^{0.94}$, corresponds to a mol.wt. of ~88,000 or a d.p. of 663.

Partial acid hydrolysis of AX-I yielded arabinose, xylose, and a mixture of (1→4)-linked β-xylo-oligosaccharides, which were identified on the basis of the relationship between d.p. and mobilities in p.c. using authentic xylo-oligosaccharides[16] as standards. As expected, arabinosyl-containing oligosaccharides could not be detected after mild acid treatment of AX-I, indicating that the arabinofuranosidic linkages had been hydrolysed.

AX-I was methylated by a modification of the method of Ciucanu and Kerek[17]. The product (88% yield), which had no i.r. absorption for hydroxyl, was hydrolysed and the products were converted into the partially methylated alditol acetates and analysed by g.l.c. and g.l.c.–m.s. The results are shown in Table II. The molar ratios of 2,3,5-tri-*O*-methylarabinose, 2,3,4-tri-*O*-methylxylose, 2,3-di-*O*-methylxylose, and 2(3)-*O*-methylxylose were 13.0:0.3:100:13.5. The arabinose was present in the furanose form as a terminal unit only, in agreement with its easy cleavage during mild acid treatment, observed also by other authors[18,19]. As the 2-*O*- and 3-*O*-methylxylose derivatives have the same retention time on SP-2340, they could not be identified by g.l.c. However, the mass spectra of the borodeuteride-reduced and acetylated samples showed that both forms were present, with the 2-*O*-methyl derivative as the main component, since the primary fragmentary patterns of the two isomers are unique[20]. The optical rotation, the products from the partial hydrolysis, and the results of the methylation analysis indicated the back-

TABLE I

ANALYTICAL DATA FOR ARABINOXYLANS AX-I AND AX-II

	Yield (% of bran)	*Rel. mol %*[a]					$[\alpha]_D^{20}$ *(degrees)*	*Xyl/Ara*	$[\eta]$ *(dL/g)*
		Ara	*Xyl*	*Glc*	*Gal*	*AU*[b]			
AX-I[14]	8.6	12.0	85.6	1.7	0	0.7	−107	7.1	2.65
AX-II	8.1	13.9	81.2	3.9	trace	1.0	−105	5.8	3.20

[a]Determined as alditol trifluoroacetates by g.l.c. on OV-225. [b]4-*O*-Methyl-D-glucuronic acid.

TABLE II

METHYLATION ANALYSIS OF ARABINOXYLAN AX-I

Methylated sugar[a]	T[b]	*Mode of linkage*	*Molar ratio*
2,3,5-Me_3-Ara	0.61 (0.59)[c]	Ara*f*-(1-	13.0
2,3,4-Me_3-Xyl	0.80 (0.77)	Xyl*p*-(1-	0.3
2,3-Me_2-Xyl	1.37 (1.43)	→4)-Xyl*p*-(1-	100
2- and 3-Me-Xyl	1.94 (1.88)	→3,4)-Xyl*p*-(1- and →2,4)-Xyl*p*-(1-	13.5
2,3,6-Me_3-Glc	1.81 (1.76)	→4)-Glc*p*-(1-	2.0

[a]2,3,5-Me_3-Ara = 2,3,5-tri-*O*-methylarabinose, *etc.* [b]Retention times of the corresponding alditol acetates on SP 2340 relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylglucitol. [c]Values in parentheses are from ref. 18.

bone of AX-I to comprise (1→4)-linked β-D-xylopyranosyl residues with L-arabinofuranosyl groups attached to O-3 (O-2) of the xylosyl residues. The amounts of terminal arabinose and monomethylated xylose correspond well, indicating only this type of branching to be present in the xylan chain. The intrinsic viscosity of AX-I was not changed during prolonged methylation, indicating that no substantial depolymerisation had occurred. However, the molar ratio of the terminal xylose residue to the other sugar residues, calculated from the methylation analysis data, was 1:422, indicating slight branching of the main chain. The majority of the xylans so far isolated from herbaceous dicotyledons contain two branches per macromolecule on average[21]. Only the xylan in soybean has been claimed to be unbranched[22].

The ^{13}C-n.m.r. spectrum of AX-I was recorded in order to establish the configuration and monomeric composition of the polysaccharide. Earlier results[23–25] permitted the unequivocal assignment of all the signals. The data in Table III show that the arabinoxylan is composed of β-xylopyranosyl residues linked at position 4. Characteristic of these linkages are the C-1,4,5 resonances[23] at δ 101.62, 75.49, and 63.14, respectively. The resonances at δ 107.20, 80.47, 85.82, and 61.79 clearly indicate[24] the presence of the α-arabinofuranosyl groups and belong to C-1″, C-2″, C-4″, and C-5″, respectively. However, there were also other signals with intensities the same as those of the arabinofuranosyl groups, which belonged to the xylopyranosyl residues substituted by arabinose at position 2′ (δ 77.31). These data confirm the structural features of AX-I established by the optical rotation, partial hydrolysis, and methylation analysis.

Arabinoxylan AX-II. — In order to avoid possible losses of the arabinose residues during the chlorite delignification, attempts were made to isolate the rye-bran hemicelluloses without treatment with acid. As shown in Scheme 1, the native bran was treated first with hot dilute alkali under nitrogen. Some of the alkali-labile lignin- or phenolic acid–hemicellulose bonds[26–28] were cleaved during this treatment, thereby solubilising the hemicelluloses. Alkaline degradation of the arabino-

TABLE III

^{13}C-N.M.R. DATA FOR ARABINOXYLANS AX-I AND AX-II

Ring	*C-1*	*C-2*	*C-3*	*C-4*	*C-5*
(C)	101.62	72.55	73.91	75.49	53.14
(C')	100.95	72.25	77.31	75.78	63.55
(C'')	107.20	80.47	77.77	85.82	61.79

TABLE IV

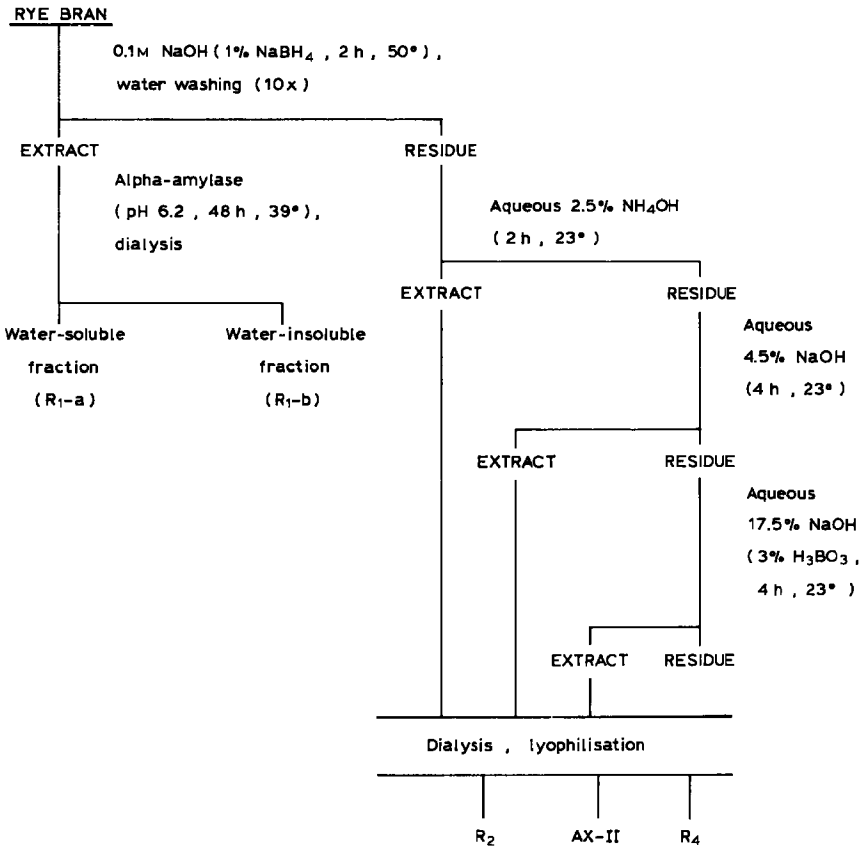
HEMICELLULOSE FRACTIONS ISOLATED FROM RYE BRAN WITHOUT CHLORITE DELIGNIFICATION (SEE SCHEME 1)

Extraction step	*Fraction*	*Yields (% of bran)*	*Molar ratio of neutral sugars*[a]					*Nitrogen (%)*
			Ara	*Xyl*	*Man*	*Glc*	*Gal*	
0.1M NaOH	R_1-a[b]	12.5	87	100	trace	9	3	3.24
	R_1-b[c]	12.0	91	100	0	7	4	8.85
2.5% NH_4OH	R_2	2.7	46	100	0	13	5	—
4.5% NaOH	AX-II	8.2	18	100	0	4	2	0.16
17.5% NaOH + 3% H_3BO_3	R_4	6.0	25	100	13	8	2	—

[a]Determined as alditol trifluoroacetates by g.l.c. on OV-225. [b]Soluble in water. [c]Insoluble in water.

xylan chains combined with losses of arabinosyl side-chains was minimised by the addition of sodium borohydride and carrying out the reaction under nitrogen[29,30]. The results in Table IV show that most of the highly branched arabinoxylan was extracted during the treatment with ammonium hydroxide. Extraction of the insoluble material with aqueous 4.5% sodium hydroxide afforded AX-II, which in yield, composition, and optical rotation was similar to AX-I, but had a considerably higher d.p. It is probable that oxidative degradation of the arabinoxylan occurs in the chlorite-delignification step without preferential cleavage of the arabinose side-chains. The ^{13}C-n.m.r. spectra of AX-I and AX-II were indistinguishable, indicating them to be fractions of the same polysaccharide.

The results confirmed the presence of an L-arabino-D-xylan with a low degree of branching in the cell-wall material of rye bran, representing ~50% of the non-starchy and non-cellulosic polysaccharides. This polysaccharide is probably located in the outer layers of the rye grain, since arabinoxylans with a similar low content of arabinose have been isolated from the vascular bundles and epidermis of grain[26] and the highly branched type has been found in the endosperm cell-walls[31]. The hitherto reported arabinoxylans from bran material (wheat[18], rice[32,33], and oat[34]) were of the highly branched type similar to the endosperm arabinoxylans of wheat[35,36], rice[37,31], and rye[11]. The structure of the rye-bran arabinoxylan fractions now reported is



Scheme 1. Fractional extraction of rye-bran hemicelluloses without chlorite delignification. Data on the various fractions are given in Table IV.

similar to that of the rice-hull[38] and wheat-straw[39] arabinoxylans.

EXPERIMENTAL

General. — Industrial rye bran (variety BRENO, C.S.S.R.) was ground to pass through a 0.2-mm mesh. The alpha-amylase was from *Bacillus subtilis* (Koch-Light).

Descending p.c. was performed on Whatman paper No. 1 with *A*, 1-butanol–pyridine–water (6:4:3); *B*, ethyl acetate–acetic acid–formic acid–water (18:3:1:4); and *C*, ethyl acetate–acetic acid–water (18:9:8); and detection with aniline hydrogenphthalate. T.l.c. was performed on Silufol (Kavalier, C.S.S.R.) with *D*, benzene–acetone (5:1) and detection by charring with sulphuric acid. The procedures for total hydrolysis, quantitative analysis of sugars, determination of uronic acid, and measurement of optical rotation have been described[14]. Partial acid hydrolysis was

achieved by heating a sample (50 mg) with 0.2M tri-fluoroacetic acid (15 mL) at 100° for 1–4 h. The hydrolysates were examined by p.c. in solvents *A* – *C*.

G.l.c. was performed on a Hewlett–Packard Instrument Model 5700 A, using *A*, a column (200 × 2 cm) of 3% of OV-225 on Chromosorb WAWDMCS (80–100 mesh) (temperature programme: 120° for 4 min, then to 170° at 2°/min); and *B*, a column (0.3 × 200 cm) of 3% of SP-2340 on Chromosorb WAWDMCS (0.15–0.17 mesh) (temperature programme: 180° for 4 min, then to 220° at 2°/min). Nitrogen was the carrier gas at 28 mL/min. G.l.c.–m.s. was performed on a JGC-20 gas chromatograph fitted with column *B*, and with helium as the carrier gas. Mass spectra were obtained at 23 eV, 300 μA, using a JMS-D 100 spectrometer.

Viscosities were determined with an Ubbelohde viscometer (U-2) for solutions in methyl sulphoxide or chloroform. Free-boundary electrophoresis of polysaccharide solutions (100 mg/mL) was performed in 0.05M sodium tetraborate buffer (pH 9.2), using a Zeiss 35 apparatus.

Methylation analysis. — The polysaccharide (100 mg) was methylated at room temperature according to the procedure of Ciucanu and Kerek[17] as modified for polysaccharides[40]. The methylated product (OMe, 38.3%) was treated with aqueous 90% formic acid for 1 h at 100° and then with M trifluoroacetic acid for 6 h at 100°. The sugars in the hydrolysate were converted into alditol acetates after reduction with sodium borodeuteride and analysed by g.l.c. on column *B* and by g.l.c.–m.s.[20]. The intrinsic viscosity of the methylated AX-I in chloroform was 2.75 dL/g. The ^{13}C-n.m.r. spectra (50.3 MHz) were recorded on solutions in $(CD_3)_2SO$ at 90° (internal Me_2SO, δ 39.7), using a Varian XL-200 spectrometer.

Arabinoxylan AX-II. — The bran (10 g) was treated with 0.1M sodium hydroxide (100 mL), containing 1% of sodium borohydride, at 50° for 2 h under nitrogen (Scheme 1), and then washed with water (10 × 200 mL) on a glass frit. The combined extract and washings were treated with alpha-amylase at pH 6.2 and 30° for 48 h. Dialysis of the mixture yielded a water-soluble (R_1-a) and a water-insoluble (R_1-b) fraction. The bran residue was extracted under N_2 with 100 mL each of aqueous 2.5% ammonium hydroxide (→ R_2), aqueous 4.5% sodium hydroxide (→ AX-II), and aqueous 17.5% sodium hydroxide containing 3% of boric acid (→ R_4). The various extracts were dialysed and freeze-dried. Data on these fractions are given in Table IV.

REFERENCES

1 F. Meuser, *Getreide, Mehl Brot*, 34 (1980) 217–222.
2 R. W. Wenlock, L. M. Sivell, and I. B. Agater, *J. Sci. Food Agric.*, 36 (1985) 113–121.
3 V. F. Rasper, *Dietary Fibers: Chemistry and Nutrition*, Academic Press, New York, 1979, pp. 93–115.
4 J. McCausland and C. W. Wrigley, *J. Sci. Food Agric.*, 27 (1976) 1197–1202.
5 H. Englist, in W. P. T. James and O. Theander (Eds.), *The Analysis of Dietary Fiber in Food*, Dekker, Basel, 1981, pp. 71–92.
6 H. J. Moss and N. L. Stenvert, *Aust. J. Agric. Res.*, 22 (1971) 547–551.
7 R. P. C. Tao and Y. Pomeranz, *J. Food Sci.*, 32 (1967) 162–168.

8 F. MEUSER, P. SUCKOW, AND ABD-ELGAWAD, *Getreide, Mehl Brot*, 35 (1981) 172–176.
9 R. R. MOD, F. L. NORMAND, R. L. ORY, AND E. J. CONKERTON, *J. Food Sci.*, 46 (1981) 571–573.
10 A. T. WARD AND R. R. MARQUARDT, *Poult. Sci.*, 62 (1983) 1054–1062.
11 G. O. ASPINALL AND R. J. STURGEON, *J. Chem. Soc.*, (1957) 4469–4471.
12 G. O. ASPINALL AND I. M. CAIRNCROSS, *J. Chem. Soc.*, (1960) 3998–4000.
13 O. THEANDER AND P. Åman, *Swed. J. Agric. Res.*, 9 (1979) 97–106.
14 Z. HROMÁDKOVÁ AND A. EBRINGEROVÁ, *Nahrung*, 31 (1987) 149–156.
15 R. G. LEBELL AND D. A. I. GORING, *J. Polym. Sci., Part C*, (1963) 29–48.
16 P. KOVÁČ AND J. HIRSCH, *Carbohydr. Res.*, 100 (1982) 177–193.
17 I. CIUCANU AND F. KEREK, *Carbohydr. Res.*, 131 (1984) 209–217.
18 J.-M. BRILLOUET, J.-P. JOSELEAU, J. P. UTILLE, AND D. LELIEVRE, *J. Agric. Food Chem.*, 30 (1982) 488–495.
19 K. A. ANDREWARTHA, D. R. PHILLIPS, AND B. A. STONE, *Carbohydr. Res.*, 77 (1979) 191–204.
20 P.-E. JANSSON, L. KENNE, H. LIEDGREN, B. LINDBERG, AND J. LÖNNGREN, *Chem. Commun. Univ. Stockholm*, 8 (1976) 1–75.
21 R. K. LYONS AND T. E. TIMELL, *Cellul. Chem. Technol.*, 3 (1969) 543–554.
22 G. O. ASPINALL, K. HUNT, AND M. MORRISON, *J. Chem. Soc. C*, (1966) 1945–1949.
23 J. HIRSCH, P. KOVÁČ, AND E. PETRÁKOVÁ, *Carbohydr. Res.*, 106 (1982) 203–216.
24 J. HIRSCH, E. PETRÁKOVÁ, AND J. SCHRAML, *Carbohydr. Res.*, 131 (1984) 219–226.
25 P. KOVÁČ, J. ALFÖLDI, P. KOČIŠ, E. PETRÁKOVÁ, AND J. HIRSCH, *Cellul. Chem. Technol.*, 16 (1982) 261–269.
26 K. C. B. WILKIE, *Adv. Carbohydr. Chem. Biochem.*, 36 (1979) 215–262.
27 Y. KATO AND D. J. NEVINS, *Carbohydr. Res.*, 137 (1985) 139–150.
28 M. M. SMITH AND R. D. HARTLEY, *Carbohydr. Res.*, 118 (1983) 65–80.
29 G. O. ASPINALL, C. T. GREENWOOD, AND R. J. STURGEON, *J. Chem. Soc.*, (1961) 3667–3674.
30 N. C. CARPITA, *Plant Physiol.*, 72 (1983) 515–521.
31 N. SHIBUYA AND A. MISAKI, *Agric. Biol. Chem.*, 42 (1978) 2267–2274.
32 N. SHIBUYA AND T. IWASAKI, *Phytochemistry*, 24 (1985) 285–289.
33 F. KURASAWA, S. ABE, Y. MANO, I. IGAVE, AND T. HAYAKAVA, *Nugata Agric. Sci.*, (1966) 158–163.
34 G. O. ASPINALL AND R. C. CARPENTER, *Carbohydr. Polym.*, 4 (1984) 271–282.
35 A. S. PERLIN, *Cereal Chem.*, 28 (1951) 382–393.
36 D. G. MEDCALF AND K. A. GILLES, *Cereal Chem.*, 45 (1968) 550–556.
37 Y. MAISUO AND A. NANBA, *Hakko Kogaku Zasshi*, 36 (1958) 190–193.
38 T. WATANABE, M. SHIDA, Y. FURUYAMA, K. I. TSUKAMOTO, T. NAKAJIMA, AND K. MATSUDA, *Carbohydr. Res.*, 123 (1983) 83–95.
39 G. O. ASPINALL AND E. G. MEEK, *J. Chem. Soc.*, (1956) 3830–3834.
40 A. EBRINGEROVÁ, unpublished results.