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ALKALI SOLUBILITY OF HEMICELLULOSES IN RELATION TO DELIGNIFICATION

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Key Word Index—*Pinus radiata*; Pinaceae; *Salix fragilis*; *Populus euramericana*; Salicaceae; *Lolium perenne*; *Chionochloa rigida*; Gramineae; bark; wood; leaves; delignification; hemicelluloses; cellulose.

Abstract—The polysaccharide composition of bark from *Pinus radiata*, *Salix fragilis*, and *Populus euramericana* has been determined. All the barks contained lower levels of cellulose and hemicellulose than the corresponding woods; cellulose:hemicellulose ratios were also lower in the barks. Alkali extracted all of the hemicellulose-A but only half of the hemicellulose-B from *P. radiata* bark without prior delignification. Similar alkaline extraction removed almost all of the hemicellulose (A + B) from ryegrass leaves without delignification. With the other samples tested only a part of the hemicellulose A and B is extracted without delignification. It is suggested that the polysaccharide so extracted represents wall hemicellulose which is not linked to lignin or other wall constituents by alkali-stable links.

INTRODUCTION

An extensive literature [1] on the non-cellulosic, alkali-soluble polysaccharides (hemicelluloses) of gymnosperm (softwood) woods is concerned with polymers extracted after chemical delignification. Without this treatment alkali extracts little hemicellulose from softwoods. With angiosperm (hardwood) woods, however, it has long been known [2] that much of the hemicellulose xylan can be dissolved by alkali without delignification. Plant tissues whose cells contain only primary walls with little lignin would naturally be expected to need no delignification for successful alkali extraction of the hemicellulose. This has been shown to be the case in, for example, lupin (*Lupinus angustifolius*) hypocotyls [3, 4], in fact delignification of such tissues can lead to serious losses of polysaccharide [3]. Nevertheless it is quite common practice in polysaccharide extraction to delignify leafy plant tissues which are rich in primary wall and relatively low (3–5%) in lignin, although in such cases the treatment may be unnecessary [5]. We have investigated this matter further by examining the alkali extraction of polysaccharides from a softwood (*Pinus radiata*) and two hardwood (*Salix fragilis* and *Populus euramericana*) tree barks (which contain cambial and phloem cells together with much lignin or tannin), from the respective woods which are also high in lignin and from grasses which contain lower levels of lignin. Possible *in vivo* relationships of polysaccharides in *P. radiata* bark have been further explored by measuring the action of microbial carbohydrases on the bark before and after a brief alkali treatment.

RESULTS AND DISCUSSION

Results from an acidic fractional analysis of total bark and wood from the three tree species are given in Table 1. While it is usual to regard the final, acid-insoluble, organic matter in plant tissues as Klason lignin, much of it in the pine bark is tannin (K. Markham, *personal commun.*). The rapid preliminary alkali extraction used to prepare the alkali-treated bark of Table 1 substantially lowers the level of this tannin fraction without removing polysaccharide. Compared with their woods the barks of each species contain lower levels of hemicellulose and substantially lower levels of cellulose. Polysaccharide compositions of the barks are similar to those recorded by Thornber and Northcote [6] for phloem tissue from pine and birch but different from those recorded from ash and sycamore.

The possible effect of lignin and high MW tannin on the alkali extractability of bark polysaccharides was investigated using *P. radiata* bark from which much of the tannin had been removed

Table 1. Carbohydrate composition of tree barks and woods

Fraction	Woods			Barks			
	Pinus radiata	*Populus euramericana*	*Salix fragilis*	*Pinus radiata*	*Pinus* radiata*	*Populus euramericana*	*Salix fragilis*
Neutral detergent soluble	7·7†	7·0	14·0	24·6	12·5	47·5	44·9
Hemicellulose	11·4	14·0	13·3	7·7	10·0	7·8	10·0
Cellulose	46·3	46·6	47·4	13·7	23·0	16·8	20·5
Cellulose : hemicellulose ratio	4·1	3·3	3·5	1·9	2·3	2·2	2·1
Acid insoluble (lignin and/or tannin)	25·4	14·4	13·3	38·4	21·8	18·5	12·7

* *P. radiata* subjected to a preliminary extraction with alkali (see Experimental).
† Results expressed as % original oven-dry weight.

by the preliminary, short, cold alkali treatment. The results (Table 2) show that all of the hemicellulose-A (long chain glucurono-xylan; precipitated from alkali with acid) was readily extractable without delignification although much of the hemicellulose-B (polysaccharide mixture not acid precipitated) was only extracted after delignification. In contrast, *P. radiata* wood hemicellulose was, as expected, almost completely resistant to alkali extraction without prior delignification.

Polysaccharide extraction by alkali, with and without delignification was also compared for the bark and wood of two hardwoods and leaf samples from two grasses; ryegrass (*Lolium perenne*) and snowtussock (*Chionochloa rigida*) containing 3 and 9% respectively of Klason lignin. The results in Table 3 show that without delignification there is almost complete extraction of hemicellulose-A and B from rye grass leaves but a very low extraction from poplar bark.

When depectinated plant cells are digested with a mixture of fungal cellulase and hemicellulase the extent of hydrolysis reflects the degree to which the cell wall polysaccharide is accessible to enzymes and not coated with, or linked to lignin or other interfering material [7]. With *P. radiata* bark digestion with three such fungal enzyme mixtures yielded reducing sugars equal to 0·8, 0·5, and 5·1% of the detergent extracted bark. When the same digests were carried out on bark which had been subjected to the short alkali treatment, reducing sugars equivalent to 3·4, 4·8 and 8·1% of the detergent-extracted bark were now liberated.

Explanations for the alkali solubility of much of the hemicellulose could well depend on the extent of its linkage to alkali-insoluble cell wall constituents by alkali-labile and alkali-stable links. At one extreme, particularly when lignin levels are very low, the extractable hemicellulose might not be linked to insoluble cell wall constituents by any alkali-stable links. Ryegrass leaves, where, without any delignification there is almost complete alkali-solubility of the hemicellulose and extensive (60–70%) enzymic hydrolysis of the cell wall polysac-

Table 2. Alkali extraction of *Pinus radiata** bark with and without chlorite delignification

Extraction sequence	(Freeze-dried, unpurified† fractions as % of original oven-dry bark) Hemicellulose-A	Hemicellulose-B
(a) *No initial delignification*		
1. 10% KOH	6·3 (0)‡	7·5 (3·1)
2. 24% KOH	0	0
3. 10% KOH after delignification	0	10·7
(b) *With initial delignification*		
1. 10% KOH	4·4 (0)	17·9 (17·9)
2. 24% KOH	0	3·8

* Solvent extracted bark subjected to a preliminary extraction with alkali.
† All hemicelluloses contained much non-polysaccharide material and gave 25–35% reducing sugars on acid hydrolysis.
‡ Results in parentheses for *P. radiata* wood.

Table 3. Effect of delignification on extraction of hemicelluloses from plant tissues

Fraction	Wood		Bark		Grass leaves	
	Populus euramericana	*Salix fragilis*	*Populus euramericana*	*Salix fragilis*	*Lolium perenne*	*Chionochloa rigida*
(a) *Without delignification*						
Hemicellulose-A*	11·6(47·6)†	6·7(43·2)	2·2(38·4)	3·5(28·9)	2·3(24·1)	3·9(49·2)
Hemicellulose-B*	6·2	5·0	3·7	4·4	12·4	17·3
(b) *With initial delignification*						
Hemicellulose-A	17·7(55·0)	15·5(66·8)	9·6(58·5)	7·8(40·3)	2·1(42·5)	5·9(73·6)
Hemicellulose-B	20·5	18·2	13·4	9·9	14·4	31·7

* Unpurified freeze-dried fractions as % of initial oven-dry plant tissue.
† In parentheses % of anhydro-reducing sugars in fractions.

charide [7], could be such an example. In lupin seed hulls, which are very low in lignin (0·5–0·9%) but high in cell wall polysaccharides, the hemicellulose is also both completely alkali soluble and almost completely enzymically hydrolysed without delignification [8]. *Chionochloa* leaves on the other hand appear to have a hemicellulose-cell wall association which permits, in the absence of delignification, ready alkali extraction of much of the hemicellulose-A but little access of enzymes to the cell walls, which are only hydrolysed to the extent of 5–6% [9]. A similar situation exists in pine bark. With the woods and barks, apart from the possible role of alkali in cleaving alkali labile links to solubilise the hemicellulose, there are two other possibilities. Firstly the pine bark is actually very low in lignin with most of the Klason lignin being in fact high MW tannin unextracted by the short alkali treatment and in no way linked to the hemicellulose. Secondly xylans of hardwoods are known to be extensively acetylated [10]; such polysaccharide will be deacetylated by alkali and dissolved or rendered accessible to enzyme attack.

Delignification as an essential pretreatment for the extraction of non-cellulosic plant cell wall polysaccharides seems to have been developed largely in terms of either extraction from gymnosperm wood or complete extraction. The present results, together with those of others [3–5, 8], suggest that delignification may be unnecessary with immature or low lignin plant tissues, even when delignification losses are small [11]. In investigating polysaccharide associations within the cell walls of plants it also seems that an extraction with and without delignification should be done to distinguish between hemicellulose polysaccharide closely associated with the other cell wall polymers, and similar polysaccharide not so closely associated. The latter polymer should be rendered digestible to fungal or rumen microbial carbohydrases by simple alkali treatment irrespective of whether it is merely acetylated or linked by alkali-labile links to lignin.

EXPERIMENTAL

Bark, wood and grass samples. Bark (2–3 kg) was separated from the trunk of a mature *Pinus radiata* tree at a local sawmill and from logs (12–15 in. dia.) of poplar (*Populus euramericana*) and willow (*Salix fragilis*) supplied by Mr. C. Van Kraayenoord, Plant Materials section, Ministry of Works, Palmerston North. Pine sawdust was from the same mill and sawdust was produced from the debarked willow and poplar logs with a chainsaw. Ryegrass (*Lolium perenne*) leaves from a pasture 6–9 cm. in height and snow tussock (*Chionochloa rigida*) leaves from subalpine growth, were harvested in the spring and summer respectively. All samples were dried for 48 hr in a freeze drier and milled to pass a 1 mm sieve.

Extractions. Before alkali extraction pine bark was extracted with boiling EtOH–C_6H_6 (1:2) and boiling H_2O–Me_2CO (1:1) to remove soluble tannins. All of the bark, wood and grass samples were then extracted with boiling neutral detergent [12] to remove solubles, protein and pectic material. Delignification was with $NaClO_2$–HOAc [13]. The bulk of the Me_2CO insoluble tannins was removed from a portion of the pine bark by stirring it for 10–15 min at 20° with 2–4 vol. of 10% NaOH, filtering and washing well with H_2O and Me_2CO (Porter, personal commun.). This rapid treatment only removed traces of carbohydrates. Alkali extractions were done with KOH (10%) or (24%) by stirring 18 hr at 20° under N_2 using 40 ml alkali per g of sample. Filtered extracts were acidified to pH 4·8 with HOAc, stood 18 hr at 2° and precipitated hemicellulose-A collected by centrifugation. Dialysis and freeze-drying of the acidified supernatant yielded hemicellulose-B.

Analyses. After solvent and detergent extraction samples were analysed for polysaccharide by sequential hydrolysis with boiling N acid and treatment with 72% acid [14]. Anhydroreducing sugars in the two hydrolysates were recorded as hemicellulose and cellulose respectively. Hemicellulose-A fractions were similarly hydrolysed with 72% acid and reducing sugars measured. Total reducing sugars in acid and enzyme hydrolysates were measured by the Nelson [15] cuprimetric method. The amount of neutral detergent soluble material was obtained

by weighing dried samples before and after extraction. Moisture contents were measured by heating at 110° for 18 hr and, except where stated, all results are expressed on an oven dry basis.

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