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POLYSACCHARIDES OF THE OAT PLANT IN RELATIONSHIP TO PLANT GROWTH

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Abstract—The molecular composition of plant hemicelluloses is considered in terms of *polymolecularity*, *polydispersity* and *polydiversity*. These, and the terms *pure* hemicellulose, *total* hemicellulose and hemicellulosic material, are defined in a way suitable for use in this and later papers on the relationship between maturity of the oat plant, *Avena sativa*, and hemicellulosic composition. Fractionation procedures have been studied and high recovery of well-fractionated hemicellulosic material obtained on a column of DEAE-cellulose.

INTRODUCTION

IN THIS and in subsequent papers, studies are reported on the polysaccharides of the oat plant, *Avena sativa*, with particular emphasis on the relationship between the growth and environment of the plant and the composition and structure of the polysaccharides in the various tissues.

It is imperative to define and discuss the terms to be used. The term hemicellulosic material is used in contexts where the terms *pure* hemicellulose and *total* hemicellulose, as defined below, would be inappropriate. A total hemicellulose is here defined as the polysaccharide in a plant tissue other than that conventionally classified as α-cellulose.[1] A pure polysaccharide, as described here and elsewhere in the literature, is a group of molecules satisfying, implicitly or explicitly, some *definition* of purity with respect to its molecular composition.

There are three major aspects that affect the molecular composition of hemicellulosic material. These are, firstly, the proportion and the range of molecular weights of structurally similar molecules (polymolecularity);[2] secondly, the co-existence of molecules of distinctly different structural type hereafter termed *polydiversity*; and, thirdly, the presence of molecules which although dominantly similar in structure yet differ in comparatively minor ways (polydispersity).[2] The last term embraces, *inter alia*, those molecules that have different proportions and distributions of sugar residues, and those in which there is a variation in the frequency of branch-points; molecules that are none the less structurally very similar. A pure hemicellulose may be defined more explicitly in the above terms. It is a hemicellulosic material where monitoring methods indicate that polydiversity has been avoided; it is not homogeneous but it has a degree of heterogeneity compatible with whatever type of work is in prospect.

By fractionation of hemicellulosic materials an attempt is made to avoid polydiversity; if the fractionation is inadequate, polydiversity may remain and erroneous structural conclusions be drawn. There is a concomitant danger in skilful fractionation of a heteroglycan.

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[1] L. E. WISE, M. MURPHY and A. A. D'ADDIECO, *Paper Trade J.* **122**, 35 (1946).

[2] G. O. ASPINALL, E. E. PERCIVAL, D. A. REES and M. RENNIE, in *Rodd's Chemistry of Carbon Compounds* (edited by S. COFFEY), Vol. 1F, p. 666, Elsevier, Amsterdam (1967).

Such a fractionation may in a sense be over-successful and lead to the isolation of a pure hemicellulose having a narrower polymolecularity and polydispersity than in the parent total hemicellulose. This danger may be accentuated if there is reason to suspect that a particular pure hemicellulose is present in the plant material. In the present work to avoid this danger, and because many tissue samples had to be studied (many of them in only small amounts), it was decided to extract the total hemicellulose without loss from each oat tissue sample and to deduce its molecular composition by studying hydrolysates qualitatively and quantitatively. Such deductions require adequate knowledge of the pure hemicelluloses present. Supplementary studies had to be carried out to determine, amongst other things, the structures of hitherto uninvestigated pure hemicelluloses in oat tissue total hemicelluloses.

The work has divided itself into two main parts. Firstly quantitative and qualitative determinations have been carried out on the sugars in hydrolysates of total hemicelluloses. Secondly, and in part concurrently, the polydiversity has been investigated by isolating hitherto unknown pure hemicelluloses from the total hemicellulose of various oat tissues. Structural studies have been carried out on these pure hemicelluloses. Maximum information on polydiversity and on the structures of pure hemicelluloses is required if the composition of each total hemicellulose is to be interpreted.

RESULTS

The first experimental problem was to investigate if there were any significant loss of total hemicellulose at any stage in its isolation. Where a procedure led to a loss the technique was modified to prevent or minimize it. It was determined whether the material lost appeared to have the same molecular composition as the material isolated and if there were a loss whether it would be significant in the comparative studies. Where the loss was insignificant or small the phrase total hemicellulose is retained to describe the material isolated.

TABLE 1. COMPOSITION OF HEMICELLULOSIC MATERIALS *a* AND *b*

Hemicellulosic material	Percentage of plant material	Acidic components	Densitometric values			
			Galactose	Glucose	Arabinose	Xylose
a	21	+	0·3	1·0	1·0	2·6
b	3·2	+	0·3	0·3	1·0	1·8

There could be some loss when hemicellulosic material is precipitated from a neutralized alkaline extract. This possibility was checked by treating oat leaf holocellulose with 5 and 24% aqueous KOH under nitrogen to extract the total hemicellulose.[1] The solution was neutralized with acetic acid and acetone added to precipitate hemicellulosic material (*a*). The mother liquor was taken to dryness and the potassium acetate dissolved in ethanol; a white flocculent residue remained in suspension (*b*). Under normal precipitation conditions some of the total hemicellulose would be lost (see Table 1). Accordingly in all later comparative studies the above modified procedure was adopted and the total hemicellulose obtained by combining hemicellulosic materials *a* and *b*. There is a higher proportion of glucose in the former than in the latter: this accords with the later isolation of a pure, non-cellulosic, glucan.

The delignification procedure was next examined. The acid chlorite method was used.[1]

It was accepted that there were dangers of oxidation[3] and of depolymerization[4] and that these could not be avoided but they were believed to be slight. It was assumed that if the polysaccharides in plant samples were identical then they would be similarly, or identically, affected; there seemed little danger that delignification would upset comparative studies unless residual lignin impeded dissolution or there were solubility losses similar to those above. There was no evidence that the duration of the delignification had any significant effect on the proportions of the sugars in hydrolysates (Table 2).

The possibility that there might be solubility losses was checked by subjecting a sample of oat leaf to delignification. After removal of dissolved gases from the solution and concentration, the solute was fractionated by gel-filtration. The material in each fraction was treated under hydrolysis conditions. The main fraction from the column yielded galactose, glucose, arabinose and xylose in the ratio of *ca.* 5:1:4:1 (compared to 1:3:3:15 in the total hemicellulose). There was also a trace of a component which later work indicates was probably fructose.[5] Less than 1 per cent of the total hemicellulose was lost and this was considered insignificant.

Fractionation studies on hemicellulosic materials were carried out with a twofold objective. The first was to achieve total accountability of all the material originally present. The

TABLE 2. HEMICELLULOSIC MATERIALS ISOLATED AFTER VARIOUS PERIODS OF DELIGNIFICATION

Period of delignification (hr)	Acidic compounds	Densitometric values				
		Galactose	Glucose	Arabinose	Xylose	Rhamnose
1	+	0·2	1·2	1·0	2·5	+
2	+	0·2	1·0	1·0	2·2	+
3	+	0·2	1·1	1·0	2·2	+
4	+	0·2	1·0	1·0	2·1	+

second was to establish the nature of any polydiversity in such materials by noting consistent and parallel variations in the composition of fractions; variations that might indicate the presence of particular homoglycans and of heteroglycans.

Structural work by Aspinall and Wilkie[6] on a pure hemicellulose from oat straw led to the conclusion that, in common with other land-plant xylans,[7] it possesses a backbone of β1—4 linked D-xylopyranose residues. There were on average forty to forty-five such residues per molecule, and one 4-*O*-methyl-D-glucopyranosyluronic acid residue (attached to the 2 position of a xylose residue) and one L-arabinofuranose residue (either directly attached to the 3 position of a xylose residue or to it through a chain of xylose residues—the former seems more probable than the latter).[7] During the fractionation the proportion of arabinose to xylose residues was reduced; this reduction could either be ascribed to an alteration in the polydisperse composition of a pure heteroglycan rich in xylose residues or be indicative of polydiversity.

Initial attempts to achieve fractionation were by use of solvent-extraction techniques.

[3] A. JEANES and H. S. ISBELL, *J. Res. Nat. Bur. Standards* **27**, 125 (1941).
[4] T. E. TIMELL and E. C. JAHN, *Svensk Papperstid.* **54**, 831 (1951).
[5] C. G. FRASER and K. C. B. WILKIE, unpublished work.
[6] G. O. ASPINALL and K. C. B. WILKIE, *J. Chem. Soc.* 1072 (1956).
[7] G. O. ASPINALL, *Advan. Carbohyd. Chem.* **14**, 437 (1959).

Such methods would be expected to give the total recovery desired. A series of fractionations were carried out in which samples of hemicellulosic material (corresponding to material *a* in Table 1) from an oat leaf holocellulose were separately treated with water or with aqueous ethanol (see Table 3). The materials extracted were proportionately richer in galactose and arabinose than was the original. The glucose again behaved in a manner consistent with it being in a homoglycan. Although the ethanolic extraction procedure gave complete accountability of all material the degree of fractionation was inadequate.

Fractionation was attempted using various ion-exchange materials. In preliminary experiments very small amounts of hemicellulosic material *a* were applied to columns of DEAE-Sephadex, aminoethyl-cellulose and DEAE-cellulose powder and to DEAE-cellulose paper. Irrigation was successively with water, increasing concentrations of potassium acetate and finally with increasing concentrations of KOH. The hemicellulosic materials eluted were directly hydrolysed and, where the amount permitted, the ratio of the sugars determined (see Table 7). There was very little hemicellulosic material in certain fractions

TABLE 3. FRACTIONATION OF HEMICELLULOSIC MATERIAL *a* USING WATER AND AQUEOUS ETHANOL AS SOLVENTS. EXAMINATION OF HYDROLYSATES

Solvent	Fraction	Acidic compounds	Densitometric values			
			Galactose	Glucose	Arabinose	Xylose
	Unfractionated material	+	0·3	1·0	1·0	2·6
Water	Soluble	+	0·3	0·6	1·0	1·8
	Insoluble	+	0	1·3	1·0	3·9
20% Ethanol	Soluble	+	0·8	0·4	1·0	1·8
	Insoluble	+	0·05	1·2	1·0	3·3
40% Ethanol	Soluble	+	0·5	0·15	1·0	1·9
	Insoluble	+	0·05	1·3	1·0	3·3
60% Ethanol	Soluble*	+	trace	trace	1·0	1·8
	Insoluble	+	0·2	1·2	1·0	2·7

* Only a trace of soluble hemicellulosic material was extracted.

and accordingly on occasion sugars present in low proportion may have escaped detection in hydrolysates (cf. Table 4). It was obvious that KOH caused degradation of the ion-exchangers. The hemicellulosic materials eluted by water and by the various concentrations of potassium acetate differed. In particular the proportion of glucose residues in a fraction appeared not to be related to the proportions of the other sugar residues (cf. Tables 3 and 7). Fractions rich in arabinose residues were also relatively rich in residues of galactose. A pure non-cellulosic glucan[5] and a pure acidic galactoarabinoxylan have since been isolated and studied.

It was discovered that hemicellulosic material *a* was soluble in saturated potassium acetate. An attempt was therefore made to fractionate it on DEAE-cellulose avoiding the use of alkali. A column was irrigated by water and by increasing concentrations of potassium acetate (see Table 4). The weight of hemicellulosic materials recovered indicated that no material had been retained on the column; but calculation of the mean composition of the recovered hemicellulosic materials indicated that selective losses had in fact occurred most notably of glucose residues (see Table 5). Probably the fractions recovered were contaminated by small amounts of occluded potassium acetate.

TABLE 4. FRACTIONATION OF HEMICELLULOSIC MATERIAL *a* ON DEAE–CELLULOSE POWDER. EXAMINATION OF ELUATES AND OF THEIR HYDROLYSATES

Irrigant	Volume (l.)	Weight of hemicellulose (mg)	Acidic compounds	Densitometric values				
				Galactose	Glucose	Arabinose	Xylose	Rhamnose
Water	1	4	+	0	4·2	1·0	3·9	0
KOAc (0·1 M)	1	3	+	0·2	0·9	1·0	2·5	trace
KOAc (0·5 M)	1	20	+	0·4	0·1	1·0	1·6	+
KOAc (1·0 M)	1	0	—	—	—	—	—	—
KOAc (5·0 M)	1	90	+	0·1	0·4	1·0	3·8	trace
KOAc (saturated)	1	17	+	0·2	0·45	1·0	3·0	0

TABLE 5. COMPOSITIONS OF HEMICELLULOSIC MATERIALS APPLIED TO AND RECOVERED FROM DEAE CELLULOSE

	Densitometric values			
	Galactose	Glucose	Arabinose	Xylose
Material applied	0·3	1·0	1·0	2·6
Material recovered*	0·2	0·5	1·0	3·3

* These values are approximate as they are based on a summation of values obtained by densitometry.

EXPERIMENTAL

General Methods

A rotary evaporator was used and all solvents taken off under reduced pressure at or below 40°. Hemicellulosic materials were hydrolysed by heating samples (*ca.* 1 mg) with N H_2SO_4 (*ca.* 3 ml) in a sealed tube at 100° for 14–24 hr. The acid was neutralized by adding $BaCO_3$ and the solids were then removed by centrifugation. Neutralized hydrolysates were concentrated in a draught of air and then examined by paper chromatography. If acidic sugars were being studied the centrifugate was treated with Amberlite IR 120 (H^+). Paper chromatography was on Whatman No. 1 paper using ethyl acetate:pyridine:water (72:20:23 v/v). Sugars were detected on chromatograms by use of triphenyltetrazolium chloride[8] and quantitative estimations made by scanning paper strips (2·5 × 20 cm) using a "Chromoscan" densitometer (Joyce Loebl Ltd.); the areas under the peaks were automatically integrated. The ratios quoted are of peak areas and are not molar; equal amounts of different reducing sugars give slightly different densitometric values.

Extraction of Hemicellulosic Materials

Leaves (*ca.* 120 g) of oats, *Avena sativa* (var. Blenda), were milled (mesh 16) and then delignified for 4 hr.[1] The air-dried holocellulose (80 g) was treated successively with 5 and 24% KOH (2 l. of each) under N_2 for 16 hr. The combined extracts were immediately neutralized by the addition of HOAc and then acetone (4 l.) was added. After washing, the precipitate (hemicellulosic material *a*) was dried over KOH (yield, 11·5 g; 21 per cent of holocellulose).

The mother liquor was taken to dryness and the crystalline KOAc dissolved in ethanol. A slight flocculent white precipitate remained—hemicellulosic material *b*—(yield 1·7 g; 3·2 per cent of holocellulose). Samples of hemicellulosic materials *a* and *b* were hydrolyzed and examined on paper chromatograms (see Table 1).

Study of Delignification Method

Four identical samples (5 g each) of milled oat leaf were delignified[1] for between 1–4 hr. The hemicelluloses were extracted and combined as described in the previous section and hydrolysates examined on paper chromatograms (see Table 2).

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

A further sample (1 g) of oat leaf was delignified for 3 hr then N_2 passed through the solution to remove dissolved gases. After concentration to *ca.* 10 ml the solution was passed through a column of Bio-Gel P6 (V_0 60 ml) irrigated with distilled water. Three fractions were collected before chloride appeared. Each fraction on freeze-drying gave a small amount of white powder which was then hydrolysed. The sugars in the hydrolysate of Fraction 1 gave a densitometric peak area which was less than that given by 1 mg of glucose. In this hydrolysate a trace of an unidentified sugar was noted on the paper chromatogram; it lay between glucose and arabinose. The hemicellulosic material in the delignification liquor was less than 1 per cent of the total hemicellulose.

TABLE 6. FRACTIONATION OF SOLUTES IN DELIGNIFICATION LIQUORS

Fraction	Eluate (ml)	Solid from eluate (mg)	Sugars in hydrolysate
1	20	8	Galactose:glucose:arabinose:xylose:5:1:4:1*
2	20	5	Trace of glucose
3	10	5	Trace of galactose and of glucose

* By visual estimation.[8]

TABLE 7. FRACTIONATION OF HEMICELLULOSIC MATERIAL *a* USING ION-EXCHANGERS. EXAMINATION OF HYDROLYSATES

Method	Ion exchanger	Irrigant	Eluate (ml)	Acidic compounds	Densitometric values			
					Galactose	Glucose	Arabinose	Xylose
1	DEAE-	Water	500	—	—	9·0	1·0	9·5
	Sephadex	KOAc (0·05 M)	500	—	+	+	+	+
	A50	KOAc (0·1 M)	500	—	0·2	0·45	1·0	4·2
	(chloride)	KOAc (0·5 M)	500	—	0·4	0·1	1·0	1·8
	powder	KOAc (1·0 M)	500	—	—	—	—	—
		KOAc (5·0 M)	500	—	—	—	1·0	6·7
		KOH (0·5 M)	500	—	4·7	14·0*	1·0	5·7
2	Aminoethyl-†	Water	100	—	—	+	—	—
	cellulose	KOAc (0·1 M)	100	—	—	—	+	+
	Whatman	KOAc (0·5 M)	100	—	+	—	+	+
	AE 50	KOAc (1·0 M)	100	—	—	—	+	+
	(acetate)	KOAc (5·0 M)	100	—	—	—	+	+
	powder	KOH (1·0 M)	100	—	—	+	+	+
3	DEAE-	Water	50	—	—	+	—	—
	cellulose	KOAc (0·1 M)	50	—	—	—	—	—
	Whatman	KOAc (0·5 M)	50	—	0·3	—	1·0	1·4
	DE 20	KOAc (1·0 M)	50	+	+	+	1·0	1·3
	(acetate)	KOAc (5·0 M)	50	+	—	—	1·0	2·8
	paper	KOH (0·1 M)	50	+	0·1	2·7*	1·0	2·0
4	DEAE-	Water	100	—	—	+	—	+
	cellulose	KOAc (0·1 M)	100	—	0·2	0·8	1·0	3·2
	Whatman	KOAc (0·5 M)	100	+	0·3	0·15	1·0	1·5
	DE 50	KOAc (1·0 M)	100	+	0·2	0	1·0	2·1
	(acetate)	KOAc (5·0 M)	100	—	—	—	1·0	4·7
	powder	KOH (0·1 M)	100	+	+	10·0*	1·0	2·4
		KOH (0·5 M)	100	+	0·2	1·8*	1·0	2·6
		KOH (1·0 M)	100	+	—	67*	1·0	3·2
		KOH (2·0 M)	100	+	—	100*	+	1·0

† Determinations were not made by densitometry.
* Glucose mainly or exclusively arising from alkaline degradation of ion exchangers.

Four samples of hemicellulosic material *a* were shaken for 2 days at room temperature with volumes (10 ml) of water and of 20, 40, and 60% aqueous ethanol. Fractionation was attempted using various ion-exchange materials (see Table 7). In each case a sample (10–30 mg) of hemicellulosic material *a* was used; it was not completely soluble and suspensions were applied to the columns and to the paper. In all cases aqueous salt-free solutions were taken to near dryness. Acetone was added to precipitate hemicellulosic material. Eluates containing KOAc were taken to near dryness and the salt dissolved in ethanol. KOH solutions were neutralized with dilute HOAc and then treated as above.

A sample (5 mg) of hemicellulosic material *a* was suspended in water (5 ml) and solid KOAc added slowly with constant mixing. At a very high concentration a clear solution was obtained. A column (2·5 × 12 cm) of DEAE-cellulose powder (Whatman DE 50—acetate form) was prepared. Hemicellulosic material *a* (131 mg) was suspended in water and added to the column which was irrigated as shown in Table 3.

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