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TOTAL HEMICELLULOSES FROM WHEAT AT DIFFERENT STAGES OF GROWTH

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Key Word Index—*Triticum vulgare*; Gramineae; wheat; hemicelluloses; xylan; β-glucan; uronic acids.

Abstract—The changes in total hemicellulose composition of leaf and stem tissues of field-grown wheat plants have been examined. In each plant tissue the percentage of xylose in the total hemicellulose increases with increasing plant maturity, that of galactose varies little and those of L-arabinose, D-glucose, and uronic acid decrease. There is a markedly higher proportion of D-glucopyranuronosyl than of 4-*O*-methyl-D-glucopyranuronosyl residues in leaf and stem tissues at all stages of maturity. The ratio of $\beta(1 \rightarrow 3)$ to $\beta(1 \rightarrow 4)$ linkages in the β-glucans, and the DP of these β-glucans decrease concommitantly with tissue maturity.

INTRODUCTION

THERE are variations in the proportion of sugar residues in total hemicelluloses[1] from the non-endospermic tissues of oat plants at different stages of growth.[2–4] These variations have been partially interpreted in terms of pure hemicelluloses[1] of known structure.[5,6] No similar studies have been reported on other cereal plants. The stem and leaf tissues of the wheat plant have now been studied.

Several D-xylans were earlier isolated from wheat straw, and others from wheat leaf[7] and bran.[8–10] All are heteroxylans and have the $\beta(1 \rightarrow 4)$ linked D-xylopyranosyl main chains typical of the xylans of higher land plants.[11] A branched homoxylan has been isolated from esparto grass, *Stipa tenacissima*,[12,13] but no homoxylan has been isolated from any wheat tissue, nor from any other species of the Gramineae. Residues of L-arabinofuranose, D-glucopyranuronic acid and 4-*O*-methyl-D-glucopyranuronic acid are commonly present in hemicellulosic xylans. The L-arabinofuranosyl residues are terminal and linked $\alpha(1 \rightarrow 3)$.[14,15] The earlier evidence[16–18] indicated that the uronosyl residues were linked

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α(1 → 3) but on some occasions[16,18] this conclusion about linkage was based exclusively on paper chromatographic identification of a derived mono-*O*-methylxylose. Other later studies have shown an α(1 → 2) linkage definitely to be present.[19] Variations in the pure xylans isolated from wheat straw require a brief survey. A methylated acidic xylan lacking arabinosyl residues was derived from a xylan which had *ca.* 0·5% L-arabinosyl, and 3% of D-glucuronosyl, residues.[18] Another xylan had 9% of L-arabinosyl residues and 3% of a mixture of D-glucuronosyl, and 4-*O*-methyl-D-glucuronosyl, residues.[19] A xylan isolated by Roudier[20,21] had *ca.* 80% of the uronosyl residues present as 4-*O*-methyl ethers. Another xylan had 13·6% of L-arabinosyl, and 10·6% of the two uronosyl, residues.[7] A non-acidic arabinoxylan was isolated by Ehrenthal *et al.*[13] It was unusual in having a small proportion of apparently C3 and C4 linked glucopyranosyl residues. Bishop,[22] after autoclaving a wheat straw xylan, isolated a branched xylooctaose. Thus, although there are obviously marked similarities in the structures of the various wheat xylans there are also noteworthy variations. Sometimes only one, and on other occasions both, of the types of uronosyl residue are reported present. Normally arabinosyl residues are present but the proportion varies. There is an indication that some xylans are branched. It has been stated, on the basis of this accumulation of evidence, that there are many xylans in wheat straw.[18] The variations between the xylans are commonly accepted as being due to the fractionation procedures used, but another factor must also be taken into account. As stated, the maturity of the non-endospermic tissues of the oat plant markedly affects their hemicellulosic composition. If the wheat plant is similar in this respect to the oat plant, then it is reasonable to conclude that the differences between xylans must, to an extent, reflect variations due to plant maturity and possibly due to the conditions of plant growth. Black and Wilkie[23] have recently isolated an acidic galactoarabinoxylan from wheat leaf. This is of interest

TABLE 1. DESCRIPTION OF FIELD-GROWN WHEAT PLANT TISSUES STUDIED

Age of plant (days from germination to harvest)	Height of plant (cm)	Description of plant tissues
56	20	Leaf
83	38	Stem, five leaves, and inflorescence
105	70	
132	90	
157*	90	Stem, five leaves, and inflorescence†

* Time of normal harvest.
† Bottom leaf withered.

both because no such hemicellulose has previously been found in wheat tissues and because it parallels the isolation of similar hemicelluloses from oat stem[24] and leaf[5] tissues. Recent studies on wheat total hemicelluloses have led to the isolation of β(1 → 3) and β(1 → 4) linked glucans.[25] Such hemicellulosic glucans were first isolated from the non-endospermic tissues

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of the oat plant. Again a parallel between wheat and oat plants is evident. Such glucans are now known also to be present in non-endospermic tissues of barley,[25,26] rye,[25] maize[27] and bamboo.[28]

RESULTS AND DISCUSSION

The plant tissues used are described in Table 1. The proportion of α-cellulose and of total hemicellulose increases in each tissue as the plants mature (Fig. 1).

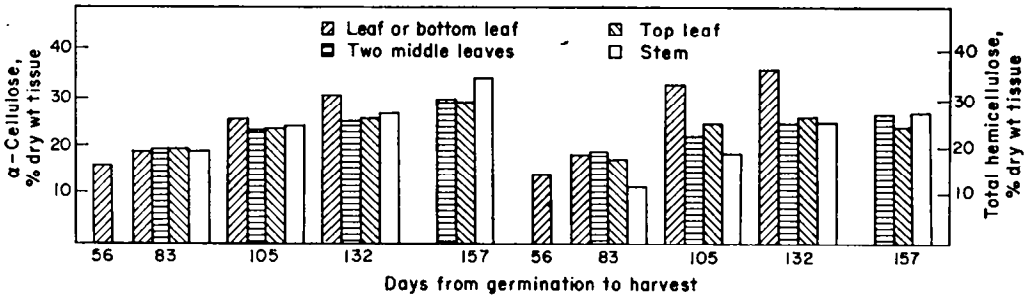

FIG. 1. PERCENTAGES OF α-CELLULOSE AND OF TOTAL HEMICELLULOSE IN TISSUES OF WHEAT PLANTS AT DIFFERENT STAGES OF MATURITY.

Quantitative estimations of the non-glucosidic residues present in the α-celluloses (Table 2)[29] and of the materials solubilized during the delignification procedure (Table 3)[29] were carried out as described in studies on oats. Each total hemicellulose was hydrolysed and the molar ratios of the neutral sugar residues were estimated by GLC examination of derived glycitol acetates;[30] uronic acids were estimated by the method of Buchala and Wilkie.[4] It is evident that the values for the sugar residues in the various total hemicelluloses (Fig. 2 and Table 4) neither need to be modified to allow for the non-glucosidic residues in the α-cellulose (Table 2), nor for the hemicellulosic material normally lost during delignification (Table 3).

TABLE 2. NON-GLUCOSIDIC RESIDUES IN THE α-CELLULOSES OF MATURING WHEAT PLANTS AND MODIFICATION OF TOTAL HEMICELLULOSE VALUES ON THE ASSUMPTION THAT THESE RESIDUES ARE HEMICELLULOSIC

	Days from germination to harvest															
	Stem				Top leaf				Middle leaves				Leaf and bottom leaf			
	83	105	132	157	83	105	132	157	83	105	132	157	56	83	105	132
Non-glucosidic residues (% in α-cellulose)	5·8	2·3	2·1	4·5	8·1	6·2	5·3	5·6	9·1	3·8	5·4	4·2	7·1	8·5	7·6	4·4
Non-glucosidic residues in α-cellulose*	9·5	3·0	2·2	6·1	9·1	5·8	5·2	6·8	9·4	4·0	5·7	4·6	8·2	8·9	6·0	3·7
Xylose/arabinose ratio in α-cellulose	5·4	2·4	3·4	9·0	6·2	2·1	2·4	5·7	3·9	1·2	1·5	3·6	3·2	3·9	1·2	1·7
Xylose/arabinose ratio in total hemicellulose	4·3	7·1	8·5	9·7	5·8	5·8	5·9	6·5	4·6	4·8	6·3	7·4	2·5	3·5	4·2	5·5
Xylose/arabinose ratio—modified value†	4·4	7·0	8·4	9·6	5·8	5·6	5·7	6·5	4·8	4·7	6·0	7·3	2·5	3·6	4·1	5·3

* Values quoted are expressed as a percentage of the plant tissue *assuming* for the calculation the residues to be in hemicellulosic material not included in the total hemicellulose.

† This ratio takes into account the amount of xylose and arabinose in the α-cellulose and shows the effect it would have if it were hemicellulosic material extracted and included in the total hemicellulose.

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TABLE 3. HEMICELLULOSIC MATERIALS PRESENT IN THE DELIGNIFICATION LIQUORS OF MATURING WHEAT

	Stem			
	83	105	132	157
Carbohydrate in delignification material*	0·23	0·22	0·57	0·51
Total hemicellulose*	11·9	18·6	25·0	25·5
Hemicellulosic material as % of carbohydrates in delignification material	97	92	92	98
Total hemicellulose including that in the delignification material*	12·1	18·8	25·5	26·0
Ratio of sugar residues in delignification material†				
xylose	1·6	0·64	0·78	0·53
galactose	0·63	0·64	0·76	0·29
Ratio of sugar residues in total hemicellulose†				
xylose	4·3	7·1	8·5	9·7
galactose	0·09	0·08	0·12	0·09
Modified values of ratio of sugar residues in total hemicellulose†				
xylose	4·2	7·0	8·3	9·5
galactose	0·10	0·09	0·13	0·09

* Expressed as % of dry plant tissue.

† These ratios show the effect of taking into account the corresponding sugar residues present in the delignification liquors (arabinose = 1·0).

It is well known that on acid hydrolysis of acidic xylans, glycosiduronic linkages commonly survive. In Table 4 no allowance has been made for the known presence of D-xylose residues in aldobiouronic acids. The results are re-evaluated in the histograms (Fig. 2) on the assumption that after acidic hydrolysis the uronosyl residues are present entirely in

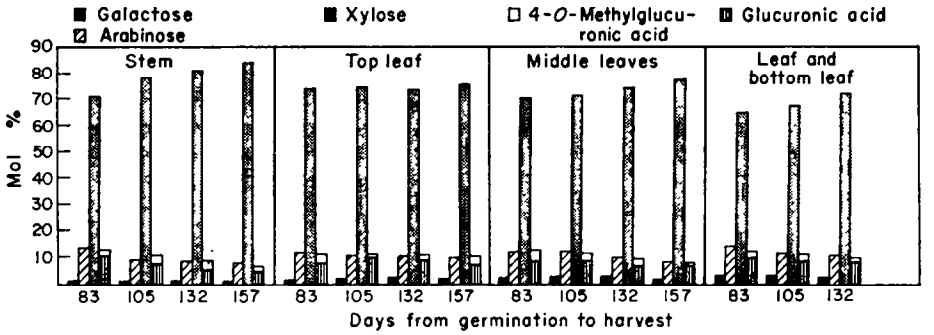

FIG. 2. COMPOSITION OF THE TOTAL HEMICELLULOSES FROM TISSUES OF WHEAT PLANTS AT DIFFERENT STAGES OF MATURITY.

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.

aldobiouronic acids. The glucose in the hydrolysates has been discounted in the histograms which show only the proportions of those sugar residues known to be present in wheat tissue heteroxylans. The histograms for top leaf tissues from plants of different maturities are very similar, but in other tissues increasing maturity is associated with an increase in the proportion of xylosyl residues and af all in the proportion of arabinosyl, glucosyl, and

PLANTS AND EFFECT OF MODIFYING TOTAL HEMICELLULOSE VALUES TO ALLOW FOR THESE MATERIALS

Days from germination to harvest											
Top leaf				Middle leaves				Leaf and bottom leaf			
83	105	132	157	83	105	132	157	56	83	105	132
0·42	0·77	0·49	0·54	0·20	0·48	0·20	0·90	0·23	0·09	0·64	0·64
17·5	24·9	26·8	23·6	19·3	21·9	24·1	26·9	14·2	18·3	32·9	36·7
89	93	96	96	91	94	94	96	90	93	96	93
17·9	25·6	27·3	24·1	19·5	22·4	24·3	27·8	14·4	18·4	33·5	37·3
0·82	0·56	0·53	1·14	0·48	0·62	0·67	0·62	2·21	0·79	0·88	1·07
0·56	0·63	0·83	0·92	0·58	0·83	1·00	0·42	0·52	0·53	0·86	0·97
5·8	5·8	5·9	6·5	4·7	4·8	6·3	7·4	2·5	3·5	4·2	5·5
0·19	0·23	0·29	0·24	0·21	0·24	0·29	0·24	0·25	0·26	0·29	0·21
5·7	5·6	5·8	6·4	4·6	4·7	6·3	7·2	2·5	3·5	4·2	5·4
0·20	0·24	0·30	0·25	0·22	0·25	0·30	0·25	0·25	0·26	0·30	0·22

uronosyl residues. The proportion of galactosyl residues varies little and exhibits no trend clearly related to tissue maturity. It is of interest to note that the proportion of D-glucuronosyl residues is markedly higher than is that of 4-*O*-methyl-D-glucuronosyl residues.

TABLE 4. COMPOSITION OF THE TOTAL HEMICELLULOSES OF MATURING WHEAT PLANTS–MOLAR PERCENTAGES (REDUCING SUGARS = 100%). VALUES DETERMINED BY GLC OF DERIVED ACETATES OF NEUTRAL SUGARS AND BY BOROTRITIDE DETERMINATION OF ACIDIC SUGARS

Tissue	Days from germination to harvest	Rhamnose	Arabinose	Xylose	Galactose	Glucuronic acid	4-*O*-Methylglucuronic acid	Glucose
Stem	83	0·2	14·3	61	1·3	11·2	2·7	8·6
	105	0·3	10·3	73	0·8	8·6	3·7	2·8
	132	0·3	9·2	78	1·1	6·0	3·4	2·6
	157	0·3	8·4	81	0·7	5·0	2·2	2·3
Top leaf	83	0·3	12·0	66	2·3	8·9	3·1	7·3
	105	0·4	12·1	69	2·8	10·9	1·5	2·7
	132	0·9	11·7	69	3·3	10·2	1·8	3·3
	157	0·3	11·2	73	2·8	7·8	4·2	2·6
Middle leaves	83	0·6	13·6	64	2·8	9·9	4·4	5·2
	105	0·7	13·8	67	3·1	10·3	2·4	3·0
	132	0·6	11·2	71	3·2	8·5	2·5	3·5
	157	0·7	10·1	74	2·3	8·8	1·4	3·4
Leaf and bottom leaf	83	1·1	16·5	58	4·2	11·8	3·0	5·5
	105	0·9	14·6	62	4·3	10·7	2·7	4·7
	132	0·6	12·8	70	2·7	9·8	2·3	2·8

* Values are not corrected for xylose residues remaining glycosidically linked to uronic acid residues after hydrolysis, but see Fig. 2.

Recently an acidic galactoarabinoxylan has been isolated by Black and Wilkie from wheat leaf tissues.[23] Its presence had been indicated in the studies now reported. When the water-soluble portion of a wheat leaf total hemicellulose was examined by free-boundary electrophoresis in 0·05 M borate buffer, four peaks were noted. One of these (mobility

$\mu = -4{\cdot}21\ 10^5 \times cm^2\ V^{-1}\ sec^{-1}$) had a mobility similar to that of the oat stem galactoarabinoxylan ($\mu = -4{\cdot}30\ 10^5 \times cm^2\ V^{-1}\ sec^{-1}$) under almost identical conditions. If the wheat acidic galactoarabinoxylans are of the types found in oat plant tissues then the trends may be *cautiously* interpreted. The results are compatible with the progressive dilution of hemicellulosic material similar to the oat acidic galactoarabinoxylan by material similar to the less substituted wheat acidic arabinoxylans mentioned earlier.

Studies have also been made of the variation in β-glucan composition with plant maturity. Smith degradation studies were carried out on the various wheat total hemicelluloses. After hydrolysis, the products were converted to the glycitol acetates, estimated and identified by GLC. This enabled the ratios of (1→3) to (1→4) linkages in the various β-glucans to be determined. With increasing plant maturity, the ratio of β(1→3) to β(1→4) linkages decreases in any one tissue (Table 5). Studies were carried out with a β-1,3-glucanase from *Cytophaga* which had no detectable β-1,4-glucan-4-glucanohydrolase activity under the conditions used. A sample of total hemicellulose, derived from the stem tissues of wheat plants harvested 132 days after germination, was incubated with the enzyme preparation under conditions already established to be optimal for the hydrolysis of oat endospermic β-glucan. When the degraded hemicellulosic material was hydrolysed, only a trace of glucose was detected on GLC examination of derived glycitol acetates.[29]

TABLE 5. β-GLUCANS FROM THE TOTAL HEMICELLULOSES OF WHEAT PLANTS

Tissue	Ratio of (1→3) to (1→4) linkages × 10^2 — Age in days from germination to harvest				DP — Age in days from germination to harvest				Percentage of glucan in dry tissue* — Age in days from germination to harvest				
	83	105	132	157	83	105	132	157	56	83	105	132	157
Stem	68	65	61	55	75	49	32	28	—	1·3	0·7	0·9	0·7
Top leaf	70	56	42	37	59	47	37	30	—	1·6	1·0	1·1	0·8
Middle leaves	—	46	49	34	65	56	53	30	—	1·3	0·9	0·9	1·2
Leaf and bottom leaf	—	50	44	27	—	46	41	32	1·3	1·2	1·9	1·3	—

* Values corrected for glucan present in delignification liquors.

It was concluded that no significant amount of cellulose or of degraded cellulose was present in the total hemicellulose. The DP's of the β-glucans in tissues from plants of different maturities were also determined. The method used for this purpose had to be specific for the β-glucans in the presence of the other hemicellulosic material from each total hemicellulose. De Wulf *et al.*[31] reduced samples of glycogen with NaB^3H_4 and determined the DP by counting the tritium in aliquots of hydrolysates. Inorganic material containing tritium complicated the analysis. In the present work the inorganic material was removed chromatographically. Rhamnose was used as an internal standard when studying each total hemicellulose. Various quantities of glucose were reduced with KB^3H_4 in the presence of a constant quantity of rhamnose and the count rates were determined for the glucitol and rhamnitol produced. The tritium was always incorporated by both sugars in equimolar amount. Samples of various dextrans of known molecular weight were reduced with KB^3H_4 in the presence of the standard. The reduced dextrans were hydrolysed and examined by PC-rhamnitol, glucitol and glucose were detected. The glucose content of an aliquot of each hydrolysate was determined by the glucose oxidase method and the rhamnitol and glucitol in another aliquot were separated by PC, eluted, and counted. There was reasonable

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agreement between the determined, and the known, values for the dextrans. The total hemicelluloses were studied as described earlier. It was found that the DP of the various β-glucans fell as each tissue matured (Table 5). The values were much lower than had been expected. Had there been any source of error due to incomplete reduction, or to the presence of modified reducing end-groups, then such errors would have caused the values to be lower than those quoted. Gel-filtration experiments indicated that the β-glucans did not have molecular weights significantly different from those of the xylan-type hemicelluloses.

The role of the β-glucans is unknown. Masuda *et al.*[32] have reported that a fungal β-1,3-glucanase promotes cell-wall elongation in oat coleoptile segments. The only known substrate in mature oat tissues on which such an enzyme could act is the β-glucan of the type now known to be present not only in oats, but in wheat and other grasses. The expansion of the cell-wall may be dependent on autolysis of polysaccharides in that wall. There is evidence that isolated cell-walls of oat and maize coleoptiles have bound enzymes possessing autolytic activity.[33,34] A maize system of this type autolysed and solubilized material equivalent to about 10% of the cell-wall weight in 8 hr at 37° and released glucose and also a glucan which on partial hydrolysis yielded 3-*O*-β-cellobiosyl-D-glucose.[35]

EXPERIMENTAL

General methods. PC was on Whatman No. 1 paper and the irrigant (v/v) was *n*-BuOH–pyridine–benzene–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 (12–16 hr) at 100°.

Plant material. The wheat plants, *Triticum vulgare* (var. Capel), were grown at the University Farm, Hillbrae, Aberdeenshire. The age of the plants was estimated from the approximate date of germination. 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, α-cellulose, and delignification material were isolated and studied as described previously.[2,4,29,30]

Determination of the DP_n *of β-glucans.* A series of solutions was prepared containing a constant quantity of rhamnose (0·527 μmol) and various quantities of glucose (0·079–0·474 μmol) in H_2O (0·5 ml). Each solution was treated with KB^3H_4 (5 μmol; 12·5 μCi) in H_2O (0·5 ml). After 12 hr the excess of borotritide was destroyed by the addition of HOAc (1 drop) and the borate was removed by passing the solution through a short column containing Borasorb (5 g). Each solution was taken to dryness in a stream of air and the sugars dissolved in H_2O (100 μl). The glucitol and rhamnitol in aliquots of each solution were separated by PC, and, after location of the sugars on side strips of the chromatograms, the sugars were eluted from the appropriate central areas into counting vials and the eluants freeze-dried. H_2O (1 ml) and 'Unisolve 1' (10 ml) were added and the emulsions were counted using a Nuclear-Chicago Liquid scintillation counter. Cpm glucitol/cpm rhamnitol plotted against mol glucose/mol rhamnose gave a straight line of gradient 1·02 ± 0·05 which showed that the tritium label had been incorporated in equimolar quantities. The irrigant had been carefully selected to ensure that no mobile tritiated inorganic material had a mobility similar to that of glucitol or of rhamnitol. Samples (*ca.* 5 mg) of each total hemicellulose were dissolved in 1 M NaOH (1 drop) and carefully neutralised with HOAc. Rhamnose (1·07 μmol) in H_2O (200 μl) was added followed by KB^3H_4 (5 μmol; 12·5 μCi) in H_2O (0·5 ml). The excess of borotritide was destroyed after 12 hr with 0·5 M H_2SO_4 (1 drop) and the reduced hemicellulose was hydrolysed with 0·5 M H_2SO_4 (2 ml). The cooled hydrolysates were neutralized with $BaCO_3$ and the borate removed as described above. Each solution was taken to dryness in a stream of air and the sugar dissolved in H_2O (200 μl). The glucose content of an aliquot (50 μl) was estimated by the glucose oxidase method. The labelled glucitol and rhamnitol in an aliquot (50 μl) were determined as described above. Determinations were carried out in duplicate with the exception of that on the material derived from 157-day-old top leaf.

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