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CHANGES IN THE HEMICELLULOSIC POLYSACCHARIDES OF RYE-GRASS WITH INCREASING MATURITY

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ABSTRACT

The extraction and fractionation of the hemicellulosic polysaccharides of rye-grass have been examined at different stages of maturity of the plant. There is evidence of the presence of a glucan, a highly branched galactoarabinoxylan, and a linear xylan. The branched xylan has a similar composition throughout growth, is considered to be homogeneous, and gradually increases in concentration during growth. The linear xylan, which is by far the major polysaccharide, exhibits considerable polydispersity, in that the xylose–arabinose ratio rises considerably during growth, but its concentration in plant tissue, as a proportion of the total hemicellulose, slowly declines during growth.

INTRODUCTION

Cell-wall carbohydrates of plants are an important source of energy to ruminant animals. Dekker *et al.*[1] have shown that the polysaccharide constituents of tropical-pasture herbage are digested at different rates in the rumen and that the rate of digestion is influenced by the method used to conserve the herbage. It was also shown that different monomer units are released from heteroglycans at different rates. Jones and Bailey[2] also observed the effect of the method of conservation on the rate of digestion, as well as showing that hemicelluloses from different species were digested at different rates.

The term hemicellulose denotes a wide variety of polysaccharides isolated from plant cell-walls[3]. Xylans have been isolated from both root[4] and aerial[5] tissue of perennial rye-grass (*Lolium perenne*). Methylation analyses and studies of partial, acid hydrolysis have indicated the similarity between the xylans, but little attempt was made in these investigations to fractionate the hemicellulosic complex. Many fractionation schemes have been described, one of the earliest being that of O'Dwyer[6], who introduced the A/B classification based on the solubility of the alkali-extracted hemicellulose after neutralisation with acid. Whistler and Lauterbach[7] used precipitation with ethanol to fractionate the B-type hemicellulosic complex from *Zea mays* cobs into an acidic branched xylan, a neutral linear xylan, and, possibly, a glucan. By

complexing the same hemicellulosic complex with iodine in strong salt solution, Gaillard[8] was able to isolate the two xylans in pure form. Reid and Wilkie[9] have isolated an acidic arabinoxylan, an acidic galactoarabinoxylan, and a β-glucan from both leaf and stem tissue of *Avena sativa*, and Buchala and Wilkie[10] have isolated similar polysaccharides from *Triticum vulgare*. Blake and Richards[11] prepared, from a B-type hemicellulosic complex isolated from a legume, both the linear and branched xylans, and showed that each could be further fractionated by ethanol precipitation.

As grasses mature, their digestibility declines and, of the cell-wall carbohydrates, it is the hemicelluloses whose digestibility declines to the greatest extent[12]. As part of an investigation into the changes in the hemicellulosic complexes of grasses during the growing season, fractionation experiments, involving both iodine complexing and ethanol precipitation, are reported on the hemicellulosic complexes isolated from cell-wall preparations which have been prepared from rye-grass cut at four different stages of maturity.

EXPERIMENTAL

Materials. — S23 Rye-grass (*Lolium perenne*) tillers were harvested at four different levels of maturity during the growing season. Immediately on harvesting, the samples were rapidly dried in a forced-draught oven at 100–110° for 45 min and were milled to pass a 0.7-mm diameter screen. Cell-wall preparations were isolated after extraction with ethanol–benzene (1:2), water at 65°, and 0.5% aqueous ammonium oxalate, and digestion with pronase[13].

Extraction of hemicelluloses. — Each of the four cell-wall preparations was divided into two equal parts. The first part was extracted overnight with M potassium hydroxide under nitrogen, the alkaline extract was neutralised with acetic acid, and the hemicellulosic complex was precipitated with 3 volumes of ethanol. The residue was delignified by the chlorite method[14] for 1 h and re-extracted with M potassium hydroxide to give a second hemicellulosic complex. The residue was further extracted as before, but with 24% aqueous potassium hydroxide, to give a third hemicellulosic complex. The second part was delignified by the chlorite method, and the residue was successively extracted with M and 24% aqueous potassium hydroxide to give two further hemicellulosic complexes. No A-type hemicellulose was obtained at any stage.

Fractionation by complexing with iodine. — The procedure of Gaillard[8] was followed. In all experiments, some material was found to be insoluble in 3.7M calcium chloride (sp. gr., 1.3).

Fractionation by ethanol precipitation. — The solutions of the linear and branched components obtained from the iodine-complexing reaction were treated with an equal volume of ethanol and then kept at 4° overnight, and the precipitate was collected by centrifugation. The centrifugate was treated with an equal volume of ethanol and left at 4° overnight, and the precipitate was collected by centrifugation. Only very small amounts of carbohydrate remained in solution.

The composition of the fractions was determined by g.l.c. of the alditol acetates[15] derived from the hemicellulosic complex following hydrolysis with trifluoroacetic acid[16]. Lignin was determined by the acetyl bromide procedure[17].

RESULTS AND DISCUSSION

The hemicellulosic complex is a mixture of a number of different polysaccharides, and the yield and composition of the complex can vary depending on the method of isolation. A prior requisite is the removal from the plant material of non-cell-wall components by extraction with organic solvents and then water, the removal of pectin by extraction with ammonium oxalate or ethylenediamine tetra-acetic acid solutions, and the removal of protein by digestion with proteolytic enzymes or extraction with detergent solutions. When isolating pure hemicelluloses, it is usual to delignify the sample but, since one of the factors being investigated was the effect of lignin on the digestion of hemicelluloses in the rumen, each plant sample was divided into two parts, and hemicellulosic complexes were extracted from the first part with M potassium hydroxide, both before and after delignification, followed by extraction with 24% aqueous potassium hydroxide. The second part was delignified and extracted successively with M and 24% aqueous potassium hydroxide. Thus, from each of the four samples of S23 rye-grass cut at different stages of maturity, five different hemicellulosic complexes were obtained. Since no material was precipitated

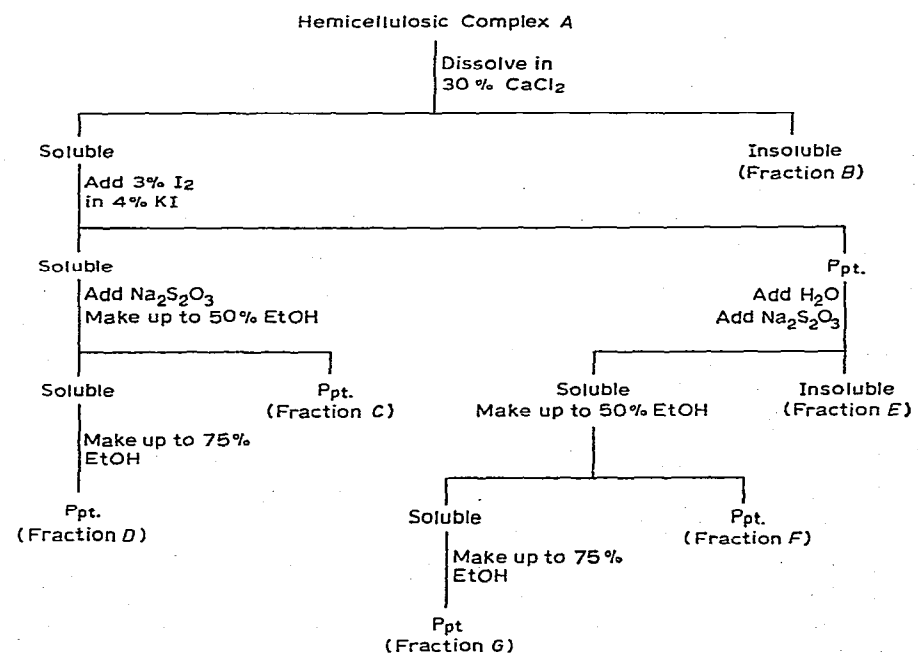

Fig. 1. Scheme for fractionation of hemicellulosic complexes.

on neutralisation of the alkaline extracts, each hemicellulosic complex was classed as B-type.

Each of the twenty hemicellulosic complexes (*A*) was fractionated by the method of Gaillard[8] into iodine-soluble and insoluble components. In virtually all the complexes, there was some material (*B*) which was not soluble in the calcium chloride solution. Ethanol precipitation of the solution of the iodine-soluble complex, after destruction of iodine with sodium thiosulphate, gave sub-fractions *C* (0–50% ethanol) and *D* (50–75% ethanol). The iodine-precipitated complex, after suspension in water and destruction of iodine, was centrifuged at high speed to separate the insoluble material (*E*), which was further fractionated by the addition of ethanol, as for the iodine-soluble complex, to give sub-fractions *F* and *G*. The fractionation scheme is outlined in Fig. 1.

The individual, neutral monosaccharides present in hydrolysates of the sub-fractions were determined, and typical results from the M potassium hydroxide extracts from delignified cell-walls of the four grass samples are shown in Table I, which also includes the yield of each fraction. Only residues of five monosaccharides, arabinose, xylose, mannose, galactose, and glucose, were found in significant quantities. Traces (<0.5% of total neutral sugar) of rhamnose and fucose, which were found in a few samples, presumably arose from contamination with pectic materials, even though grasses have a low content of pectin and the samples had been pre-extracted with solutions of ammonium oxalate.

As in previous findings, the total hemicellulose extracted, both as a proportion of the plant dry-matter and of the cell wall, increased with increasing maturity of the plant. During this period, the cellulose content, as a proportion of the cell wall, decreases with increasing maturity. The proportion of xylose residues in the samples extracted with M alkali rose with increasing maturity, whilst the proportion of arabinose and glucose residues declined. The proportion of galactose residues remained relatively constant, and only traces of mannose residues were detected. The decline in the proportion of glucose residues was very marked, since it accounted for ~20% of the neutral sugars at the earliest stage of growth but less than 5% at the most-mature stage. The hemicellulosic complex extracted with 24% aqueous potassium hydroxide comprised a relatively small proportion of the total hemicellulose, ranging from 10% for the youngest plant sample to ~3% for the most mature. These extracts contained a higher proportion of glucose residues than the M alkali extracts; the proportion declined with increasing maturity. Low, but significant, proportions of mannose were found in these extracts. Although the proportion of xylose residues rose, and that of arabinose residues declined, with increasing maturity, the ratio of xylose to arabinose, for each grass sample, was lower than for the M alkali extracts. Assuming that all the arabinose residues arise from arabinoxylans, this result suggests that the more highly branched arabinoxylans are less readily extracted by alkali than the more-linear polymers.

The yield of hemicellulosic complex extracted from grass cell-walls by M alkali was lower than that obtained from delignified cell-walls. However, when the residue

TABLE I

YIELDS AND COMPOSITIONS OF HEMICELLULOSIC FRACTIONS OBTAINED FROM DELIGNIFIED, GRASS CELL-WALLS AT DIFFERENT STAGES OF MATURITY BY EXTRACTION WITH M ALKALI

Cut No.	*Fraction*	*Yield (%)*[a]	*Carbohydrate composition (% of neutral sugar)*			
			Arabinose	*Xylose*	*Galactose*	*Glucose*
	A	36	15.7	60.1	3.3	20.8
	B	4	13.6	55.3	6.2	24.9
	C	3	29.7	55.6	10.1	4.6
1	*D*	1	30.1	56.1	11.8	2.0
	E	16	12.4	66.7	1.8	19.1
	F	76	16.8	67.9	2.1	13.2
	G	—				
	A	38	14.9	68.5	5.3	11.4
	B	6	13.5	69.8	4.6	12.1
	C	8	30.9	55.9	10.4	2.8
2	*D*	1	31.0	56.9	11.1	1.0
	E	17	10.4	76.1	1.7	11.8
	F	68	14.5	73.5	2.0	10.0
	G	—				
	A	39	11.2	76.9	3.6	8.3
	B	7	12.9	73.0	3.8	10.3
	C	11	31.3	52.6	12.4	3.7
3	*D*	1	30.1	55.5	10.9	3.5
	E	16	9.4	82.5	1.8	6.3
	F	64	11.7	78.4	2.0	7.9
	G	1	11.5	78.0	2.4	8.1
	A	40	10.1	77.7	3.5	8.7
	B	9	12.9	73.9	3.1	10.1
	C	11	31.6	52.2	13.6	2.6
4	*D*	1	31.1	53.0	13.5	2.4
	E	15	8.1	81.4	3.1	4.5
	F	62	9.2	81.8	1.7	7.3
	G	2	9.4	80.3	1.9	8.4

A is a percentage of the cell wall; *B* to *G* are percentages of *A*.

obtained after alkaline extraction of the cell walls was delignified and then re-extracted with M alkali, more hemicellulosic complex was obtained and, furthermore, the combined yield of hemicellulosic complexes, on a lignin-free basis, was greater than that obtained from delignified cell-walls. Although the general trends, with increasing maturity, in the xylose, arabinose, and glucose proportions in the extracts obtained before delignification were similar to those reported above for extracts obtained after delignification (see Table I), there were significant differences. The

glucose contents of the samples before delignification were lower, and the xylose–arabinose ratio was higher. The reason for the increased, overall yield of lignin-free, hemicellulosic complex is not clear. Buchala, Fraser, and Wilkie[18] reported that up to 8% of the total hemicellulose from different oat tissues was lost on delignification, and this loss is of the same order as the increased yields obtained in our experiments. It is possible that the hemicelluloses that are lost on delignification are recovered in the prior extraction with alkali.

The fractionation procedure (Fig. 1) showed that the concentration and composition of different polysaccharide fractions altered with increasing maturity of the plant. This is shown in Table I for M potassium hydroxide extracts from delignified cell-walls. Results for the other extracts were very similar. Fraction *B* showed a gradual increase in concentration with increasing maturity. The content of glucose residues was high, suggesting that it could contain a non-cellulosic β-glucan similar to those reported by Reid and Wilkie[9] from oat tissue and by Buchala and Wilkie[10] from wheat tissue. The iodine-soluble fractions (*C* and *D*) also increased with increasing maturity. The compositions of the two fractions were very similar and there was little change in the composition throughout growth. The content of glucose residues was very low, and the xylose–arabinose and xylose–galactose ratios were also low. These fractions are therefore highly branched galactoarabinoxylans of very similar structure. The second, iodine-soluble fraction (*D*) was a very minor component.

The iodine-precipitated material, which was by far the major polysaccharide fraction, declined as a percentage of the total complex. Fraction *G* was frequently absent and was a very minor component when present. Both fractions *E* and *F* contained low proportions of glucose and galactose residues, while the xylose–arabinose ratios were high, that from *E* being the higher. The ratio of xylose to arabinose rose strongly with increasing maturity, from $\sim$4 to 7 for fraction *F*. These fractions presumably comprise more-linear arabinoxylans, which are polydisperse in having a wide spectrum of xylose–arabinose ratios.

The results presented above show that rye-grass is similar to maize[7], oats[9], and wheat[10], in containing a highly branched, but homogeneous, galactoarabinoxylan, a wide spectrum of more-linear arabinoxylans having different xylose to arabinose ratios, and, possibly, a glucan. Acidic sugars were found in the hydrolysates of most fractions but it was not possible to assign their role. The structural significance of the mannose present in the 24% potassium hydroxide residues is also unknown. The relative proportions of these polysaccharides in the hemicellulosic complex show considerable variation with increasing maturity, as does the composition of the different polysaccharides. It has also been shown that extraction of hemicelluloses with alkali, both before and after delignification, can be used to isolate fractions that are richer in certain of the hemicellulose components.

The various hemicellulosic fractions have been examined by molecular-sieve chromatography. Each was eluted as an apparently single component, and the xylose–arabinose ratio for each iodine-soluble fraction was very similar throughout the carbohydrate peak. However, each iodine-precipitated sample gave a range of

values throughout the carbohydrate peak, further confirming that the linear arabinoxylans are polydisperse.

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