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URONIC ACID RESIDUES IN THE TOTAL HEMICELLULOSES OF OATS

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Abstract—A new method for the estimation of uronic acid residues is described. The changes in *total hemicellulose* composition of the leaf and stem tissues of field-grown oat plants have been further examined. In any one such tissue, increased plant maturity is accompanied by an increase in the percentage of xylose residues and by decreases in the percentages of arabinosyl, glucosyl, and uronosyl residues. The ratio of 4-*O*-methyl-D-glucuronosyl to D-glucuronosyl residues increases with maturity.

INTRODUCTION

THERE are variations in the composition of the total hemicelluloses[1] from oat plant tissues at different stages of maturity.[2,3] When oat total hemicelluloses are isolated, there are no significant losses of non-glucosidic residues due to their having been retained in the α-celluloses[4] or due to such residues passing into the delignification liquors.[5] On the other hand, as much as 10% of β-glucan may be lost during delignification.

There is a lack of information on variations with plant maturity in the proportions of the two types of acidic residues known to be present in acidic xylans of the *Gramineae*. Such studies have now been carried out on field-grown oat plants.

RESULTS AND DISCUSSION

The plantstuffs and the total hemicelluloses have been described.[4] The molar proportions of the neutral sugars in hydrolysates were determined by GLC of derived glycitol acetates. Blake and Richards[6,7] have discussed analytical difficulties caused by incomplete removal of acidic sugars after hydrolysis. Lactonization is extensive during hydrolysis and the lactones are not hydrolysed under the conditions normally employed. Norstedt and Samuelson[8] showed that under certain conditions D-glucuronic acid and D-glucurono-3,6-lactone were efficiently removed from aqueous solution by a strongly basic anion-exchange resin in the bicarbonate form. In the present studies, hydrolysis conditions were simulated using a solution of a mixture of 10% D-glucuronic acid and 90% of neutral sugars. The uronic acid was completely removed within 4 hr.

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There are many problems in the determination of D-glucuronosyl and 4-*O*-methyl-D-glucuronosyl residues in hemicelluloses. The acidic conditions required for complete hydrolysis of the glycosiduronic linkages lead to extensive degradation of the uronic acids. Many colorimetric methods have been described[9] but they are not based on stoichiometric reactions and interference is caused by neutral sugar residues. Uronosyl residues can be estimated by determining the carbon dioxide released on decarboxylation. Under the conditions used, partial degradation of neutral sugars takes place and is accompanied by the release of some carbon dioxide. Perry and Hulyalkar[10] attempted, during hydrolysis, to reduce uronic acids *in situ* with platinum and hydrogen but reproducible results were neither obtained then nor when diborane was used as reductant.[11]

A different method has been used. The uronosyl residues in total hemicelluloses were esterified. The uronate residues were then reduced by sodium borotritide, and the labelled products determined. Before esterification, it was necessary to reduce the latent aldehydic groups of the end residues of β-glucan and xylan molecules in the various total hemicelluloses, otherwise labelled glucitol and xylitol from these residues would complicate the analyses. In earlier work quantitative esterification and reduction was rarely achieved. In the present work, on the other hand, the hemicellulosic carboxylic groups were completely esterified by a two-stage process. After treatment with saturated aqueous propylene oxide[12] the product was treated twice with diazomethane in ether. Reduction of the derived esterified total hemicelluloses with NaB^3H_4 followed by hydrolysis and PC examination of the hydrolysate showed that the only tritiated products were D-glucose, and to a lesser extent, 4-*O*-methyl-D-glucose.

Reductions by $NaBH_4$ carried out under alkaline conditions lead to partial saponification of uronates and consequently to low values for the uronic acid residues in the hemicelluloses.[13] Methyl methyl-D-galactopyranosiduronate was included as a standard which enabled corrections to be made, to allow for saponification, when estimating D-glucuronic acid and 4-*O*-methyl-D-glucuronic acid residues by the above procedure. It was realised that the standard would be more accessible to the reductant than would the esterified residues in each total hemicellulose.

Samples of the reduced and esterified total hemicelluloses were further reduced using a 5-fold excess of NaB^3H_4 in the presence of the above standard. In later work KB^3H_4 was used, as it is more stable in aqueous media. After hydrolysis, the labelled D-glucose, 4-*O*-methyl-D-glucose, and galactose were separated by PC and each was eluted from the paper and counted. The L-arabinose was also recovered from the chromatograms and estimated by the phenol/H_2SO_4 method. The quantity of uronic acid was determined relative to the standard. The proportion of uronic acid in the total hemicellulose was calculated from the amount of arabinose present.

The results show distinct trends (Table 1). In nearly all cases, it was found that as a particular plant matures the proportion of D-xylose in a tissue increases whereas the proportions of L-arabinose and of D-glucose decreases. The proportion of galactose remains constant or varies slightly. The results presented separately in histogram form (Fig. 1) differ from those determined directly (Table 1) in two respects. Firstly, glucose values have been omitted as these residues are not in the heteroxylans but in another pure non-acidic hemicellulose, a

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β-glucan.[14] Secondly, it has been assumed that all uronic acid residues after hydrolysis are present in the hydrolysates as aldobiouronic acids having D-xylosyl residues. The xylose values (Fig. 1) are accordingly increased above those determined directly (Table 1). It is apparent that the proportion of uronic acid in the xylans from any one type of tissue decreases as the plant ages. These results are in accord with those obtained by Waite and Gorrod[15] in their studies on rye, cocksfoot, and timothy grasses. It is of interest to note that the quantitative results for the neutral sugars are very similar to those obtained in earlier studies by Reid and Wilkie.[2] Such differences as these might at least in part be due to environmental factors affecting growth.

TABLE 1. COMPOSITION OF THE TOTAL HEMICELLULOSES OF MATURING OAT PLANTS IN 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 sowing to harvest	Rhamnose	Arabinose	Xylose	Galactose	Glucuronic acid	4-*O*-Methylglucuronic acid	Glucose
Stem	81	0·8	11·7	61·2	0·9	9·1	5·6	10·6
	106	0·4	11·2	62·8	0·9	9·4	4·4	10·6
	137	0·5	10·2	67·8	1·1	7·4	8·2	8·2
	162	0·4	10·8	70·7	1·2	7·6	3·3	6·1
Top leaf	106	0·5	16·5	58·7	1·9	11·4	5·0	5·9
	137	1·0	16·2	58·7	3·7	13·9	4·9	1·5
	162	0·7	15·1	62·0	3·0	8·6	7·5	3·0
Middle two leaves	106	1·0	18·0	54·1	5·3	10·5	4·6	6·3
	137	0·1	16·3	55·3	5·7	10·6	3·7	8·4
	162	0·3	14·3	58·4	3·4	10·5	6·1	6·9
Leaf and bottom leaf	56	0·8	19·5	34·5	3·6	16·5	3·8	20·9
	81	1·0	18·9	41·9	3·0	11·8	2·8	20·4
	106	1·3	17·2	48·0	3·3	14·5	2·9	12·6
	137	0·9	16·4	56·8	5·9	7·3	4·6	8·1

Values are not corrected for xylose residues remaining glycosidically linked to uronic acid residues after hydrolysis, but see Fig. 1.

As yet a degree of uncertainty must surround the interpretation of Fig. 1 in terms of the pure hemicelluloses isolated earlier, namely the pure acidic galactoarabinoxylan[16,17] and the pure arabino(4-*O*-methylglucurono)xylan.[18] But, the results are compatible with the predominance in the young tissues of hemicellulosic material similar to the acidic galactoarabinoxylan. In the more mature tissues the results are compatible with the dilution of this material by hemicellulosic material more similar to the acidic arabinoxylan. It is of interest to note that the proportion of 4-*O*-methyl-D-glucuronic acid in the leaf tissues increases relative to D-glucuronic acid as the plant matures. This is consistent with the work of Kauss and Hassid[19] who showed that 4-*O*-methylation of D-glucuronic acid residues probably occurred *in vivo* at the molecular level.

Samples of the soluble hemicellulosic materials derived from the leaf and bottom leaf tissues of the oat plants were examined by free-boundary electrophoresis. Using a 0·05 M borate buffer, a series of complex Schlieren curves was obtained. In some cases it was possible to distinguish up to six peaks but nearly all of these were generally poorly resolved.

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Peaks with mobilities similar to those obtained for the oat leaf and oat stem acidic galactoarabinoxylans and the oat endospermic β-glucan were observed. The complexity of the electrophoretograms suggests that it is inappropriate to completely interpret trends observed in hemicellulose composition in terms of the three known types of *pure* hemicellulose. Possibly the two pure xylans are members of a highly polydisperse, rather than of a polydiverse, population of molecules.[1] A study of the molecular population of xylans from oat tissues is now in progress.

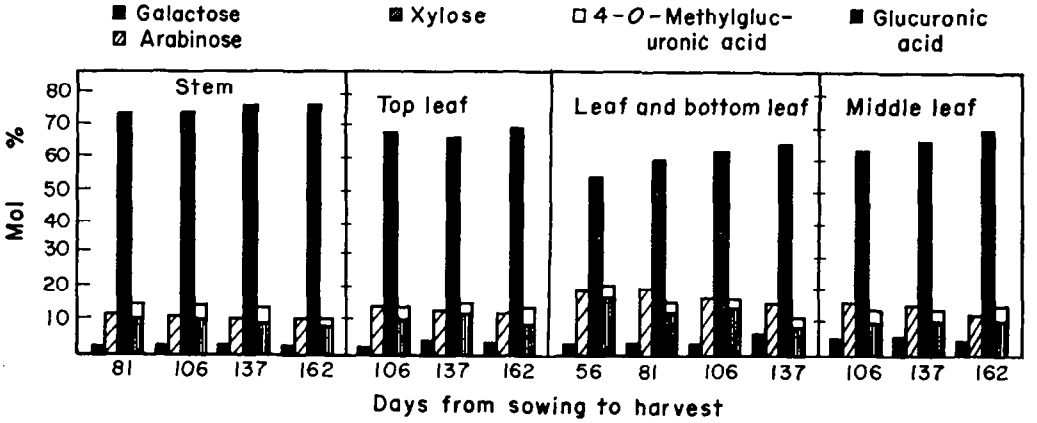

FIG. 1. COMPOSITION OF THE TOTAL HEMICELLULOSES FROM TISSUES OF OAT PLANTS AT DIFFERENT STAGES OF MATURITY.

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.

EXPERIMENTAL

General methods. PC was on Whatman No. 1 paper. The irrigants were: (A) EtOAc–pyridine–H_2O (72:20:23) and (B) *n*-BuOH–pyridine–benzene–H_2O (5:3:1:3). Alkaline $AgNO_3$ was used as chromatographic detection reagent. A Perkin–Elmer F-11 gas chromatograph was used for GLC and the columns (2 m × 3 mm i.d.) contained 3% ECNSS-M on Gas Chrom Q (100–120 mesh). Free-boundary electrophoresis was in a Tiselius–Svensson type apparatus at 4°. Radioactive samples were counted at a counting efficiency of *ca.* 21% in a Nuclear-Chicago liquid scintillation apparatus. The samples were emulsions of the aqueous solutions of sugars (1 ml) and of the scintillant 'Unisolve 1' (10 ml; supplied by Koch-Light Ltd.).

Isolation of the total hemicelluloses. The total hemicelluloses were isolated by the method of Reid and Wilkie[16] from the plant tissues described earlier.[4]

Examination of the total hemicelluloses. Neutral sugar residues. The relative neutral sugar compositions were determined by GLC of derived glycitol acetates.[4] *Acidic sugar residues.* A sample (*ca.* 20 mg) of each total hemicellulose was reduced with $NaBH_4$. After 48 hr the excess of borohydride was destroyed by HOAc and the solution was then dialysed against running water until free of inorganic material. The non-diffusible material was esterified by treatment with propylene oxide (37% v/v)[12] for 7 days at room temp. The propylene oxide was removed by evaporation under reduced pressure and the material was freeze-dried. Preliminary experiments showed that a further 2 treatments with ethereal CH_2N_2 were necessary for complete esterification of the hemicellulose. To a sample (5 mg) of each modified total hemicellulose suspended in H_2O (0·5 ml) was added methyl methyl-D-galactopyranosiduronate (0·46 μmol) and a solution (1 ml) of NaB^3H_4 (25 μCi) with a specific activity of 2·5 mCi/mmol. After 12 hr the excess of borotritide was destroyed by the addition of 0·5 M H_2SO_4 (1 drop). A further addition of M H_2SO_4 (2 ml) was made and the material was hydrolysed (100°; 12 hr) in a sealed tube. The cooled hydrolysate was passed through a short column containing Deacidite FF-IP (HCO_3^- form; 5 g) and Borasorb (5 g) and the exchange-resins were washed with H_2O (2 × 5 ml). The combined washings and eluate were reduced in vol. and aliquots examined by PC (irrigant B). In some irrigants tritiated inorganic material caused some difficulty. Glucose, galactose, 4-*O*-methylglucose, arabinose, and xylose were located on side-strips of each chromatogram. The central area of each chromatogram was excised appropriately to give strips carrying individually glucose, galactose and

4-*O*-methylglucose. The sugars were eluted quantitatively into counting vials with H_2O and were then freeze-dried. The arabinose was also eluted from the paper and estimated by the phenol/H_2SO_4 method.[20] To each of the counting vials H_2O (1 ml) was added to dissolve the sugar, and then scintillant (10 ml) was also added. The mixture was shaken and then counted to a relative standard error of 1%. Corrections were made for background count and quenching. The quantity of uronic acid was calculated from the following equation: D-glucuronic acid (μmol) = standard (μmol) × cpm *D*-glucose/cpm standard. The quantity of 4-*O*-methyl-D-glucuronic acid was calculated similarly. The proportion of each uronic acid in the total hemicellulose samples was calculated from the quantity of arabinose present.

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