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THE RATIO OF $\beta(1\rightarrow3)$ TO $\beta(1\rightarrow4)$ GLUCOSIDIC LINKAGES IN NON-ENDOSPERMIC HEMICELLULOSIC β-GLUCANS FROM OAT PLANT (*AVENA SATIVA*) TISSUES AT DIFFERENT STAGES OF MATURITY

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Abstract-The β-glucans in non-endospermic tissues of field-grown oat plants have been studied in relation to plant maturity. There is a fall in the ratio of $\beta(1\rightarrow3)$ to $\beta(1\rightarrow4)$ D-glucosidic linkages in the β-glucans *in* the total hemicelluloses isolated from stem, leaf and hull tissues of increasing maturity.

INTRODUCTION

THIS paper is the eighth in a series concerned with the relationship between the maturity of oat plant (*Avena sativa*) tissues and the hemicellulosic composition of such tissues. The terms ***pure*** and ***total*** hemicelluloses have been defined earlier.[1]

It was established by Fraser and Wilkie[2] that a β-glucan was present in oat leaf. It had $\beta(1\rightarrow3)$ and $\beta(1\rightarrow4)$ glucosidic linkages in the ratio of 1 to 1·65, was unbranched and had a high D.P. The glucan isolated accounted for 3·3 per cent of the total hemicellulose and for about one fifth of the glucosidic residues present in the total hemicellulose. Similar glucans were isolated from oat stem, coleoptile and root tissues and have also been isolated by Buchala and Wilkie from fully mature barley, rye and wheat stem tissues.[3] Fraser and Wilkie[4] isolated *pure* glucans from the water-soluble portion of total hemicelluloses from oat leaf and stem tissues at different stages of maturity with the object of investigating any relationship between plant maturity and β-glucan composition. It was concluded that in this, as in other cases, the procedures that lead to the isolation of pure hemicelluloses yield materials that are atypical in their polydispersity,[1] and probably in their polymolecularity,[1] of the cognate hemicelluloses as they occur in the parent total hemicelluloses. Pure glucans were accordingly concluded to be unsuitable for comparative studies of the type envisaged. There was no evidence in any of the studies carried out in our laboratories on oat leaf, stem, coleoptile and root tissues of any glucoarabinoxylan of the type isolated from oat hull by Hay.[5] Also there was neither any indication of the presence of any other hemicellulose containing glucosidic residues nor of cellulose, degraded cellulose nor starch. This being the case, it appeared to be appropriate to translate the amount of glucosidic residues in total hemicelluloses in terms of glucans of the type isolated earlier.[1] Reid and Wilkie[6] noted that with increasing plant maturity there was a decrease in the *amount* of glucosidic

[1] J. S. G. REID and K. C. B. WILKIE, *Phytochem.* **8,** 2045 (1969).
[2] C. G. FRASER and K. C. B. WILKIE, *Phytochem.* 10, 199 (1971).
[3] A. J. BUCHALA and K. C. B. WILKIE, *Naturwissenschaften 57,496 (1970).*
[4] C. G. FRASER and K. C. B. WILKIE, *Phytochem.* **10,** 1539 (1911).
[5] G. W. HAY, Ph.D. Thesis, University of Minnesota (1959).
[6] J. S. G. REID and K. C. B. WILKIE, *Phytochem.* **8,** 2059 (1969).

residues in any one type of non-endospermic tissues (leaf, stem, coleoptile and root) from field and laboratory grown oat plants. It is possible that the β-glucan is metabolized as the plant matures.

RESULTS AND DISCUSSION

In the present studies the ratio of β(1→3) to β(1→4) linked glucosidic residues in total hemicelluloses has been determined for various tissues at different stages of maturation.

Three types of pure hemicellulose are present in non-endospermic oat total hemicelluloses namely a (4-*O*-methyl-glucurono)arabinoxylan,[7] a (4-*O*-methyl-glucurono)galactoarabinoxylan[8] and a β-glucan.[2] On periodate oxidation of β-glucans followed by reduction and acidic hydrolysis the β(1→3) linked residues would yield D-glucose, and the β(1→4) linked residues, erythritol. Neither of the other types of hemicellulose would yield either erythritol or D-glucose under such conditions. Periodate oxidation of total hemicelluloses in the above way followed by determination of the D-glucose and erythritol should therefore enable the ratio of (1→3) to (1→4) glucosidic links to be established for all the glucan present in each total hemicellulose. Such a procedure would avoid the loss inescapable when the earlier approach was employed involving the isolation of pure glucans. Enzymic studies reported below showed the absence of cellulose and of degraded cellulose and there was no indication of the presence of any starch on testing with iodine.

Studies were carried out using a β-1,3-glucanase from *Cytophaga*. This enzyme preparation did not catalyse the hydrolysis of cellulose, carboxymethylcellulose, hydroxypropylmethylcellulose, water-soluble cellulose dextrins, cellobiose, cellotriose or cellotetraose but it catalysed the hydrolysis of laminaran, oat endospermic glucan, and the β-glucans in the total hemicelluloses. The enzyme preparation lacked β-1,4-glucan 4-glucanohydrolase activity. A sample of oat stem total hemicellulose was treated for 120 hr with the β-1,3-glucanase under the conditions established to be optimum for the catalytic hydrolysis of oat endospermic glucan.[9] After this, the surviving total hemicellulose on recovery yielded only traces of glucose on acidic hydrolysis; determinations were made by GLC examination of TMS derivatives. It was concluded that, at most, the total hemicellulose contained only a trace of cellulose or of degraded cellulosic material-too little to be of any significance in the subsequent comparative studies on the various total hemicelluloses.

A sample of one oat stem total hemicellulose was oxidized by $NaIO_4$ and after reduction of the resultant polyaldehyde with $NaBH_4$ the product was hydrolysed by CF_3COOH.[10] The hydrolysate was reduced again by $NaBH_4$ and the derived glucitol hexaacetate and erythritol tetraacetate determined by GLC;[11] they were present in the molar ratio of 1 to 2·40. This value was confirmed by an independent method based upon the determination of HCHO released by the glucitol and erythritol present in the above hydrolysate-a molar ratio of 1 to 2·3 was obtained. The GLC method was concluded to be fully satisfactory and was used in the study of all the total hemicelluloses listed in Tables 1 and 2. It was found that for any one tissue the ratio of β(1→3) to β(1→4) glucosidic linkages in the total hemicelluloses *decreased with increasing tissue maturity.*

[7] G. O. ASPINALL and K. C. B. WILKIE, *J. Chem. Soc.* 1072 (1956).
[8] J. S. G. REID and K. C. B. WILKIE, *Phytochem.* 8.2053 (1969).
[9] A. J. BUCHALA and K. C. B. WILKIE, To be published.
[10] P. ALBERSHEIM, D. J. NEVINS, P. D. ENGLISH and A. KARR, *Carbohydrate Res.* 5,340 (1967).
[11] A. J. BUCHALA, C. G. FRASER and K. C. B. WILKIE, *Phytochem.* 10, 1285 (1971).

TABLE 1. PERCENTAGE OF β-GLUCAN IN PLANT TISSUES OF FIELD-GROWN PLANTS AND RATIO OF $(1\rightarrow3)$ TO $(1\rightarrow4)$ GLUCOSIDIC LINKAGES"

Tissue	Age-days from sowing to harvest	Percentage of glucose in total hemicellulose	Ratio of $(1\rightarrow3)$/ $(1\rightarrow4)$ glucosidic linkages*
Stem	81	12.4	0·57
	106	12.3	0·45
	137	9·4	0·42
	162	6·9	0·35
Leaf and bottom leaf	56	26.3	0·55
	81	23·9	0·50
	106	15·3	0·40
	137	9·2	0·26
Top leaf	106	7·1	0.48
	137	1·9	0·41
	162	3.6	0·29
The two middle leaves	106	7·4	0·37
	137	9·8	0·27
	162	8.3	0.30
Hulls	106	4.8	0·51
	137	5·0	0·43
	162	7·5	0.39

* Ratio based on GLC determinations of glucitol hexaacetate and erythritol tetraacetate in periodate-oxidized, reduced, hydrolysed and reduced total hemicelluloses after acetylation of the final products.

Having now established that there is a definite relationship between these ratios and plant maturity it was concluded that it would be desirable to carry out further studies under controlled growth conditions; such conditions were impossible in the field work so far undertaken. It is appreciated that the age of the plant may not be the best parameter for the study of the relationship between maturation and total hemicellulose composition.

A high β-1,3-glucan 3-glucanohydrolase activity was noted in oat coleoptiles.[12] It is possible that there is a comparable type of activity in the various other, and more mature, tissues; an activity that leads to a progressive reduction, with increasing tissue maturity, of $\beta(1\rightarrow3)$ glucosidic linkages in the total hemicellulosic glucans. Similar studies to those reported are now being carried out on non-endospermic glucans in total hemicelluloses from barley and wheat plants at different stages of maturation. It is possible that the non-endospermic glucans may be cell-wall reserve polysaccharides but preliminary experiments do not confirm or contradict this postulation. A check was made to ascertain if there were any obvious effects on the ratio of the two glucosidic linkages in plants unable to photosynthesise carbohydrates necessary for growth. Oat plants, 64 days after sowing, were deprived of normal daylight for 71 hr and then the glucans in the total hemicelluloses from the top leaf and stem tissues were compared against those in the total hemicelluloses of plants grown under normal conditions of illumination. On such comparatively gross

[12] A. N. J. HEYN, *Arch. Biochem. Biophys.* 132,442 (1969).

TABLE 2. PERCENTAGE OF β-GLUCANS IN PLANT TISSUES OF LABORATORY-GROWN PLANTS AND RATIO OF (1→3) TO (1→4) GLUCOSIDIC LINKAGES"

Tissue	Age-days from germination to harvest	Percentage of glucose in total hemicellulose	Ratio of (1→3)/(1→4) glucosidic linkages*
Leaf	5	24.9	0.63
	8	21.3	0.47
	10	18.3	0.26
Coleoptile	5	18.7	0.85
	8	17·2	0·60
	10	14·9	0.42
Root	5	17.1	075
	8	24.4	0·71
	10	21.9	0·31

* Ratio based on GLC determinations of glucitol hexaacetate and erythritol tetraacetate in periodate-oxidized, reduced, hydrolysed and reduced total hemicelluloses after acetylation of the final products.

structures as fairly mature stems and leaves no differences were noted; it is concluded that it would be more appropriate to check on the possibility that these glucans are reserve polysaccharides by studying meristematic tissues or tissues undergoing cell-wall elongation. Such studies are now being undertaken.

EXPERIMENTAL

General methods. Paper chromatography was on Whatman No. 1 paper using EtOAc–pyridine–H_2O (72:20:23 v/v) as solvent. Chromatograms were treated with alkaline $AgNO_3$. A Perkin-Elmer F-11 gas chromatograph was used with a column (2 m x 0·3 cm i.d.) packed with *A*, 3% ECNSS-M on gas-chrom Q (100-120 mesh) at 170" with a nitrogen flow rate of ca. 75 ml/min or *B* 3% E301 (silicone gum rubber) on AW-HMDS celite (100–120 mesh) at 150" with a nitrogen flow rate of cu. 60 ml/min. Solvents were removed by rotary evaporation below 40". Polysaccharides were hydrolysed in sealed tubes with 0·5 M H_2SO_4 (12-16 hr, 100°) and the hydrolysate neutralized by $BaCO_3$.

Plant tissues and total hemicelluloses. The plant tissues used and the derived total hemicelluloses are fully described earlier." None of the total hemicelluloses gave a starch-iodine coloration.

Periodate oxidation of a sample of total hemicellulose. [13] A sample (50 mg) of total hemicellulose from 137-day-old-stem tissues" was dispersed in 0·05 M $NaIO_4$ (50 ml) and the mixture kept in the dark at 5" with daily shaking. Samples (0.10 ml) were withdrawn at intervals, diluted to 100 ml and the amount of periodate reduced determined by measurement of the absorbance at 222.5 nm. Moles x 10^{-3} of periodate reduced per g of total hemicellulose were 2·05 (17 hr); 4.96 (43 hr); 7.48 (83 hr); 8·03 (134 hr); 8·40 (185 hr); 8.62 (331 hr) and 9·10 (400 hr) corresponding to an extrapolated value of 7·5 at zero time. The oxidation mixture was dialysed against running water for 4 days and the non-diffusible solute reduced by the addition of $NaBH_4$ (100 mg). After 48 hr the excess of $NaBH_4$ was destroyed by the addition of a slight excess of CH_3COOH and the borate was removed as the volatile methyl ester by codistillation with successive volumes of CH_3OH.

An aliquot (*ca.* 10 mg) of the polyalcohol was treated with 0·05 M $NaIO_4$ (10 ml) as described[14] but no further oxidant was reduced within 48 hr. It was concluded that the oxidation limit was reached and this was an unnecessary additional step.

The remaining polyalcohol was hydrolysed with 3 M CF_3COOH[10] (3 ml, 100°, 24 hr). The hydrolysate was taken to dryness in a stream of air, dissolved in H_2O (5 ml) and $NaBH_4$ (50 mg) added. After 12 hr the excess of $NaBH_4$ was destroyed by the addition of a slight excess of CH_3COOH, the solution evaporated,

[13] G.O. ASPINALL and R. J. FERRIER, *Chem. & Ind.* 1216 (1957).
[14] B. LARSEN and T. J. PAINTER, *Carbohydrate Res.* 10, 186 (1969).

and the borate removed as before. Half the product was acetylated by adding Ac_2O (5 ml) to the NaOAc already present and heating at 120" for 4 hr in a sealed tube. The acetylated products were examined by GLC (column A). The following compounds were identified by comparison with authentic samples—ethylene glycol diacetate, glycerol triacetate, erythritol tetraacetate, threitol tetraacetate, rhamnitol pentaacetate, arabinitol pentaacetate, xylitol pentaacetate, galactitol hexaacetate and glucitol hexaacetate. The molar ratio of glucitol hexaacetate to erythritol tetraacetate was determined." This was found to be 1 to 2.40.

The remaining aliquot (cu. 10 mg) of the reduced hydrolysate was dissolved in H_2O (20 ml) and treated with Zeo-Karb 225 (H^+) ion-exchange resin to remove Na^+. The filtered solution was reduced in volume and applied as a streak to Whatman No. 1 paper. After 18 hr irrigation the erythritol was located near the lower end of the chromatogram; the lower part was cut off and the area carrying the erythritol excised. Irrigation of the shortened chromatogram was continued for another 18 hr to obtain complete separation of glucitol from galactitol. The area corresponding to glucitol was then excised and the components were eluted from the paper with H_2O (15 ml). The eluates were treated with 0·05 M H_2SO_4 (1 ml) and 0·1 M $NaOI_4$ (2 ml). After 15 min M $NaAsO_2$ (2 ml) was added and the mixture set aside for 10 min and then diluted to 25 ml. Samples (2 ml) were treated with chromotropic acid reagent (10 ml),[15] heated at 100" for 30 min, cooled, and the optical density at 570 nm measured. The molar ratio of glucitol to erythritol, obtained by reference to appropriate calibration curves, was found to be 1 to 2.3.

Study of glucans in total hemicelluloses. The glucan composition of the various total hemicelluloses listed in Tables 1 and 2 was determined by the method described.

Enzymic hydrolysis of the β-glucans. A sample (100 mg) of the total hemicellulose (T), derived from the top leaf tissues of plants harvested 162 days after sowing, was dispersed in H_2O (80 ml) and KH phthalate buffer (20 ml; pH 5.4) and β-1,3-glucanase (20 mg) added. The mixture was gently stirred for 2 min and then incubated at 37" for 120 hr. After boiling the mixture for 5 min, the insoluble material (I) was centrifuged off. The soluble material (S) was applied to a column of Bionel P2 (25 cm x 1·5 cm i.d.) and the high molecular weight material eluted with H_2O (100 ml) and freeze-dried. The insoluble material, I,' after aqueous washing was dispersed in H_2O and freeze-dried. Samples of each material (*ca.* 5 mg) were hydrolysed and the hydrolysates taken to dryness. The ratio of the components of the hydrolysates was estimated by the ratios of the peak areas of the trimethylsilyl (TMS) derivatives on GLC examination" (column B).

TABLE 3. GLC PEAK-AREARATIOS OF SUGAR TMS DERIVATIVESFROMA SAMPLE OF TOP LEAF TOTAL HEMICELLULOSE

Sample*	Arabinose	Galactose	Glucose	Xylose
T	10	2	2	41
I	10	1	trace	59
S	10	3.7	trace	30

* See text.

Studies on the influence of light on glucan composition. Young oat plants, 64 days after sowing, were deprived of all sources of light for a period of 71 hr in a well ventilated cloche. During this period 11·1 hr of direct sunlight was recorded in the area. A sample was harvested before the cloche was put in position and another sample after 71 hr of growth in the dark. Another harvest was made after the light-deprived oats had been exposed for a further 71 hr to normal daylight. Similar harvests were made of plants grown in the light and comparisons were made between the tissues of comparable ages. Only the total hemicelluloses from the top leaf and stem tissues were studied in all the ways described earlier. No significant differences were noted between the samples compared.

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[15] G. W. HAY, B. A. LEWIS and F. SMITH, in ***Methods in Carbohydrate Chemistry*** (edited by R. L. WHISTLER), Vol. 5, p. 377, Academic Press, New York (1965).
[16] C.C. SWEELEY, R. BENTLEY, M. MAKITA and W. W. WELLS, *J. Am. Chem. Soc.* **85**, 2497 (1963).