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β-GLUCANS FROM OAT LEAF TISSUES AT DIFFERENT STAGES OF MATURITY

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Abstract-Non-cellulosic β-glucans have been isolated from tissues of the oat plant, *Avena sativa* (var. Blenda), at different stages of maturity. They are deceptively similar. It is concluded that in this, and in other cases, the selective fractionation used is inadequate when seeking to isolate polysaccharides for comparative studies of any type. A different approach is specifically mentioned.

THE STRUCTURES have been determined of a β-glucan,[1] an acidic arabinoxylan[2] and an acidic galactoarabinoxylan[3] in non-endospermic tissues of field-grown oat plants, *Avena sativa.* Studies in recent years have been carried out on the relationship between the maturity of tissues of field-grown and laboratory-grown *Avena sativa* (var. Blenda) and polysaccharide composition.[4] The terms *pure* and *total* hemicelluloses have been defined in relation to the terms *polydispersity* and *polydiversity*.[5]

The β-glucan isolated by Fraser and Wilkie[1] from young oat leaf tissues has β(1 → 3) and β(1 → 4) D-glucopyranosidic residues only; similar glucans are in other oat tissues.[6,7] It was determined that there was a decrease in the *amount* of glucose residues in the total hemicelluloses in any one type of tissue (root, coleoptile, leaf or stem) as the tissue matured. The object of the present work was to investigate this change which is believed to be a direct reflection of a change in the composition of parent glucan material. There was no evidence of the presence of a glucoarabinoxylan in the tissues studied; Hay[8] isolated a glucoarabinoxylan from oat hulls.

The simplest approach to the proposed study seemed to be to isolate a pure hemicellulosic glucan from each total hemicellulose and then to compare the various glucans. Earlier work on oat acidic galactoarabinoxylans indicated, however, that results obtained in this way might be of less significance than is apparent. By a selective fractionation procedure, *typical* of that employed in the process of isolating many other polysaccharides for structural study, *pure* acidic galactoarabinoxylans were isolated from various tissues of field-grown oat plants at different stages of maturity. These pure hemicelluloses were markedly similar in composition although the parent total hemicelluloses were in many cases very different. It was concluded that selectivity during fractionation had led to the isolation of only a part of the polydisperse spectrum of the acidic galactoarabinoxylan present in each total hemicellulose; such pure hemicelluloses are of considerable structural interest but are so atypical

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with respect to polydispersity, and possibly polymolecularity,[5] that they are unsuitable for comparative studies. In studying the relationship between plant tissue maturity and β-glucan composition, the approach was modified. The various β-glucans were isolated by procedures that, it was thought, would lead to a less selective fractionation with respect to polydispersity while enabling hemicellulosic materials to be obtained that lacked polydiversity. The fractionation technique gave high accountability of all the hemicellulosic materials in each total hemicellulose. It involved graded precipitation of the water-soluble fraction of each total hemicellulose by the addition of EtOH. From each total hemicellulose (0·30–1·5 g) the amount of β-glucan isolated was normally low-it ranged from ~ 1 to 3 mg with one exception of 19 mg (leaf tissue sample 3; Table 1). Other hemicellulosic materials isolated also contained glucose residues (Table 2). Each β-glucan was studied by oxidising a sample with $NaIO_4$ and after reduction of the resultant polyaldehyde with $NaBH_4$ the product was hydrolysed by CF_3COOH.[9] The products were glucose, erythritol and glycolaldehyde. The glucose was assumed to derive from those residues in the glucan that carried 3-*O*-glucopyranosidic residues and the erythritol from those residues that carried 4-0-glucopyranosidic residues. The ratio of (1 → 3) to (1 → 4) linkages in the various β-glucans was calculated from GLC quantitative determinations on derived acetates and by densitometric determinations of the erythritol and glucose on paper chromatograms; the results are shown in Table 1. In the earlier structural studies[1] particular care was taken to recover a high proportion of the β-glucan from young oat leaf tissue total hemicellulose. It was then found that the ratio of (1 → 3) to (1 → 4) linkages was 1·00:1·65; in the present work, by contrast, the procedure led to the isolation of a lower proportion of β-glucan and the ratio of (1 → 3) to (1 → 4) linkages was reduced to ~1:0·8–1·0. As remarked some glucose residues remained in other hemicellulosic fractions (Table 2). The reduced ratio indicated that even this method of fractionation was too selective; it favoured the isolation of β-glucans relatively impoverished in (1 → 4) linked residues.

TABLE 1. RATIO OF (1 → 4) TO (1 → 3) GLUCOSIDIC LINKAGES IN β-GLUCANS FROM TISSUES OF OAT PLANTS AT DIFFERENT STAGES OF MATURITY

Plant tissue[4]		Days from sowing to harvest	Ratio of (1 → 4) to (1 → 3) linkages A*	B*
Leaf	1	39	0·85	—
	2	76	0·85	0·86
	3	107	1.10	1.12
	4	122	0.80	0.76
	5	142	—	—
Bottom leaf	4	122	0·80	0.80
	5'	142	1·0	0.83
Stem	4"	122	0·95	1.21
	5"	142	—	1·70

*A = Ratio of erythritol to glucose in periodate oxidized and reduced glucans after hydrolysis-determinations by densitometry.

*B = Ratio of erythritol tetraacetate to glucitol hexaacetate in periodate oxidized, reduced and hydrolysed glucansderived glycitol acetates were determined quantitatively by GLC.

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TABLE 2. RATIOS OF MONOSACCHARIDES IN HYDROLYSATES OF HEMICELLULOSIC FRACTIONS OBTAINED DURING THE PROCEDURE LEADING TO THE ISOLATION OF GLUCANS*

Plant tissue[4] (see Table 1)	Fraction?	Weight (mg)	Sugars in hydrolysates estimated by densitometry		
			galactose	arabinose	xylose
1 (Leaf)	TH	300	4	8	15
	1	73	3	6	21
	S	199	4	21	31
	W	7	trace	4	9
2 (Leaf)	TH	400	5	16	29
	I	180	1	5	19
	S	171	6	14	46
	W	51	3	7	29
3 (Leaf)	TH	1500	5	23	37
	I	377	4	14	120
	S	892	5	30	45
	W	62	5	23	45
4 (Leaf)	TH	1500	6	12	24
	I	369	trace	21	190
	S	887	10	41	80
	W	69	8	36	80
5 (leaf)	TH	1000	8	23	67
	I	348	4	25	40
	S	584	9	28	49
	W	14	5	31	75
4’ (Bottom leaf)	TH	1000	8	24	57
	I	264	6	16	52
	S	512	11	32	46
	W	20	7	21	60
5’ (Bottom leaf)	TH	1500	8	24	53
	I	546	10	13	160
	S	749	4	19	64
	W	34	4	25	54
4” (Stem)	TH	700	2	13	80
	I	237	3	20	160
	S	391	14	22	28
	W	22	1	6	31
5” (Stem)	TH	1200	trace	23	90
	I	499	trace	43	270
	S	568	3	14	46
	W	53	3	13	69

* Determinations by densitometry on paper chromatograms relative to glucose (= 10) in hydrolysates.

† TH-total hemicellulose. I, S and W are sub-fractions-for procedures leading to their isolation see text.

The results re-emphasise the dangers inherent in carrying out comparative studies on polysaccharides from different plant species, or from different parts of a plant of one species, or from the same part of a plant species at different stages of maturity. The danger arises where the procedure used in isolating the materials for study is either undesirably selective or inadequately reproducible. It was concluded from the studies on the β-glucans that future work should be directed towards comparative studies of the glucose residues in the various *total* hemicelluloses as there was good reason to believe that they were present in β-glucans only and not in cellulose or degraded cellulose. Buchala and Wilkie have carried out such studies and the results appear to vindicate both the viewpoint and the methods employed. The ratio of (1 → 3) to (1 → 4) linkages in the glucans from any one tissue falls as the plant matures.[6,7]

EXPERIMENTAL

General Methods

Paper chromatography was on Whatman No. 1 paper using EtOAc–pyridine–H_2O (72:20:23, v/v) as irrigant. Chromatograms were treated with alkaline $AgNO_3$. Densitometric determinations were on a calibrated Joyce Loebl Chromoscan; peak areas were integrated automatically. A Perkin-Elmer F11 gas chromatograph was used with a column (2 m x 0·3 cm i.d.) packed with 3 % ECNSS-M on Gas-Chrom Q (100-120 mesh) at 185” with N_2 flow rate of 75 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 $BaCO_3$.

Isolation of the Glucans

The total hemicelluloses were isolated as described earlier.[4] In the isolation of the glucans water was removed at all stages by freeze-drying. A sample (0·30–1·50 g) of each total hemicellulose was dispersed in H_2O (10-30 ml), the insoluble material (I) separated and EtOH (6-18 ml) added to the solution to give a final concentration of 37.5%. Each precipitate (E) was separated and then washed with H_2O (3 x 25 ml); the supernatant liquors were taken to dryness and gave a solid (S) and the wash liquors on being taken to dryness left a solid (W). The washed precipitate E was dispersed in H_2O (10 ml) and on freeze-drying gave a homoglucan. Details relating to the various fractions are given in Table 2. The glucans gave glucose alone on hydrolysis. The glucans all gave IR peaks at ~ 890 cm-’ and the spectra of all the glucans were similar both to one another and to the spectrum of the oat leaf glucan studied earlier.’

Periodate Oxidation of the Glucans and Quantitative Studies

A sample (1-3 mg) of each glucan was dispersed in 0·05 M $NaIO_4$ (10 ml) and the suspension stored at 5 ° in the dark for 21 days with frequent shaking. The insoluble material was separated,. washed with H_2O (3 x 2 ml), dispersed in H_2O (5 ml) and $NaBH_4$ (100 mg) was then added. After 3 days the excess of $NaBH_4$ was destroyed by addition of Zeo-Karb 225 (H^+) ion-exchange resin; the resultant solution was evaporated to dryness. Borate was removed by co-distillation with CH_3OH and the residue dissolved in H_2O (3 ml) and the solution freeze-dried. The polyalcohol was hydrolysed with 3M CF_3COOH[9] (2 ml: 100”. 30 hr) and the hydrolysates examined chromatographically. The ratio of glucose to erythritol was estimated densitometrically. The hydrolysate was further treated with 0·05 M H_2SO_4 (3 ml; 100°, 12 hr) and the solution neutralized with De-Acidite FF-IP (HCO_3^- form) and $NaBH_4$ (10 mg) added. After 16 hr the excess of $NaBH_4$ was destroyed by addition of a slight excess of CH_3COOH, and the solvent evaporated. Acetylation was carried out using Ac_2O–AcONa, and the products were examined by GLC. Peak areas were measured by triangulation and absolute amounts calculated from calibration curves prepared using xylitol pentaacetate as an internal standard. A sample (16·3 mg) of the glucan derived from leaf harvested after 107 days was used to standardize the above procedure.

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