Carbohydrate Research, 150 (1986) 290–294
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Studies of some arabinoxylans from barley endosperm walls*

G. MURRAY BALLANCE†, R. STUART HALL, AND DAVID J. MANNERS
Department of Brewing and Biological Sciences, Heriot-Watt University, Edinburgh EH1 1HX (Great Britain)

(Received October 3rd, 1985; accepted for publication, December 11th, 1985)

Previous studies from these[1] and other laboratories[2,3] have shown that barley endosperm walls are composed of a mixture of polysaccharides. The major component (~70%) is a mixed-linkage β-D-glucan containing (1→3) and (1→4) linkages in the ratio of 3 to 7. The next largest component (~25%) is a pentosan containing L-arabinose and D-xylose. The walls also contain small amounts (2–4%) of cellulose and glucomannan. We now describe the results of a methylation analysis of the pentosan from two cultivars of barley, Julia and Tern, which differ significantly in malting properties.

Extraction of barley endosperm walls with alkali gave small amounts of pentosan which, after digestion with β-D-glucanases to remove contaminating β-D-glucan, were subjected to Hakomori-type methylation analysis. The results are shown in Table I.

All three samples contained the normal backbone of (1→4)-linked D-xylosyl residues and side chains consisting of single L-arabinofuranosyl groups. In sample II (75), the molecule had a d.p. of ~33, and about one-half of the xylosyl residues were substituted at O-2 and/or O-3 with a single arabinofuranosyl group. Sample II (18) had a greater d.p., 86% of the xylosyl residues were substituted with single arabinofuranosyl groups, and ~8% carried two arabinosyl groups. In sample C, which also had a higher d.p. than sample II (75), ~60% of the xylosyl residues were unsubstituted, 12% carried a single arabinofuranosyl group, and ~25% carried two such groups. The overall results show the presence of a family of arabinoxylans in the endosperm cell walls which vary in solubility, in the relative proportions of L-arabinose and D-xylose, and in the degree of substitution of (1→4)-linked xylosyl residues by L-arabinofuranosyl groups.

Two features of the endosperm wall pentosan merit comment. Firstly, the relative content of arabinose is higher than in similar material from other parts of the same plant. In barley husk, the pentosan contains[4] xylose and arabinose in the

*Dedicated to Professor N. K. Kochetkov.
†Present address: Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada.

0008-6215/86/$ 03.50

TABLE I

METHYLATION ANALYSIS OF ARABINOXYLANS

Product (mol %)	*Arabinoxylan*		
	Tern 18[a]	*Tern 75*[a]	*Julia C*[b]
2,3,5-Tri-*O*-methyl-L-arabinose	50	31	41
2,3,4-Tri-*O*-methyl-D-xylose	—	3	—
2,3-Di-*O*-methyl-D-xylose	3	33	37
2(3)-Mono-*O*-methyl-D-xylose	43	33	7
D-Xylose	4	—	15

[a]See Scheme 2. [b]See Scheme 1.

molar ratio of 6:1, whilst a water-soluble pentosan from barley flour had[5] a ratio of 5:3. Secondly, the presence of quadruply-linked xylose, *i.e.* a (1→4)-linked residue substituted at *both* O-2 and O-3, is an interesting feature which was relatively uncommon when these studies were carried out. In recent years, several workers have reported the presence of such residues in pentosans from the husk of *Plantago ovata* Forsk[6], the bark of *Cinnamomum iners*[7], endosperm walls from wheat[8], and aleurone cell walls from wheat and barley[9]. The biosynthesis of these particular residues is an intriguing problem.

EXPERIMENTAL

Analytical methods. — The general methods used for the determination of protein, starch, monosaccharides, and methylated sugars, and for the methylation and hydrolysis of polysaccharides were as described by Ballance and Manners[1].

Materials. — Endosperm cell walls were prepared from pearled barley flour (var. Tern and Julia), using the method devised by Palmer[10] and described by Ballance and Manners[1].

The preparations of salivary alpha-amylase and amyloglucosidase were those used previously[1]. A mixture of endo-(1→3)-β-D-glucanase and endo-[1→3(4)]-β-D-glucanase was prepared from malted barley by Dr. R. W. Gordon, as described by Manners and Wilson[11]. Purified endo-(1→4)-β-D-glucanase was prepared from a commercial enzyme preparation (Sigma Cellulase Type II derived from *Aspergillus niger*) by a combination of chromatography on DEAE-cellulose, BioGel P-60, and QAE-Sephadex. Appropriate column fractions were analysed for protein and hydrolytic activity towards arabinoxylans, barley β-D-glucan, and CM-cellulose. Details of the purification will be given elsewhere.

Extraction of cell walls. — The cell-wall preparation (~1 g), which had been stored under aqueous 70% ethanol, was centrifuged to remove most of the ethanol, and the resulting pellet was washed with distilled water (45 mL) and recentrifuged. The compressed walls were suspended in 10mM sodium acetate buffer (pH 5.0, 100

mL) containing amyloglucosidase (0.2 mL) and salivary alpha-amylase (0.2 mL). The extract was incubated at 45° in a shaking water-bath for 1 h and then centrifuged. The recovered walls were re-extracted three times in a similar manner, with homogenisation in a Potter–Elvejham homogeniser between each extraction. The combined extracts were treated with 3 vol. of ethanol and cooled to 3° overnight, to precipitate the water-soluble polysaccharides (Fraction I).

The water-extracted walls were then treated with M potassium hydroxide (100 mL) containing 0.5% of potassium borohydride. This initial extraction was carried out at room temperature since the extract rapidly became viscous. Three subsequent extractions were carried out at 55° following the same procedure as for the water extraction. The combined extracts were neutralised with 2M acetic acid, and 3 vol. of ethanol were then added at 3° to precipitate the alkali-soluble polysaccharides (Fraction II).

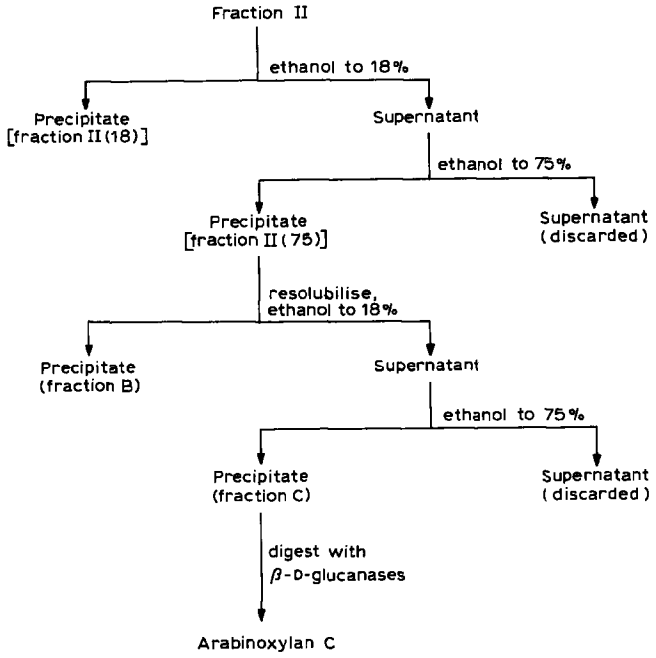
The alkali-insoluble material was not examined further.

Purification of arabinoxylan from Julia barley. — Fraction II (600 mg) was further fractionated using ethanol at 3°, as shown in Scheme 1. Fractions II (18) and B were β-D-glucan-rich fractions from which purified β-D-glucan II was eventually isolated[1]. Fraction C, on acid hydrolysis, contained approximately equal amounts of glucose, arabinose, and xylose as indicated by paper chromatography. The β-D-glucan from fraction C was removed by digestion with a mixture of endo-(1→3)-β-D-glucanase and endo-[1→3(4)]-β-D-glucanase (prepared from malted barley) at pH 5.0 and 20° for 24 h, followed by incubation with a purified endo-(1→4)-β-D-glucanase from *Aspergillus niger* under the same conditions. Control experiments had shown that the enzyme preparations were free of activity against wheat-flour and barley-husk arabinoxylans. The arabinoxylan from fraction C was recovered by precipitation with ethanol to 60%. It was free of β-D-glucan and was examined by methylation analysis.

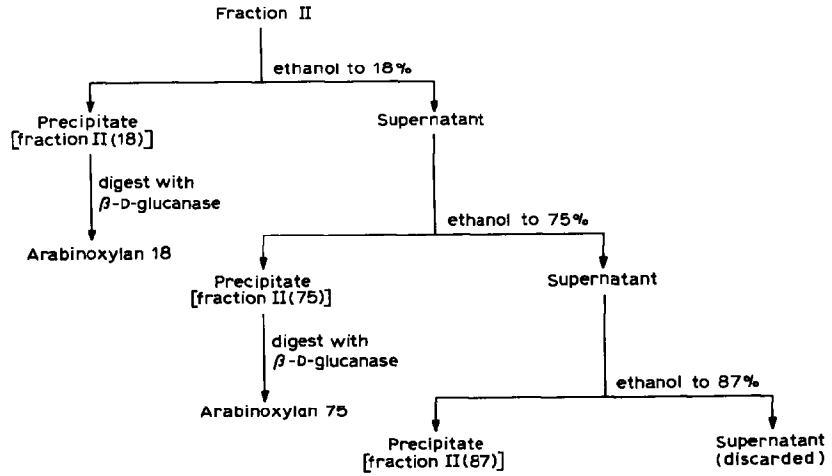
Purification of arabinoxylans from Tern barley. — Fraction II (560 mg) was dissolved in water (200 mL) by stirring overnight. The solution was then made 50mM with respect to sodium hydroxide and stirred for a further 30 min to facilitate dissolution of residual material. The solution was then neutralised with 2M acetic acid, filtered, and fractionated using ethanol at 3° as shown in Scheme 2. After the addition of ethanol to 75%, the supernatant solution was still quite viscous; on addition of further ethanol, a third precipitate was obtained. The various precipitates were dried by using ethanol and ether, to give the following yields: II (18), 213 mg; II (75), 163 mg; and II (87), 98 mg. The total recovery from the original alkali-soluble material was 83%.

The monosaccharide compositions of the various sub-fractions are shown in Table II. The results indicate the presence of pentosan of varying arabinose–xylose ratios. Since sub-fractions II (18) and II (75) contained larger amounts of pentosan than II (87), the former two sub-fractions were examined further.

Fractions II (18) and II (75) were each suspended in 0.1M sodium acetate buffer (pH 5.0, 100 mL) and stirred overnight at room temperature. Fraction II



Scheme 1. Isolation of arabinoxylan from Julia barley.



Scheme 2. Isolation of arabinoxylan from Tern barley.

(18) did not readily dissolve under these conditions, and insoluble material was removed by centrifugation (1500*g*, 10 min). The polysaccharide solutions were then digested with endo-(1→4)-β-D-glucanase from *Aspergillus niger* (8 mL) and, after 3 days, further enzyme (6 mL) was added to each digest and incubation continued for a further 5 days.

TABLE II

MONOSACCHARIDE COMPOSITION OF SUB-FRACTIONS OF FRACTION II FROM TERN BARLEY

Fraction	*Composition (%)*			*Arabinose–xylose ratio*
	Arabinose	*Xylose*	*Glucose*	
II (18)	28	29	43	0.97
II (75)	18	26	56	0.70
II (87)	6	12	82	0.50

The digests were dialysed against running tap-water for 48 h, and ethanol (3 vol.) was then added to precipitate the polysaccharides, which were collected by centrifugation (5000*g*, 15 min) and dried with ethanol and ether. Paper chromatographic analysis of acid hydrolysates showed that Fraction II (18) still contained some glucose, and Fraction II (75) only a faint trace of glucose, in addition to the arabinose and xylose.

The glucan contaminant in Fraction II (18) could not be removed by further digestion with endo-(1→4)-β-D-glucanase. Since the two sub-fractions were as free from digestible glucan as possible, methylation analysis was carried out.

ACKNOWLEDGMENTS

We thank the Eda, Lady Jardine Charitable trust and the Science and Engineering Research Council for the award of research studentships (to G.M.B and R.S.H., respectively).

REFERENCES

1 G. M. BALLANCE AND D. J. MANNERS, *Carbohydr. Res.*, 61 (1978) 107–118.
2 G. B. FINCHER, *J. Inst. Brew., London*, 81 (1975) 116–122.
3 R. G. THOMPSON AND D. E. LABERGE, *Tech. Q., Master Brew. Assoc. Am.*, 18 (1981) 116–120.
4 G. O. ASPINALL AND R. J. FERRIER, *J. Chem. Soc.*, (1957) 4188–4194.
5 G. O. ASPINALL AND R. J. FERRIER, *J. Chem. Soc.*, (1958) 638–642.
6 J. F. KENNEDY, J. S. SANDHU, AND D. A. T. SOUTHGATE, *Carbohydr. Res.*, 75 (1979) 265–274.
7 J. P. GOWDA, D. C. GOWDA, AND Y. V. ANJANEYALU, *Carbohydr. Res.*, 87 (1980) 241–248.
8 A. BACIC AND B. A. STONE, *Carbohydr. Res.*, 82 (1980) 372–377.
9 A. BACIC AND B. A. STONE, *Aust. J. Plant Physiol.*, 8 (1981) 475–495.
10 G. H. PALMER, *Proc. Am. Soc. Brew. Chemists*, 33 (1975) 174–180.
11 D. J. MANNERS AND G. WILSON, *Carbohydr. Res.*, 48 (1976) 255–264.