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# QUANTITATIVE STUDIES ON THE POLYSACCHARIDES IN THE NON-ENDOSPERMIC TISSUES OF THE OAT PLANT IN RELATION TO GROWTH

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**Abstract**—It has been established by studying *total hemicelluloses* that the hemicellulosic composition of root, coleoptile, leaf and stem tissues of laboratory-grown and of field-grown oat plants, *Avena sativa* (var. Blenda), depends on tissue maturity. Studies on the composition of the α-celluloses and of the total hemicelluloses from tissues of different maturity confirm that statements made on total hemicellulose composition do not require re-evaluation to take account of non-glucosidic residues remaining in the α-celluloses. A reliable and accurate quantitative method for estimating neutral sugar residues in polysaccharides has been developed.

## INTRODUCTION

THIS paper is the sixth in a series dealing with the relationship between the composition of the hemicelluloses in non-endospermic tissues of the oat plant and the effect of tissue maturity on hemicellulosic composition. The terms *pure* and *total hemicelluloses* have been defined earlier.[1] Statements made on the composition of the various total hemicelluloses could be subject to qualification if there were losses of any cell-wall polysaccharides during the extraction procedures leading to isolation of total hemicelluloses, or if in the residual cellulosic material there were significant quantities of non-glucosidic residues.[2] It is convenient to retain the commonly used term 'α-cellulose' for the residual material but in the sense modified by the definition of total hemicellulose.[3]

Total recovery of all cell-wall polysaccharides has been sought.[1] It has been found that the α-cellulose and the total hemicellulose from each tissue together account for practically all the cell-wall polysaccharides. The small amount of polysaccharide material normally lost during delignification has been isolated and studied.[4] There is very little of it, and the loss of this material in no significant way affects the earlier results or statements made on the relationship between plant growth and hemicellulosic composition.

The total hemicelluloses from any one part of the oat plant at different stages of maturity vary in composition; there are also, as is to be expected, variations in the hemicellulosic composition of different parts of the plant at one stage of maturity.[2] In any one part of the plant with increasing maturity there is an increase in the percentage of xylose and a decrease both in the percentage and in the *total* amount of glucose residues. These changes, and changes in the proportions of arabinose, galactose and xylose residues, have been interpreted in terms of two pure hemicelluloses—an acidic arabinoxylan[5] and an acidic

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[1] J. S G. REID and K. C B. WILKIE, *Phytochem* 8, 2045 (1969)
[2] J S. G REID and K C B. WILKIE, *Phytochem* 8, 2059 (1969).
[3] H. J ROGERS and H R. PERKINS, *Cell Walls and Membranes*, Spon, London (1968).
[4] C. G FRASER and K. C. B. WILKIE (to be published)
[5] G. O ASPINALL and K. C. B. WILKIE, *J Chem. Soc.* 1072 (1956).

galactoarabinoxylan.[6] At least a high proportion, possibly all, of the glucose residues found in total hemicelluloses are now known to be present in a $\beta(1 \rightarrow 4')$ and $\beta(1 \rightarrow 3')$ linked glucan.[7,8] The results obtained earlier for field-grown and young laboratory-grown plants have been parallelled by results obtained for plants grown in a subsequent year.[9]

The present report deals with the various α-celluloses remaining after the isolation of total hemicelluloses, and the work is an essential corollary to the earlier studies on these total hemicelluloses.[1,2] Certain of the quantitative methods have been improved and although in the present report the concern is primarily with α-celluloses the methods developed have been used in studies on total hemicelluloses; these will be reported later.[9]

## RESULTS AND DISCUSSION

Field-grown plants were harvested at different stages of growth and dissected to isolate the tissues shown in Table 1. The various tissues specified were delignified and the α-celluloses isolated after the removal of the various total hemicelluloses. Before satisfactory

TABLE 1. DESCRIPTION OF OAT PLANTS GROWN IN THE LABORATORY AND IN THE FIELD AND OF THE TISSUES SUBJECTED TO STUDY

| Age of plant (days) | Height of plant (cm) | Description of plant tissues |
|---|---|---|
| *Laboratory-grown plants** | | |
| 5 | 6 | Root, leaf and coleoptile |
| 8 | 8 | Root, leaf and coleoptile |
| 10 | 11 | Root, leaf and coleoptile |
| *Field-grown plants*† | | |
| 56 | 18 | Leaf and coleoptile |
| 81 | 29 | Leaf, very short stem and inflorescence |
| 106 | 60 | Bottom, top and two middle leaves |
| 137 | 120 | As above; plant green and mature |
| 162 | 120 | As above but bottom leaf withered |

* Days from germination to time of harvest

† Days from sowing to time of harvest (inflorescence at 81 days and bottom leaf at 162 days not studied).

studies could be carried out on these α-celluloses it was necessary to examine the complete sequence of methods that collectively or individually might affect quantitative results. Arabinose, galactose, glucose, rhamnose and xylose residues are present in the polysaccharides in the cell-wall. The five corresponding free sugars were subjected to the analytical procedures to be employed in the study of polysaccharides, that is, the monosaccharides were treated under hydrolysis conditions, subjected to borohydride reduction and acetylation, and to normal but minimal procedures of handling. It is appreciated that the degradation of a sugar residue present in a polysaccharide may not parallel precisely the degradation of the same sugar in the free state. No studies were carried out specifically on the oligouronic acids under the acidic condition of hydrolysis.

[6] J S. G. REID and K C B WILKIE, *Phytochem* **8**, 2053 (1969)
[7] C. G FRASER and K C B WILKIE, *Phytochem* **10**, 199 (1971)
[8] A J BUCHALA and K C. B. WILKIE, *Naturwiss* **10**, 496 (1970)
[9] A J BUCHALA and K C B. WILKIE, *Phytochem.* (in press)

All qualitative and quantitative determinations of glycitol acetates were carried out by GLC; the quantitative estimations were based upon measurement of peak areas by triangulation. The molar response of the flame ionization detector to each glycitol acetate was determined with reference to erythritol tetraacetate.

In the studies on the free sugars it was found that no correction factor need be applied when carrying out quantitative studies on arabinose, galactose or xylose but that in the case of glucose and rhamnose the amount of sugar estimated was consistently lower than the amount of sugar present. There was, however, a linear relationship between the proportion lost and the amount of sugar originally present. Relative to arabinose (= 1·0) the correction factors used in this work are 1·05 for glucose and 1·09 for rhamnose. It is of interest to note that when the acetylation of D-glucitol was carried out at 100° a correction factor of 1·43 had to be employed whereas when the acetylation was carried out at 120° the

TABLE 2. QUANTITATIVE DETERMINATION OF MONOSACCHARIDES EMPLOYING CORRECTION FACTOR

| | Molar ratios of monosaccharides in mixtures* | | | | |
|---|---|---|---|---|---|
| | Arabinose | Galactose | Glucose | Rhamnose | Xylose |
| Quantity present | 1·00 | 0 81 | 0 83 | 0 93 | 3·51 |
| Determined value | 1 00 | 0 84 | 0 84 | 1 00 | 3 48 |
| Quantity present | 1 00 | 0·32 | 0·17 | 0 19 | 0 35 |
| Determined value | 1 00 | 0·34 | 0 16 | 0·21 | 0 34 |
| Quantity present | 1 00 | 0 44 | 1 34 | 0 50 | 0 94 |
| Determined value | 1 00 | 0 45 | 1 32 | 0 49 | 0 89 |
| Quantity present | 1 00 | 0 81 | 8 27 | 0 93 | 0 87 |
| Determined value | 1·00 | 0 83 | 8 29 | 0·94 | 0 86 |

* Determinations by GLC; total amount of the monosaccharides was *ca* $5 \times 10^{-7}$ moles in 2 $\mu$l.

correction factor was reduced to 1·05; acetylation was accordingly carried out in all cases at 120°. This temperature change did not affect the correction factors for other glycitols. Crowell and Burnett[10] deduced that it was more difficult to acetylate borohydride reduced D-glucose and L-rhamnose in the presence of borate than it was to acetylate xylose and arabinose; the present work agrees with these results but there was no evidence that it was difficult to acetylate D-galactitol fully. The validity of employing the correction factors given above to correct quantitative values obtained by GLC was vindicated by carrying out quantitative estimations on mixtures of the five sugars and correcting the values obtained (Table 2); a good correlation was obtained in all cases.

After delignification of the plant tissues they were treated with 5% and 24% potassium hydroxide and the total hemicelluloses and α-celluloses were then isolated. It will be noted in Fig. 1 that the proportion of α-cellulose and of total hemicellulose increased in each tissue in nearly all cases as the plant matured.

The α-celluloses and total hemicelluloses were hydrolysed and the hydrolysates quantitatively studied by GLC after conversion of the sugars to the corresponding glycitol acetates

[10] E. P. CROWELL and B B BURNETT, *Analyt Chem.* **39**, 121 (1967).

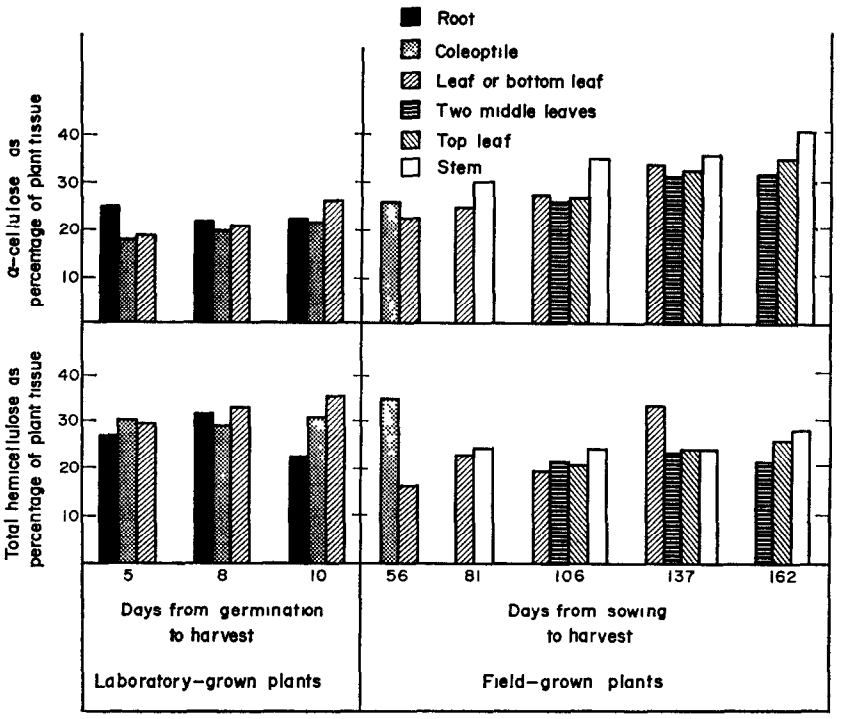


FIG 1.

TABLE 3 NON-GLUCOSIDIC RESIDUES IN THE α-CELLULOSE OF LABORATORY-GROWN OAT PLANTS

| | Days from time of germination to harvesting | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 5 | | | 8 | | | 10 | | |
| | Root | Coleoptile | Leaf | Root | Coleoptile | Leaf | Root | Coleoptile | Leaf |
| Non-glucosidic residues (% in α-cellulose) | 6 80 | 6 05 | 7·74 | 7 27 | 10 2 | 6·50 | 6 89 | 7 50 | 10 4 |
| Non-glucosidic residues in α-cellulose* | 6 54 | 2 97 | 4 14 | 4 68 | 6 01 | 3·88 | 6 47 | 5 05 | 7·20 |
| Xylose/arabinose ratio in α-cellulose | 0 72 | 0 91 | 0 97 | 0 84 | 1 14 | 1·12 | 1 01 | 1 08 | 0 98 |
| Xylose/arabinose ratio in total hemicellulose | 1·36 | 1 31 | 2 13 | 1 88 | 1 34 | 2·16 | 2 04 | 1 36 | 2 16 |
| Xylose/arabinose ratio —modified value† | 1·32 | 1·30 | 2·11 | 1 83 | 1 33 | 2 12 | 1 97 | 1 35 | 2 08 |

* 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

TABLE 4. NON-GLUCOSIDIC RESIDUES IN THE α-CELLULOSES OF FIELD-GROWN OAT PLANTS

| | Days from time of sowing to harvesting | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 56 | | 81 | | 106 | | | | 137 | | | | 162 | | |
| | Coleoptile | Leaf | Leaf | Stem | Bottom leaf | Two middle leaves | Top leaf | Stem | Bottom leaf | Two middle leaves | Top leaf | Stem | Two middle leaves | Top leaf | Stem |
| Non-glucosidic residues (% in α-cellulose) | 11 5 | 2 19 | 5 07 | 9 70 | 8 42 | 4 82 | 3 56 | 3 22 | 6 00 | 4 25 | 4 68 | 1 86 | 3·96 | 2 30 | 1·89 |
| Non-glucosidic residues in α-cellulose* | 8 52 | 3 31 | 5 56 | 12·1 | 12 4 | 5 77 | 4·81 | 4 63 | 6 12 | 5 88 | 6 57 | 2 75 | 5·66 | 5·12 | 2·77 |
| Xylose/arabinose ratio in α-cellulose | 1 12 | 1 82 | 2 33 | 1·81 | 1 75 | 1 63 | 1·59 | 1·66 | 1 55 | 1 49 | 1 61 | 1·92 | 1·46 | 2 24 | 2·20 |
| Xylose/arabinose ratio in total hemicellulose | 1 62 | 1·77 | 2 21 | 5 22 | 2 79 | 3 00 | 3·56 | 5·58 | 3 46 | 3 40 | 3 63 | 6·58 | 4 08 | 4 11 | 6·54 |
| Xylose/arabinose ratio —modified value† | 1 58 | 1·77 | 2 10 | 4 81 | 2 66 | 2·92 | 3·47 | 5 40 | 3·34 | 3·29 | 3 50 | 6 45 | 3·91 | 4 01 | 6 42 |

*, † See footnotes to Table 3.

(Tables 3 and 4). At high sensitivity a number of unidentified peaks were observed; together they accounted for less than 0·5% of the total area under the peaks of each chromatogram. Perhaps they derive from acetylated degradation products.

In Tables 3 and 4 quantitative values are listed. It will be noted that non-glucosidic residues were present in the various α-celluloses. The ratio of xylose to arabinose residues in the α-cellulose and the corresponding ratio in the total hemicellulose differed; in some cases this was quite marked, but the important conclusion of the work is that even if the non-glucosidic residues in the α-cellulose were present in hemicellulosic type material then a correction factor applied to the total hemicellulose to take into account this unextracted material would in no significant way alter the xylose to arabinose ratio in what has been satisfactorily defined as the total hemicellulose[1] (see the last two lines of Tables 3 and 4).

## EXPERIMENTAL

*Quantitative Estimations by GLC*

Quantitative separations and qualitative identifications were carried out using a Perkin-Elmer gas chromatograph F11 fitted with a dual column flame ionization detector and a Hitachi Perkin-Elmer recorder 159. The glass columns (3 mm i d × 2 m) were packed with 3% ECNSS-M on 100–120 mesh Gas Chrom Q (Applied Science Laboratories, State College, Pa, U S A) $N_2$ was the carrier gas at *ca* 75 ml/min and 185° All quantitative determinations were carried out in triplicate with excellent reproducibility.

The glycitol acetates used as standards in this investigation were (relative molar response on GLC in brackets)—erythritol tetraacetate, m p. 85° (1 00), L-rhamnitol pentaacetate (a syrup) (0 90); L-arabinitol pentaacetate, m p. 75–76° (0 88); xylitol pentaacetate, m p 58–59° (0 91); 2-deoxy-D-*arabino*hexitol pentaacetate, m p. 85–86° (0 87); D-galactitol hexaacetate, m p 170–171° (0 92) and D-glucitol hexaacetate, m p 98–99° (1 16). The glycitols, other than erythritol, were prepared from the corresponding free sugar and converted to the fully acetylated derivatives.

Erythritol tetraacetate was used to calibrate the GLC detector and this acetate and all glycitol acetates subsequently studied gave linear responses The molar quantities of the various acetates studied aimed to cover the range within which subsequent work was carried out The relative molar response (RMR) of erythritol tetraacetate and of the other glycitol acetates are given in brackets above. The molar values were based in all cases on measurement of peak areas on the chart recorder and the RMR calculated as follows·

$$\text{RMR} = \frac{(\text{area of glycitol acetate})(\text{moles of erythritol tetraacetate})}{(\text{area of erythritol tetraacetate})(\text{moles glycitol acetate})}.$$

A series of mixtures of the five monosaccharides was made up using standard solutions After preliminary studies to establish optimum conditions for the quantitative work, a standard procedure was adopted as follows An aliquot (1 ml) of each solution containing a total of 5–10 mg of monosaccharides was heated in a sealed tube for 12 hr at 100° with $H_2SO_4$ (0 5 M; 3 ml) The cooled hydrolysate was taken to pH 7 by the addition of an excess of Deacidite FF-IP ($HCO_3^-$ form) (*ca* 10 g) The resin was filtered off on a glass-wool pad and washed with water (2 × 2 ml) To the filtrate and wash liquors $NaBH_4$ (60 mg) was added After 18 hr the solution was neutralized by the addition of a slight excess of glacial HOAc and the solvent removed under reduced pressure $H_3BO_4$ was removed by codistillation with successive volumes of MeOH (5 × 3 ml) $Ac_2O$ (2 ml) was added, the mixture heated in a sealed tube at 120° for 5 hr, and allowed to cool A standard erythritol tetraacetate solution ($1{\cdot}335 \times 10^{-2}$ moles) was then added plus $H_2O$ (5 ml) and the mixture taken to dryness. Further $H_2O$ (5 ml) was added to complete the hydrolysis of the $Ac_2O$ and the mixture again taken to dryness The products were treated with hot $CH_2Cl_2$ (*ca* 5 ml) and the extract reduced in volume to *ca* 0 1 ml. Samples (2 μl) were withdrawn and examined immediately by GLC

*Study of Oat-plant Polysaccharides*

*Field-grown plants* The oat plants, *Avena sativa* (var Blenda) were harvested from one part of a field at the University Farm, Tillycorthie, Aberdeenshire, in 1969 Although this was a later year, the growth conditions were similar to those of the plants studied earlier [1,2,6] Immediately after harvesting, the plants were dissected into the parts detailed in Table 1. The tissues were boiled in EtOH for 20 min, air-dried, passed through a Casella Mill (No 16 mesh, British Standard, square hole mesh 0 16 cm per side) and stored at −4°.

*Laboratory-grown plants* Oat seeds (var Blenda) were steeped for 6 hours in saturated $Ca(OCl)_2$ to avoid the danger of fungal contamination during growth [11] The seeds were then thoroughly washed and

[11] E. J. HEWITT, *Sand and Water Culture Methods used in the Study of Plant Nutrition*, Commonwealth Agricultural Bureaux, London (1966)

allowed to germinate over a period of 2–3 days in the dark on acid-washed sand at 24°. Growth was then continued in clean air in the laboratory but under otherwise normal conditions of day and night. Immediately after laboratory harvesting, the plants were dissected (Table 1) and the tissues treated as previously.

*Isolation of the total hemicelluloses and α-celluloses.* The moisture content of each plant tissue was determined and, after delignification of the tissues, each was treated successively with 5% and 24% KOH solutions After quantitative isolation, the moisture and ash contents[12] of each total hemicellulose were determined and the moisture content of the corresponding α-cellulose was also determined. A small proportion of several volatile degradation products was detected when the hydrolysates of the various α-celluloses were quantitatively examined. A convenient standard, 2-deoxy-D-glucose, was introduced after the hydrolysis of each α-cellulose and before the reduction. The entire hydrolysis of each α-cellulose sample (*ca.* 20 mg) was carried out in a single hydrolysis tube thereby avoiding handling losses. To each sample, $H_2SO_4$ (72%, w/w; *ca.* 0·5 ml), was added and the tube heated at 37° for 1 hr by which time dissolution of each α-cellulose was complete The solution was diluted to *ca.* 3 ml and the heating continued at 100° for 12 hr.

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[12] L. E. Wise, M. Murphy and A. A. D'Addieco, *Paper Trade J.* 122, 35 (1946).