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TOTAL HEMICELLULOSES FROM *HORDEUM VULGARE* PLANTS AT DIFFERENT STAGES OF MATURITY

ANTONY J. BUCHALA* and KENNETH C. B. WILKIE

Department of Chemistry, University of Aberdeen, Old Aberdeen, Scotland

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Abstract—The changes in the composition of the total hemicelluloses of leaf and stem tissues of field-grown barley plants have been examined at different stages of maturation In each plant the proportion of xylose residues in the total hemicellulose increases with tissue maturity, that of galactose varies little, and the proportions of arabinose, glucose and uronic acid residues decrease. The ratio of $\beta(1\rightarrow3)$ to $\beta(1\rightarrow4)$ linkages in the β-glucans decreases with tissue maturity and there is a decrease in the $\overline{DP}_n$ of these β-glucans.

INTRODUCTION

THE POLYSACCHARIDE composition of the non-endospermic tissues of the oat[1–3] and wheat[4] plants alter as the plants mature. Similar changes are now reported to occur in barley. The object of the work has been to interpret the composition of total hemicelluloses from the various plants in terms of structural features established to be present in pure hemicelluloses isolated from the plant tissues.[5] There is much information on wheat hemicelluloses,[4,6] but a comparative dearth of information on the hemicelluloses of barley, other than that of the husks[7] and mature leaves;[8] however, much work has been carried out on the polysaccharides in barley seeds before and after germination because of the importance of such information to the distilling and brewing industries.[9]

Homoxylans have been prepared by autoclaving barley stem heteroxylans.[10,11] The degraded xylans had the $\beta(1\rightarrow4)$ linked D-xylopyranosyl residues typical of land plant xylans. There is a little evidence that the homoxylan core of barley husk heteroxylan[7] may

* Present address Institut de Recherche Cardioangéiologique, Chemin du Musée, CH 1700 Fribourg, Switzerland

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be very slightly branched but the evidence is easily interpreted otherwise and is less persuasive than in the case of one wheat heteroxylan.[12] The structure of many simple heteroxylans from the Gramineae have been established Commonly these have low proportions of L-arabinofuranosyl residues on C3 positions and of either 4-*O*-methyl-D-glucopyranuronosyl, or of D-glucopyranuronsyl, residues, or of both the latter, on C2 positions

The xylan isolated from mature leaves[8] has a $\overline{DP}_n$ of *ca* 96 and, in addition to 8 1 L-arabinofuranosyl residues and 4 4 4-*O*-methyl-D-glucopyranuronosyl residues, has 3·8 galactosyl-(1 → 4)-D-xylopyranosyl-(1 → 2)-L-arabinofuranosyl side-chains at C3 positions This last structural feature has been noted in the acidic galactoarabinoxylans from oat stem,[13] perennial rye grass roots[14] and maize hulls.[15] There is also evidence of the presence of a more complex xylan which, as noted later, may be similar to the acidic galactoarabinoxylans found in oat leaf[16] and stem[13] and in wheat leaf[4]

Some workers have chosen to fractionate the hemicellulose of barley into hemicelluloses A and B;[17] the fractionation is dependent upon solubility characteristics and, unless particular care is taken, these may vary markedly.[18] It is probable that the less fully substituted xylans are present in hemicellulose A and the more fully substituted in hemicellulose B The heteroxylans containing galactose residues have commonly not been isolated Terent'ev[19] has concluded that the proportion of hemicellulose A reaches a maximum of 33% in the barley stem during the period of waxy ripeness while hemicellulose B reaches a maximum of 12 5% during the period of milky ripeness[20] and the ratio of the former to the latter ranges from 2 5–3 5 to 1

RESULTS AND DISCUSSION

The plants (see Table 1) were grown under conditions similar to those for the wheat plants studied earlier[4] The *total hemicelluloses*[5] were isolated and the delignification liquors and the α-celluloses were retained. The proportion of α-cellulose and of total hemicellulose increases in each tissue as the plants mature. Quantitative estimations were made of the neutral sugar composition in various α-celluloses,[21] in the materials solubilized dur-

TABLE 1 DESCRIPTION OF FIELD-GROWN BARLEY PLANT TISSUES STUDIED

Age of plant (days from sowing to harvest)	Height of plant (cm)	Description of plant tissues
36	15	Leaf
63	35	
85	60	Stem, five leaves and inflorescence
126	75	
137*	75	Stem, five leaves and inflorescence

*Time of normal harvest Bottom leaf withered

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ing delignification[22] and in the total hemicelluloses. The uronic acid composition of each total hemicellulose was also estimated (Fig. 1 and Table 2).[3] The values obtained showed, as in the cases for oat[21,22] and wheat,[4] that the total hemicellulose compositions do not require to be modified to allow either for the non-glucosidic residues in the α-celluloses[21] or for the small amount of hemicellulosic material that passes into solution during delignification.[22]

TABLE 2 COMPOSITION OF THE TOTAL HEMICELLULOSES OF MATURING BARLEY PLANTS IN MOLAR PERCENTAGES

Tissue	Days from sowing to harvest	Arabinose	Xylose	Galactose	Glucuronic acid	4-*O*-Methyl-glucuronic acid	Glucose
Stem	63	9·4	68·9	1·3	3·1	7·0	10·2
	85	10·7	72·6	1·5	4·2	5·7	5·2
	126	10·2	74·0	1·3	3·5	6·0	4·9
	137	9·2	77·0	1·9	3·8	3·5	4·4
Top leaf	63	13·2	61·9	2·3	4·5	6·1	12·2
	85	13·9	68·3	2·9	4·0	6·4	4·4
	126	13·0	71·9	2·7	3·9	5·6	2·9
	137	10·4	75·5	2·5	3·8	5·2	2·8
Middle leaves	63	16·0	58·8	4·5	6·0	6·6	8·2
	85	13·3	63·5	5·0	5·6	6·6	5·9
	126	13·9	66·8	4·7	4·5	5·8	4·3
	137	13·4	67·5	4·5	4·5	5·8	4·3
Leaf and bottom leaf	36	16·6	41·7	4·7	9·5	5·7	21·8
	63	19·0	52·5	6·4	6·8	7·2	8·0
	85	18·0	58·5	6·3	5·7	6·3	5·1
	126	14·5	61·4	7·7	4·7	5·8	5·8

Reducing sugars = 100%. Values determined by GLC of derived acetates of neutral sugars and by borotritide determination of acidic sugars. Values are not corrected for xylose residues remaining glycosidically linked to uronic acid residues after hydrolysis, but see Fig. 1.

In Table 2, no allowance has been made for the neutral sugar residues present in oligouronic acids. Glucose was released during hydrolysis of the total hemicelluloses (Table 2) but it derives from β-glucans[23–26] and has been omitted from the histograms which are designed to show only the sugar residues in barley heteroxylans. In barley tissues, as the plants mature, there is an increase in the proportion of xylosyl residues and a fall in the proportions of arabinosyl, glucosyl and uronosyl residues. The changes in the proportion of galactosyl residues do not show a clear trend related to plant maturity. Glucuronic acid and 4-*O*-methylglucuronic acid are present in similar proportions in barley tissues whereas in oat[3] and wheat[4] tissues throughout the period of growth and in maturity glucuronic acid predominates.

The results of the present study indicate the presence of an acidic galactoarabinoxylan in barley tissues similar to that isolated from oat stem.[13] Free-boundary electrophoresis of the water-soluble portion of a barley leaf total hemicellulose in 0·05 *M* borate buffer gave four peaks. One of these (mobility $\mu = -3{\cdot}99\ 10^5 \times cm^2\ V^{-1}\ sec^{-1}$) has a mobility similar to that of the oat stem acidic galactoarabinoxylan ($\mu = -4{\cdot}30\ 10^5 \times cm^2\ V^{-1}$

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sec^{-1}) under almost identical conditions.[13] If it is assumed that barley acidic galactoarabinoxylans are similar to those found in oat tissues then the above changes in the proportions of sugar residues would accord with the progressive dilution of hemicellulosic material of the galactoarabinoxylan type mentioned above by material similar to the less complex xylan isolated from barley.[8] The changes in composition of the barley total hemicelluloses are similar to those that take place in oat[1,3] and wheat[4] tissues. Comparable changes may occur during the maturation of other temperate grasses.

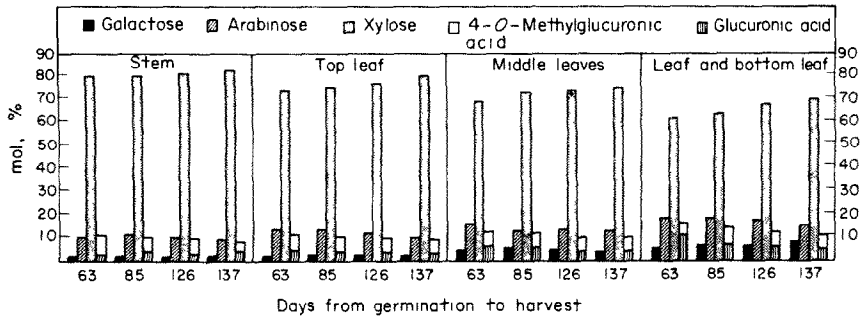

FIG. 1. COMPOSITION OF THE TOTAL HEMICELLULOSES FROM BARLEY PLANTS AT DIFFERENT STAGES OF MATURITY.

It is assumed that all uronic acid residues were present after hydrolysis in aldobiouronic acids containing a D-xylose residue. The proportions of D-xylose found in hydrolysates have been altered to allow for these non-liberated xylose residues.

A sample of barley leaf total hemicellulose was incubated with an enzyme preparation from *Cytophaga*. Under similar conditions employed in studying the barley total hemicellulose the enzyme preparation catalysed the hydrolysis of laminaran, laminaribiose and laminaritriose but did not lead to the hydrolysis of cellulose, cellobiose, cellotriose, cellotetraose or oat stem acidic galactoarabinoxylan. It is known that the enzyme acting on $\beta(1 \rightarrow 3)$ linked glucans will hydrolyse adjacent $\beta(1 \rightarrow 4)$ linkages.[27] After treatment with the *Cytophaga* enzyme preparation acidic hydrolysates of the total hemicellulosic materials contained only traces of glucose. It was concluded that no significant amount of cellulose, or of degraded cellulose, was present in the total hemicellulose and starch was known to be absent. The glucose residues were in $\beta(1 \rightarrow 3)$ and $\beta(1 \rightarrow 4)$ linked glucans of the type found in the non-endospermic tissues of oats[2,23,26] wheat,[28] rye,[24] maize[25] and bamboo.[29] The variation in β-glucan composition with plant maturity was determined. Degradation studies on the various barley total hemicelluloses showed that there was a decrease in the ratio of $\beta(1 \rightarrow 3)$ to $\beta(1 \rightarrow 4)$ linkages as the plants matured. The values for the $\overline{DP}_n$ of the β-glucans in the tissues from the plants of different maturity were also determined by a procedure involving labelling the reducing end groups by use of sodium borotritide. It was found that the $\overline{DP}_n$ of the various β-glucans fell as each tissue matured (Table 3). The biological function of the β-glucan is of interest and is being further investigated. Studies have been carried out on the changes in the levels of β-glucan hydrolase activities in an internode of the stem of the developing oat plant[26] and these and the above results are in accord with changes which occur during the growth of decapitated maize root tips (Pilet; private communication).

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TABLE 3 β-GLUCANS FROM THE TOTAL HEMICELLULOSES OF BARLEY PLANTS

	Age in days from sowing to harvest											
	Ratio of (1 → 3) to (1 → 4) linkages × 10^2				DP				Percentage of glucan in dry tissue*			
Tissue	63	85	126	137	63	85	126	137	63	85	126	137
Stem	70	61	34	25	73	67	50	45	2·4	1·6	1·4	1·3
Top leaf	68	44	—	24	76	46	46	35	3·1	0·9	1·0	1·1
Middle leaves	41	38	27	26	67	53	51	37	1·6	1·8	1·3	1·6
Leaf and bottom leaf	47	36	33	23	67	43	35	31	4·3	1·8	1·3	2·0

* Values corrected for glucan present in delignification liquors

EXPERIMENTAL

General methods. PC was on Whatman No 1 paper and the irrigant was *n*-BuOH–pyridine–C_6H_6–H_2O (5 3·1.3) Alkaline $AgNO_3$ was the chromatographic detection reagent. Hemicellulosic samples were hydrolysed by heating with 0·5 M H_2SO_4 for *ca* 12 hr. The neutral sugars in the hydrolysates were determined by GLC of their glycitol acetates. Uronic acids were estimated by the method of Buchala and Wilkie,[3] and the $\overline{DP}_n$ of each β-glucan was determined as previously described.[4]

Plant material The barley plants, *Hordeum vulgare*, (var. Ymer) were grown at the University farm, Hillbrae, Aberdeenshire Immediately after harvesting, the plants were dissected, boiled in EtOH for 20 min, air-dried and stored at 0° until required

Examination of the total hemicelluloses, α-celluloses and delignification materials. Each plant tissue was delignified and the total hemicellulose[3] (Table 2), α-cellulose[21] and the delignification material[22] were isolated and studied

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