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TOTAL HEMICELLULOSES FROM OAT PLANTS AT DIFFERENT STAGES OF GROWTH

J. S. G. REID* and K. C. B. WILKIE

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

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Abstract—Total hemicelluloses have been isolated from the leaves and stems of field-grown oat plants and from the leaves, roots and coleoptiles of plants grown in the laboratory. In any one part of the plant, with increasing maturity there is an increase in the percentage of xylose and a decrease in the percentage of glucose residues. These changes and accompanying changes in the percentages of galactose and arabinose residues are considered in terms of three pure hemicelluloses known to be present—an arabinoxylan, an acidic galactoarabinoxylan and a non-cellulosic glucan. Pure galactoarabinoxylans were isolated from the leaves of field-grown oat plants at different stages of growth. The results indicate that it is inappropriate to study the effect of growth on hemicellulosic composition solely by considering *pure* hemicelluloses.

INTRODUCTION

THREE pure hemicelluloses have been isolated from the leaf and stem tissues of the oat plant. They are an acidic arabinoxylan,[1] an acidic galactoarabinoxylan,[2] and a glucan;[3] there is as yet no evidence in these tissues of the presence of a glucoarabinoxylan of the type found by Hay in oat hulls.[5] Preliminary studies established that the total hemicellulose could be isolated without significant loss.[4] This being the case, hereafter it is assumed that any difference in the composition of the total hemicelluloses of oat plants at different stages of growth reflect variations due to the growth and are not merely caused by incidental fractionation or loss. Two series of growth studies were made. The first study was on plants grown in the field and harvested at various stages of maturation; the second was on plants grown in the laboratory. The object of the latter study was to investigate younger plants and also to study the roots of the plants uncontaminated by soil.

RESULTS

After collection, plants were stored under conditions to avoid modification of the total hemicelluloses.[4] Each plant was then divided into parts which were studied separately. In the case of the field-grown plants the first two collections consisted of leaf tissue only. The third collection was of plants where the stems and heads were enclosed within the inner leaves. Leaves and stems were separated and studied. Tissues of the fourth and fifth collections (Table 1) were approaching maturity. They were divided into three parts—the top leaves, the bottom leaves and the part of the stem between the head and the first node. It was not

* Present address: Department of Botany, University of Fribourg, Fribourg, Switzerland.

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2 J. S. G. REID and K. C. B. WILKIE, *Phytochem.* **8**, 2045 (1969).
3 C. G. FRASER and K. C. B. WILKIE, work to be published.
4 J. S. G. REID and K. C. B. WILKIE, *Phytochem.* **8**, 2053 (1969).
5 G. W. HAY, Ph.D. Thesis, University of Minnesota (1959).

practicable to study the whole of the stem as beneath the first node it was ensheathed by leaf tissue which was difficult to remove completely.

Table 1. Sampling details of field-grown oat plants

Sample	Height of plants at harvest *ca.* (cm)	Days after planting	Tissue
1	5	39	Leaf
2	30–40	76	Leaf
3	80	107	Leaf
3″			Stem
4	110	122	Top leaf
4′			Bottom leaf
4″			Stem
5	120	142	Top leaf
5′			Bottom leaf
5″			Stem

The various plant samples were delignified as outlined earlier using the modified procedure to obtain each total hemicellulose.[4] The content of total hemicellulose (Table 2) rose from 16 per cent in the youngest leaves to 36 per cent in the top leaves of the almost mature plant and fell to 28 per cent in the top leaves of the mature plant.

Table 2. Percentage total hemicellulose in oat plant tissues

	Leaf					Bottom leaf		Stem	
Sample	1	2	3	4	5	4′	5′	4″	5″
% Total hemicellulose in plant tissue	16	22	28	36	28	25	18	39	29

The results show distinct trends. In any one part of the plant it was found that increase of plant maturity was accompanied by an increase in the percentage of xylose and a decrease in the percentage of arabinose and glucose residues in each total hemicellulose. In the leaf total hemicelluloses the percentage of galactose residues also decreased. It was noted that the total hemicellulose from the top leaves, stems and the bottom leaves of plants of any one age differed in the percentage of non-acidic sugar residues they contained. The results are shown in the histogram (Fig. 1).

The decrease in the percentage of glucose residues is probably due to a change in the amount of the non-cellulosic glucan.[3] In the second histogram (Fig. 2) the results for glucose are omitted. The histogram shows that considering any one part of the plant the proportion of xylose residues rises greatly with increasing plant maturity whilst the proportion of arabinose residues decreases. The percentage of galactose residues either remains constant (stem and bottom leaf tissue) or decreases slightly (leaf tissue).

No simple interpretation of Fig. 2 is possible on the assumption that the sugars derive only from mixtures of the pure acidic galactoarabinoxylan (galactose:arabinose:xylose: 2·8:10:18·5)[2] and the pure acidic arabinoxylan (arabinose:xylose:3:97)[1] isolated earlier. But the results are compatible with the predominance in the young tissues of hemicellulosic material similar to the galactoarabinoxylan; in older tissue this material appears to have been diluted by hemicellulosic material more similar to the arabinoxylan. At the moment there is little quantitative information on the acidic residues, but this aspect is being investigated.[3, 6] It will be noted that there is a difference in the proportion of the sugar residues in

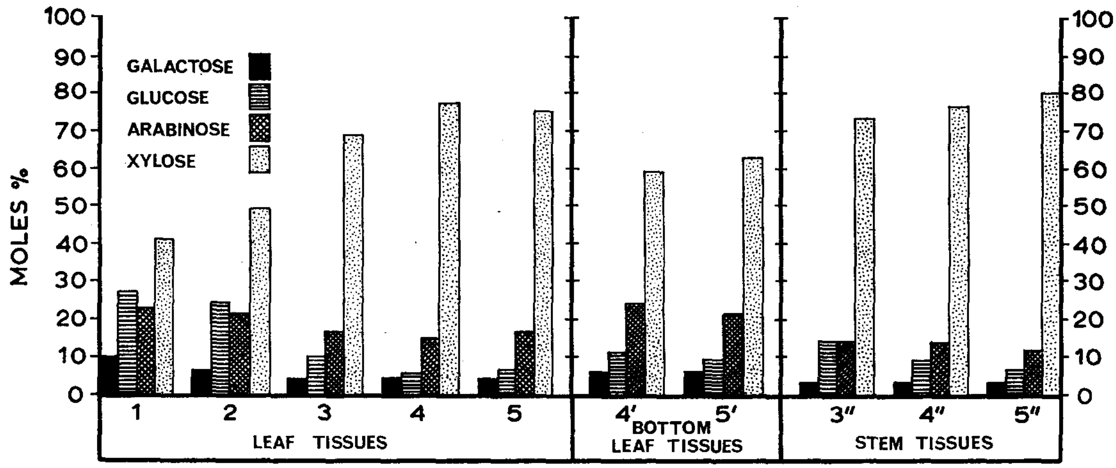

FIG. 1. SUGAR RESIDUE COMPOSITION OF TOTAL HEMICELLULOSES FROM FIELD-GROWN OAT PLANTS.*

* For details of samples see Table 1.

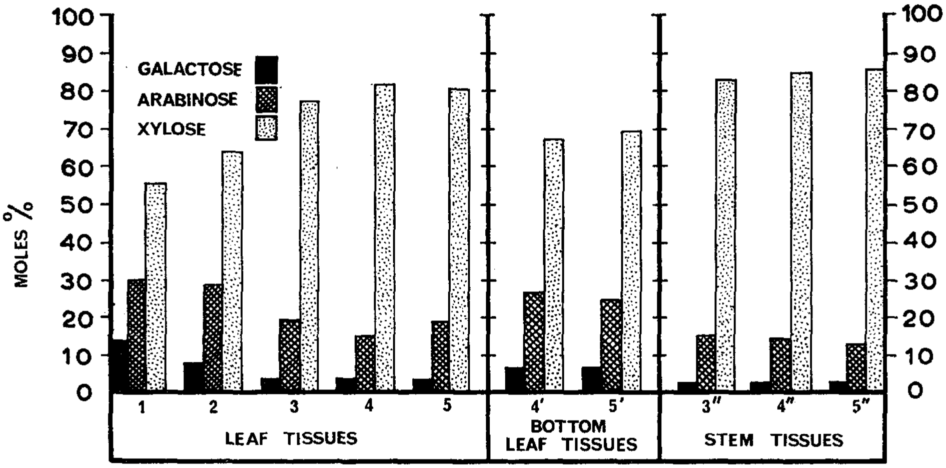

FIG. 2. SUGAR RESIDUE COMPOSITION OF TOTAL HEMICELLULOSES FROM FIELD-GROWN OAT PLANTS EXCLUDING GLUCOSE RESIDUES.*

* For details of samples see Table 1.

the bottom leaves compared to the top leaves in older plants. There is an apparent slight reversal in the growth-related trend with respect to xylose and arabinose in the total hemicellulose from sample 5 (top leaf)—this apparent anomaly may be due to the inclusion of leaves between those at the top and the bottom.

[6] A. J. BUCHALA and K. C. B. WILKIE, work to be published.

The studies on field-grown oat plants having shown that the composition of the total hemicellulose from different parts of the plant changes with plant growth, the trend in hemicellulose composition in even younger plants was investigated. Young oat plants were grown in the laboratory either on damp glass-fibre paper or on sand (Table 3).

The youngest seedlings (Y_1) consisted of the seed and root only. The other three samples (Y_2, Y_3 and Y_4) had distinct leaf, coleoptile and root tissues which were studied separately. The plants Y_1, Y_2, Y_3 were grown in the dark for the period stated. The plants Y_4 after germination in the dark were allowed to grow indoors under otherwise normal conditions of day and night until they were the same height as the plants in sample Y_3. The object was to determine whether plants which were photosynthesizing and those that were not differed in their total hemicellulose.

All tissue samples were delignified and the total hemicellulose isolated as described earlier. Chromatographic examination of a hydrolysate of a sample of each total hemicellu-

TABLE 3. SAMPLING DETAILS OF OAT PLANTS GROWN IN THE LABORATORY

Plant sample	Height at harvest	Days after planting	Tissue	Weight (mg)	Total hemicellulose (mg)
Y_1	0	5	Root	136	33
Y_2	2–4	6	Root	*	56
			Leaf	114	16
			Coleoptile	94	18
Y_3	8–13	7	Root	*	26
			Leaf	140	28
			Coleoptile	19	4
Y_4	8–13	—	Root	*	16
			Leaf	120	33
			Coleoptile	20	7

* Weights could not be determined due to presence of firmly adhering sand.
Plants Y_2–Y_4 were grown at 24° on sand and Y_1 on glass-fibre paper. Plants Y_1–Y_3 were grown in the dark and Y_4 in daylight.

lose showed that galactose, glucose, arabinose, xylose and acidic sugars were present. The relative proportions of the non-acidic sugars are shown in Fig. 3. Commonly there were also traces of rhamnose. The total hemicelluloses from leaves, roots and coleoptiles differed markedly at any one stage of growth. Considering any one part of the plant, there were again trends related to the growth of the plant. As in the case of field-grown oat plants, it was found that the more mature the plant the higher the proportion of xylose and the lower the proportion of glucose in each total hemicellulose. Within each part studied there was little change in the percentages of galactose and arabinose with increasing age. The total hemicellulose in each of the tissues of the plants grown in the dark and under more normal conditions of light differed very little. The growth-related trend earlier noted in field-grown plants is noted also in laboratory-grown plants.

Non-glucose residues were present in small proportion in the α-celluloses from both field- and laboratory-grown plants. The relative proportions of these residues in no way required re-evaluation of the data.

In order to extend this study, pure hemicelluloses were isolated from the tissues of plants at different stages of growth. Field-grown plants at different stages of growth were separated as appropriate into stem and leaf tissues. The procedures that led earlier to the isolation of a pure acidic galactoarabinoxylan (fraction *c*) from the leaf[2] were applied to these tissues also. The hemicellulosic materials isolated are referred to as "the galactoarabinoxylans" below

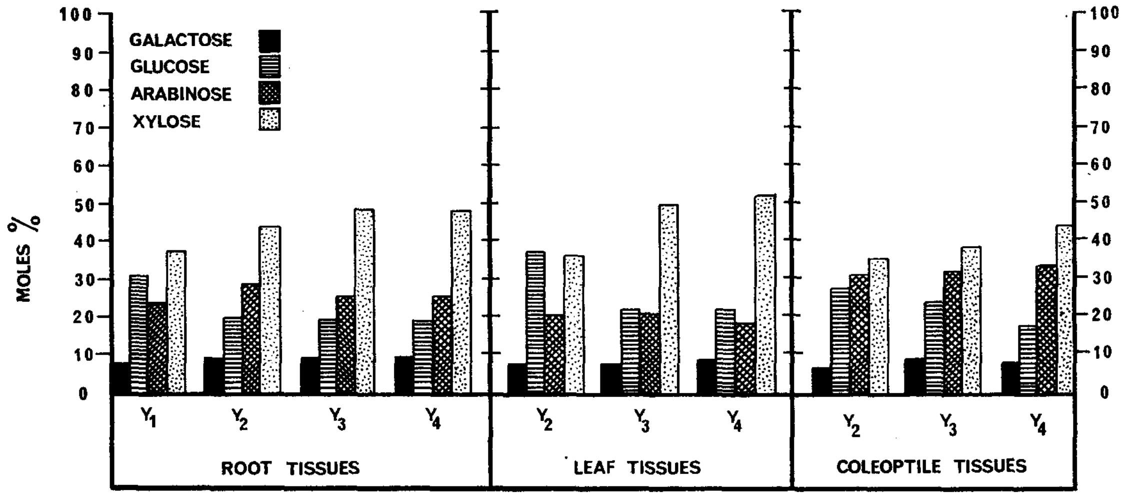

FIG. 3. SUGAR RESIDUE COMPOSITION OF TOTAL HEMICELLULOSES FROM LABORATORY-GROWN OAT PLANTS.*

* For details of samples see Table 3.

TABLE 4. NON-ACIDIC SUGAR COMPOSITION OF THE GALACTOARABINOXYLANS FROM THE TISSUES OF OAT PLANTS AT DIFFERENT STAGES OF GROWTH

Tissue	Composition of the galactoarabinoxylan (densitometric values)			
	Galactose	Arabinose	Xylose	(Glucose)*
Leaf				
1	5	10	16	3
2	4	10	15	0
3	5	10	14	2
4	5	10	15	2
5	4	10	15	2
Bottom leaf				
4′	4	10	14	3
Stem				
4″	2	10	15	2
5″	3	10	22	4

* Probably derived from a contaminating non-cellulosic glucan.

and in Table 4. It is of interest to note that when the water-soluble part of the various total hemicelluloses from the leaves of plants at different stages of growth were fractionated on DEAE-cellulose, under the conditions that led to the isolation of fraction *c*, galactoarabinoxylans were obtained that were very similar in their composition with respect to galactose, arabinose and xylose residues. On the other hand, under the same conditions of fractionation, the two stem hemicellulosic materials yielded galactoarabinoxylans that differed from one another and from the galactoarabinoxylans from the leaf tissues. The glucose found in the various hydrolysates probably derived from a non-cellulosic glucan of the type already isolated.[3] Acidic compounds were present in the hydrolysates and are under study.[3,6] A trace of rhamnose was detected in all hydrolysates other than that from tissue sample 5″.

Ultracentrifugal studies were carried out on the galactoarabinoxylans comparing them against the parent water-soluble part of the total hemicelluloses. The peak maximum given by the former always differed from that given by the latter. There were no irregularities in the Schlieren patterns indicative of polydiversity[4] such as might be expected from a galactoarabinoxylan and an arabinoxylan. It is probable that by varying the fractionation conditions other pure galactoarabinoxylans could be isolated that differed in their polymolecular and polydisperse composition (cf. Table 5). Five xylans have been isolated from wheat straw.[7–11] It may be that the precise molecular composition of these and other pure hemicellulosic heteroglycans is significantly influenced by the method of fractionation.

TABLE 5. PROPORTIONS OF SUGARS ESTIMATED BY DENSITOMETRY IN HYDROLYSATES OF HEMICELLULOSIC MATERIALS OBTAINED FROM THE WATER-SOLUBLE PART OF TOTAL HEMICELLULOSES BY FRACTIONATION ON DEAE CELLULOSE USING AQUEOUS KOAc AS ELUANT

	Galactose KOAc				Xylose KOAc				Glucose KOAc			
Plant sample	0·1 M	0·5 M	1 M	5 M	0·1 M	0·5 M	1 M	5 M	0·1 M	0·5 M	1 M	5 M
Leaf												
1	5	5	4	2	13	16	21	25	8	3	4	3
2	4	4	4	3	14	15	16	23	4	0	4	5
3	4	5	4	3	14	14	17	19	3	2	3	2
4	4	5	4	2	16	15	20	24	6	2	3	2
5	5	4	3	3	14	15	18	20	5	2	2	2
Bottom leaf												
4′	5	4	5	4	14	14	17	21	6	3	3	3
Stem												
4″	2	2	2	2	19	15	26	27	6	2	4	3
5″	2	3	3	2	23	22	27	29	5	4	5	2

Arabinose was present in all hydrolysates and is given the densitometric value of 10.

Whilst it is clear that for the investigation of molecular structure it is highly desirable that pure hemicelluloses be isolated it is equally clear that for comparative work of the present type it is more appropriate to study the total hemicellulose and to carry out supplementary studies on pure hemicelluloses.

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[11] G. O. ASPINALL and E. G. MEEK, *J. Chem. Soc.* 3830 (1956).

EXPERIMENTAL

Plant Material

Field-grown plants. The oat plants, *Avena sativa* (var. Blenda), were always taken from the same part of a field at the University Farm, Tillycorthie, Aberdeenshire. Analysis of the soil, a sandy loam, indicated that from an agricultural point of view its lime content was assessed as satisfactory, its potash content was satisfactory to slightly low and its phosphate content was slightly low. It contained 10 per cent of organic matter. The pH of the soil was 6·6. Immediately after harvesting, the plants (*ca.* 1 kg) were immersed in boiling ethanol, air-dried and stored at $-4°$ until required.

Laboratory-grown plants. Oat seeds (var. Blenda) were allowed to germinate at 24° in the dark either on damp glass-fibre paper or alkali-washed (24% KOH) sand. Immediately after harvesting the plants were treated with ethanol and studied.

Isolation of the Total Hemicellulose

Each plant tissue was delignified and the total hemicellulose isolated as described earlier.[4]

Fractionation of the Total Hemicelluloses of Field-Grown Oat Plants

Each total hemicellulose (140–190 mg) was dispersed in water (40 ml) using an MSE homogenizer. The insoluble material was separated and the solution fractionated on a column of DEAE-cellulose powder (Whatman DE 11—acetate). The column was successively irrigated with 0·1, 0·5, 1, and 5 M KOAc. The compositions of the hemicellulosic materials eluted are shown in Table 5.

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