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EXTRACTION OF HEMICELLULOSE FROM OAT TISSUES DURING THE PROCESS OF DELIGNIFICATION

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Abstract—The solutes in liquors after delignification of the coleoptile, leaf, stem, and root tissues of oat plants, *Avena sativa*, have been studied. Under the conditions used to isolate total hemicelluloses, for studies of polysaccharide composition in relation to plant maturity, no significant loss of arabinose, xylose, or galactose residues occurs and there is only a small loss of glucan.

INTRODUCTION

THE PRESENT studies complement earlier work on the relationships between the maturity of non-endospermic tissues of oat plants, *Avena sativa*, and the composition of the *total hemicelluloses*[1] derived from each of these tissues. Both field and laboratory grown plants have been studied.[2,3] The sugar residue composition of each total hemicellulose has been translated in terms of *pure hemicelluloses*[1] previously isolated from non-endospermic oat tissues and subjected to structural study; these pure hemicelluloses are an arabino(4-*O*-methylglucurono)xylan,[4] an arabinogalacto(4-*O*-methylglucurono) xylan,[5,6] and a non-cellulosic glucan having $\beta(1\rightarrow3)$ and $\beta(1\rightarrow4)$ glucosidic linkages.[7]

The isolation of total hemicelluloses requires very high and ideally total accountability of all cell-wall polysaccharides. In earlier studies it was established that statements made on the relationship between total hemicellulose composition and plant tissue maturity did not require to be altered to take into account non-glucosidic residues remaining in the various α-celluloses after delignification and alkaline extraction of the plant tissues.[8] The present study reports an investigation of hemicellulosic materials that are solubilized and normally lost during the delignification procedure employed prior to the isolation of each total hemicellulose.

RESULTS AND DISCUSSION

A sample of each tissue was delignified,[5,9] the polysaccharide material isolated and the high salt content reduced by ultrafiltration. The residual ash was found to be due to silicate, which is at a high level in many grasses.[10,11]

A sample of the material from each delignification liquor was hydrolysed and the amount

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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] A. J. BUCHALA and K. C. B. WILKIE (to be published).
[4] G. O. ASPINALL and K. C. B. WILKIE, *J. Chem. Soc.* 1072 (1956).
[5] J. S. G. REID and K. C. B. WILKIE, *Phytochem.* **8**, 2053 (1969).
[6] A. J. BUCHALA, C. G. FRASER and K. C. B. WILKIE, *Phytochem.* to be published.
[7] C. G. FRASER and K. C. B. WILKIE, *Phytochem.* **10**, 199 (1971).
[8] A. J. BUCHALA, C. G. FRASER and K. C. B. WILKIE, *Phytochem.* **10**, 1285 (1971).
[9] L. E. WISE, M. MURPHY and A. A. D'ADDIECO, *Paper Trade J.* **122**, 35 (1946).
[10] E. BLACKMAN, *Can. J. Bot.* **49**, 769 (1971).
[11] K. A. HANDRECK and L. P. H. JONES, *Plant and Soil* **29**, 449 (1968).

TABLE 1. FIELD GROWN PLANT MATERIALS: HEMICELLULOSIC

	Days from time of sowing to harvesting						
	56	81		106			
	Leaf	Leaf	Stem	Bottom leaf	Two middle leaves	Top leaf	Stem
Carbohydrate in delignification material*	0·37	1·60	0·73	1·42	1·27	0·92	0·57
Total hemicellulose*	15·2	23·4	24·6	18·4	21·1	19·7	25·5
Hemicellulosic material as percentage of carbohydrates in delignification material	90·3	85·5	84·7	88·2	81·3	85·9	88·3
Total hemicellulose including that in the delignification material*	15·5	24·8	25·2	19·7	22·2	20·5	26·0
Ratio of sugar residues in delignification material†							
xylose	1·42	0·56	1·49	0·74	0·46	1·42	1·13
galactose	0·63	0·55	0·53	0·37	0·46	0·32	0·72
Ratio of sugar residues in total hemicellulose†							
xylose	1·77	2·21	5·22	2·79	3·00	3·56	5·58
galactose	0·18	0·16	0·07	0·19	0·30	0·11	0·08
Modified values of ratio of sugar residues in total hemicellulose†							
xylose	1·76	2·11	5·12	2·65	2·88	3·47	5·49
galactose	0·19	0·18	0·08	0·20	0·31	0·12	0·09

* Expressed as percentage 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).

of total reducing sugar estimated[12] (Tables 1 and 2). In the case of field grown plants *ca.* 1% of the plant tissues was normally not accounted for, due to solubility losses during the delignification procedure. For the laboratory grown plants the values were similar in the case of leaf tissues, and slightly higher in the case of coleoptile and root tissues. The delignification procedure used was that employed in the earlier studies in this series; milder delignification procedures led to the extraction of less hemicellulosic material.

Chromatographic examination of hydrolysates of the materials isolated showed that arabinose, fructose, galactose, glucose, and xylose were the major sugars. There was also a smaller proportion of acidic sugars and a trace of rhamnose. The various delignification materials isolated had very similar sugar residue compositions; arabinose, fructose, galactose, glucose, and xylose were present, with a mean ratio of *ca.* 1·6:1·0:1·0:4·4:2·2.[13] The laboratory grown plants differed from those grown in the field in that the former had a higher proportion of galactose, and a lower proportion of fructose, residues.

Tables 1 and 2 show the ratios of galactose, arabinose, and xylose residues in the hemi-

[12] Z. DISCHE, in *Methods in Carbohydrate Chemistry* (edited by R. L. WHISTLER and M. L. WOLFROM), Vol. 1, p. 478, Academic Press, New York (1962).

[13] F. G. FISCHER and H. DÖRFEL, *Z. Physiol. Chem.* **297**, 164 (1954).

MATERIALS PRESENT IN THE DELIGNIFICATION LIQUORS

Days from time of sowing to harvesting									
106	137					162			
Hull	Bottom leaf	Two middle leaves	Top leaf	Stem	Hull	Two middle leaves	Top leaf	Stem	Hull
0·44	0·70	0·72	0·55	0·49	3·62	0·89	0·56	0·36	2·18
36·7	33·3	22·9	23·8	23·8	17·7	21·3	25·5	28·4	23·2
90·9	82·8	81·0	78·4	90·9	90·9	86·8	85·5	83·4	93·2
21·7	33·9	23·5	24·2	24·3	21·7	22·1	26·0	28·7	25·4
1·36	0·67	0·79	0·87	1·37	1·15	0·83	0·99	1·35	2·23
0·53	0·21	0·22	0·22	0·61	0·43	0·21	0·27	0·21	0·16
2·60	3·46	3·40	3·63	6·58	3·22	4·08	4·11	6·54	4·00
0·04	0·36	0·35	0·23	0·11	0·20	0·24	0·20	0·11	0·15
2·59	3·41	3·33	3·58	6·48	2·84	3·96	4·05	6·48	3·84
0·07	0·36	0·35	0·23	0·12	0·24	0·24	0·20	0·11	0·15

cellulosic material from each delignification liquor and the ratios of these residues in the corresponding total hemicelluloses from the field grown and laboratory grown plants. The effect of modifying the value for each total hemicellulose to take into account the material in the delignification liquor is shown. In calculating the corrected values, the fructose in the delignification material has not been taken into account as there is no evidence of the presence of fructose residues in any of the hemicellulosic materials studied. It can be seen that the loss of hemicellulosic material during delignification has a negligible effect on the ratio of sugar residues. No correction is required when considering the relationship between plant tissue maturity and compositional variation in xylan-type molecules in the various total hemicelluloses. Free-boundary electrophoresis of one delignification material showed the presence of components with very similar mobilities to those of the components in the water-soluble fraction of the corresponding total hemicellulose; this is compatible with both containing similar molecular species.

The proportion of glucose in each of the hydrolysates was high (average *ca.* 35%); less than 10% of the hemicellulosic β-glucan was solubilized during the delignification procedure carried out on field grown plant tissues and on the leaf tissues of laboratory grown plants. None of the materials gave a coloration with iodine. Enzymic studies, similar to

TABLE 2. LABORATORY GROWN PLANT MATERIALS: HEMICELLULOSIC MATERIALS PRESENT IN THE DELIGNIFICATION LIQUORS*

	Days from germination to harvesting								
	5			8			10		
	Coleoptile	Leaf	Root	Coleoptile	Leaf	Root	Coleoptile	Leaf	Root
Carbohydrate in delignification material	1·75	1 04	2 34	1·04	0 52	2 07	3 90	1·02	3 83
Total hemicellulose	31·2	31·0	26·0	30·7	33 3	32 3	32 1	35·4	23·2
Hemicellulosic material as percentage of carbohydrates in delignification material	95	90	92	90	92	92	87	92	92
Total hemicellulose including that in the delignification material	32 9	32 0	28 4	31 6	33 8	34·3	35·9	36·4	26 1
Ratio of sugar residues in delignification material									
xylose	0 82	0·94	0·83	0 85	0 78	0·85	1 13	1·00	0·88
galactose	0 55	0·55	0 83	0·40	0 39	0 65	0·62	0·52	0·88
Ratio of sugar residues in total hemicellulose									
xylose	1·13	2·13	1·36	1·34	2·16	1 88	1 36	2·16	2 04
galactose	0 11	0 14	0 07	0 15	0 15	0 12	0 18	0 15	0 18
Modified values of ratio of sugar residues in total hemicellulose									
xylose	1·28	2 09	1 32	1 33	2 14	1 82	1 34	2 13	1·91
galactose	1·13	0·15	0 13	0 16	0·15	0·15	0 23	0·16	0 26

* See footnotes to Table 1.

those described earlier,[14] using a β-(1,3)-glucanase from *Cytophaga* showed that the glucan was non-cellulosic. The proportion of hemicellulosic glucose residues in any one plant tissue decreases with plant maturity and there is a parallel decrease in the ratio of $\beta(1\rightarrow3)$ to $\beta(1\rightarrow4)$ linkages in the β-glucan in the total hemicellulose.[14] The number average degree of polymerization of the β-glucans also decreases with increasing plant maturity for any one plant tissue.[3] Tables 3 and 4 show the percentage of glucan in the plant tissues, and the

TABLE 3. FIELD GROWN PLANTS: HEMICELLULOSIC β-GLUCANS IN DELIGNIFICATION LIQUORS

	Tissue and days from sowing to harvesting of plant												
	Stem				Leaf and bottom leaf			Top leaf			Middle leaves		
	81	106	137	162	81	106	137	106	137	162	106	137	162
Carbohydrate in delignification material*	0 73	0·57	0·49	0·36	1·60	1 42	0·70	0 92	0·55	0 56	1·27	0·72	0·89
Glucose residues†	31 9	43 8	46·3	32 5	43·3	48·2	52·5	33·4	30·1	39·9	34·6	34·3	40·4
Glucan normally unaccounted*	0·2	0 2	0·2	0·1	0·7	0·7	0·4	0·3	0 2	0·2	0·4	0·2	0·3
Glucan in total hemicellulose*	3·1	3·1	2·2	2·0	6·3	3·5	3 3	1·7	0·6	1·1	1·9	2·4	2·1
Glucan—modified value*	3·3	3·3	2·4	2 1	7 0	4 2	3·7	2·0	0·8	1·3	2·3	2 6	2·4

* Expressed as percentage of plant tissue.
† Expressed as percentage of carbohydrate material from delignification material.

corrected values for glucan obtained when account is taken of the glucan in the delignification materials. Irrespective of whether or not an allowance is made for the glucose residues in the delignification liquors, the proportion of glucan from field grown plant tissues and from leaf tissues of laboratory grown plants decreases with increasing plant maturity.

Attempts to study the materials by the Smith degradation procedure were not successful, probably due to the presence of inorganic material. A preliminary methylation analysis, however, indicated that the polysaccharides present in the delignification materials were similar to those in the total hemicelluloses and that for the particular sample examined the

[14] A. J. BUCHALA and K. C. B. WILKIE, *Phytochem.* **10**, 2287 (1971).

TABLE 4. LABORATORY GROWN PLANTS: HEMICELLULOSIC β-GLUTANS INDELIGNIFICATION LIQUORS*

	Tissue and days from germination to harvesting of plant								
	Coleoptile			Root			Leaf		
	5	8	10	5	8	10	5	8	10
Carbohydrate in delignification material	1·73	1·04	3·90	2·34	2·07	3·83	1·04	0·52	1·02
Glucose residues	43	45	43	44	42	45	45	42	44
Glucan normally unaccounted	0·7	0·5	1·7	1·0	0·9	1·3	0·5	0·2	0·4
Glucan in total hemicellulose	5·8	5·3	4·8	4·4	7·9	5·1	7·7	7·1	6·5
Glucan—modified value	6·5	5·8	6·5	5·4	8·8	6·4	8·2	7·3	6·9

* See footnotes to Table 3.

ratio of β(1→3) to β(1→4) linkages (which is equivalent to the ratio of 2,4,6-tri-*O*-methylglucose to 2,3,6-tri-*O*-methylglucose) was 1·0:1·4. Values of this order have no significant effect on the trends observed previously.[14]

EXPERIMENTAL

General methods. Paper chromatographic solvents (Whatman No. 1 paper) were: (A) EtOAc–pyridine–H_2O (360:100:115); (B) EtOAc–HOAc–HCO_2H–H_2O (18:3:1:4); (C) EtOAc–HOAc–H_2O (3:1:3); (D) *n*-BuOH–pyridine–benzene–H_2O (5:3:1:3). Alkaline $AgNO_3$ was used as chromatographic detection reagent. A Perkin–Elmer F-11 GLC apparatus was used with columns (2 m × 3 mm i.d.) containing (A) 3% ECNSS-M on Gas-Chrom Q (100–120 mesh) and (B) 10% *m*-bis(*m*-phenoxyphenoxy)benzene on AW-DMCS Chromosorb W (100–120 mesh). The N_2 carrier-gas flow-rate was 50–80 ml/min. Free-boundary electrophoresis was conducted in a Tiselius-Svensson type apparatus at 4°. Hemicellulosic samples were hydrolysed in sealed tubes by heating with 0·5 M H_2SO_4 (12–16 hr at 100°); the hydrolysates were neutralized with $BaCO_3$ and, where appropriate, were treated with Zeo-Karb 325 (H^+) resin.

Plant material. The field-grown and laboratory-grown oat plants (var. Blenda) have been described earlier.[8]

Delignification of the plant tissues and isolation of the total hemicelluloses. A sample of each plant tissue was delignified by the method of Wise *et al.*[9] and each total hemicellulose was isolated as described previously.[2] Each delignification liquor was combined with the wash liquors, the resultant solution taken to near dryness, and EtOH (10 vol.) added. After centrifuging, each ppt. was washed with EtOH (4 × 50 ml), dissolved in H_2O, and the solution desalted in an Amicon Diaflow ultrafiltration apparatus using a UM 05 anion-exchange membrane (exclusion limit M_n *ca.* 500). The solution retained in the apparatus after 48 hr was freeze-dried.

Examination of the materials obtained from the delignification liquors. Carbohydrate content. A weighed sample of each material was hydrolysed and the volume of the neutralized hydrolysate adjusted to 25 ml. The total reducing sugar content was determined as glucose by the 1-naphthol/H_2SO_4 method.[12] The individual sugars in the hydrolysates were estimated by the method of Fischer and Dörfel.[13]

Inorganic material. A sample (*ca.* 100 mg) of one of the delignification materials was heated in a Pt crucible at *ca.* 900° for 5 min. After cooling, the ash was dissolved in H_2O (10 ml), and acetone (5 ml) and molybdate/H_2SO_4 reagent[15] (10 ml) were added. A yellow coloration was produced on adding 10% mannitol (10 ml), indicating the presence of silicate in the original material.

Methylation of a sample of delignification material. An aqueous solution of the material (1 g) derived from the leaf tissue of oat plant harvested 81 days after sowing, was dialysed against running-water for 2 days and freeze-dried. The material was subjected to methylation procedures by using successively the methods of Hakomori,[16] Kuhn,[17] and Purdie[18] until the methylated material showed negligible absorption in its IR spectrum (KBr disc) attributable to hydroxyl. The methylated material was boiled under reflux in light petroleum (b.p. 60–80°) and the soluble material discarded. The material insoluble in the light petroleum but soluble in $CHCl_3$ was concluded to be methylated polysaccharide (*ca.* 100 mg) and corresponded to *ca.* 65% of the polysaccharide present in the starting material.

Examination of the products in a hydrolysate of the methylated material. A sample of the material was hydrolysed by the HCO_2H/H_2SO_4 method[19] and, after neutralization ($BaCO_3$), the hydrolysate was reduced

[15] R. A. CHALMERS and A. G. SINCLAIR, *Anal. Chim. Acta* **34**, 412 (1966).
[16] S. HAKOMORI, *J. Biochem., Tokyo* **55**, 205 (1964).
[17] R. KUHN, H. TRISCHMAN and I. LÖW, *Angew. Chem.* **67**, 32 (1955).
[18] T. PURDIE and J. C. IRVINE, *J. Chem. Soc.* **83**, 1021 (1903).
[19] H. O. BOUVENG, H. KIESSLING, B. LINDBERG and J. E. MCKAY, *Acta Chem. Scand.* **16**, 615 (1962).

with $NaBH_4$. After 12 hr the excess of $NaBH_4$ was destroyed by the addition of HOAc and the borate was removed by passing the solution through a column of a borate-specific anion exchanger (Borasorb). The eluate was taken to dryness under reduced pressure at room temp. and heated with Ac_2O (1 ml) and pyridine (1 ml) in a sealed tube (120°; 30 min). The excess of Ac_2O was destroyed by the addition of H_2O (2 ml) and the reaction-mixture extracted with $CHCl_3$. The products were examined by GLC (column A) and the identities of the components were established by comparison with authentic compounds; certain minor components were not identified. The following compounds were identified as the methylated glycitol peracetates —2,3,5-tri-*O*-methyl-L-arabinose, 2,3,4-tri-*O*-methyl-D-xylose, 2,3-di-*O*-methyl-D-xylose, 2-*O*-methyl-D-xylose, 2,3,4,6-tetra-*O*-methyl-D-galactose, 2,3,4,6-tetra-*O*-methyl-D-glucose, 2,3,6-tri-*O*-methyl-D-glucose, and 2,4,6-tri-*O*-methyl-D-glucose.

A sample of the methylated material was also heated with 4% MeOH/HCl (100°; 16 hr) in a sealed tube and the cooled methanolysate examined directly by GLC (columns A and B). The chromatograms were complex but the results were compatible with those obtained when the methylated and acetylated glycitol derivatives were examined.

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