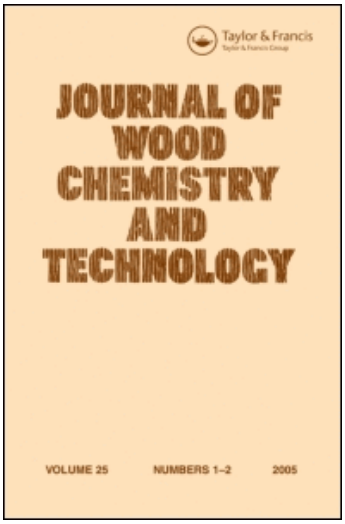

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ISOLATION AND ANALYSIS OF THE HEMICELLULOSES OF THE FOSSIL TREE (*METASEQUOIA GLYPTOSTROBOIDES*)

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ABSTRACT

The hemicelluloses of the fossil tree (*Metasequoia glyptostroboides*) were isolated from a mildly-prepared acid chlorite holocellulose. Arabinoxylan, glucomannan, and galactoglucomannan were isolated from the holocellulose by extraction and fractionation techniques. The sugar compositions of the polysaccharides were analyzed by quantitative thin layer chromatography and gas chromatography. The hemicelluloses accounted for 21-22% of the wood; the arabinoxylan about 10%, the glucomannan about 6.3%, and the galactoglucomannan about 4.8%. The glucose:mannose ratio for the glucomannan and galactoglucomannan were approximately 1:1.6 and 1:1.7, respectively, in contrast to a ratio of 1:3 commonly found in coniferous woods.

INTRODUCTION

The fossil tree (*Metasequoia glyptostroboides*) is a coniferous relic species indigenous to China which has recently been introduced to other countries. Some investigations of its physical properties have appeared in the literature. For example, Saka Kechi Iora Bi[1] found that it has low longitudinal compressive strength and shearing strength, and that it is soft and brittle, but it is suitable for pulp manufacture. It has also been demonstrated that because its fibers are long, with a high length to width ratio and a low wall to lumen ratio, it is a potential raw material for paper production.[2]

No studies of the hemicelluloses of the fossil tree have been reported. A scheme (Figure 1) involving the separation of the glucomannan family of poly-

saccharides developed by Timell[3], and the separation of arabinoxylans by Dutton[4] was adapted for this purpose. The fractions isolated by this scheme were then analyzed by quantitative thin-layer and gas chromatographic techniques.

EXPERIMENTAL PROCEDURES

Holocellulose Preparation

The holocellulose was prepared by a method recommended by Li Zhongzheng.[5] Extractive-free wood meal (40-60 mesh) was reacted with aqueous acetic acid (10ml/L, pH 4-5) containing sodium chlorite (50 g/L) at a liquor-to-wood ratio of 10:1 for about 25 days at room temperature. During the preparation, the brown liquid containing soluble lignin was removed at random intervals and fresh reagent was added to the wood meal. The reaction was terminated when the lignin content of the residue was less than 4%. The solid was filtered from the liquor, washed with water, and air-dried, resulting in a white holocellulose (Table 1).

Extraction

The procedures of Timell[3] and Dutton[4] for separation and purification of hemicellulose are satisfactory for obtaining high purity hexosan or pentosan, respectively, but neither of them is capable of separating the total hemicellulose from the wood. Therefore to isolate and purify the hemicelluloses completely, a method combining the two procedures was used (Fig. 1). The holocellulose was first extracted with 24% KOH under nitrogen at room temperature. The mixture was shaken for 10 hours and allowed to settle overnight before the reddish-brown floc was recovered by filtration and washed with distilled water. The combined filtrate and washings were added to four volumes of 95% ethanol containing acetic acid (pH 6). A yellowish-gray precipitate slowly settled from the medium.

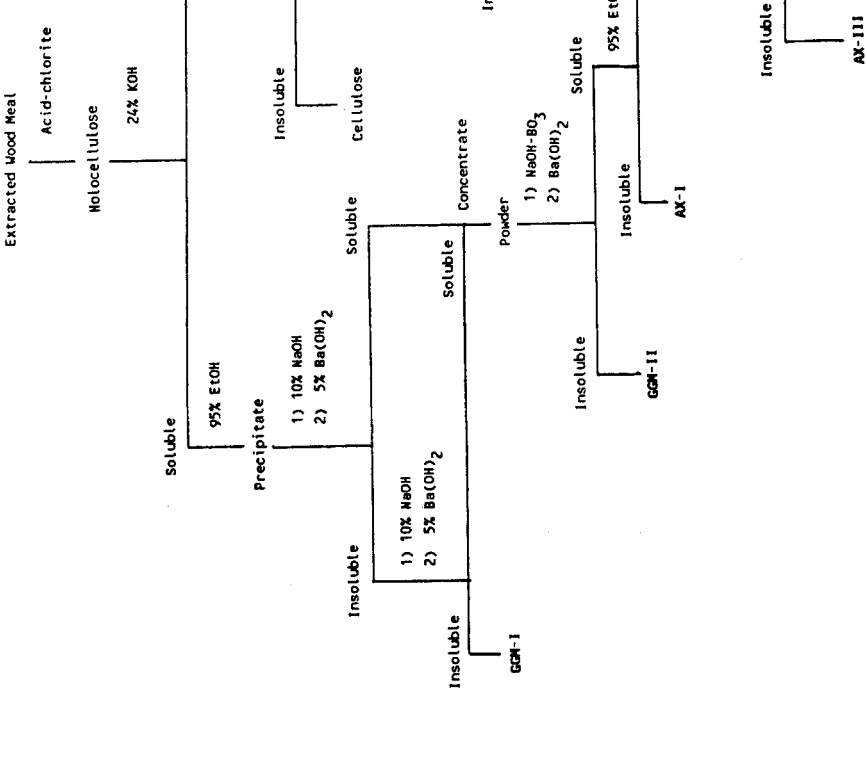

Figure 1. Hemicellulose Isolation and Purification

The solid remaining after extraction of the holocellulose with 24% KOH was extracted with 17.5% NaOH containing 4% H_3BO_3. A precipitate was isolated from the yellow extract as described above.

Fractional Purification

The precipitate from the NaOH-borate extract was dissolved in 10% NaOH (1:20, w:v) and two volumes of 5% aqueous barium hydroxide were added dropwise to precipitate the crude polysaccharide. The precipitate was allowed to settle, concentrated by centrifugation, washed with 5% NaOH, filtered, and dissolved by stirring with 50% acetic acid. The glucomannan (GM, Fig. 1) was isolated as a white powder from this solution.

The precipitate from the 24% KOH extraction was similarly dissolved in 10% NaOH and crude glactoglucomannan was precipitated by addition of two volumes of 5% aqueous $Ba(OH)_2$. Galactoglucomannan (GGM-I, Fig. 1) was obtained from the crude material by repeating the fractionation. The supernatants from the isolation of GGM-I were combined with other washings and concentrated *in vacuo* to yield a white powder, rich in pentosans. The powder was dissolved in NaOH-borate solution and two volumes of 5% $Ba(OH)_2$ were added dropwise to precipitate the polysaccharide. This procedure was repeated on the precipitate to yield another galactoglucomannan (GGM-II, Fig 1).

Anhydrous ethanol was added to the supernatants from the preparation of GGM-II until no further precipitation occurred. An arabinoxylan fraction (AX-I, Fig. 1) was isolated from the precipitate, and another arabinoxylan fraction (AX-II, Fig. 1) was isolated from the supernatant by precipitation with ethanol. The latter fraction was redissolved in NaOH-borate and the arabinoxylan fractions (AX-III and AX-IV, Fig. 1) were isolated by similar techniques.

Polysaccharide Hydrolysis

The tlc and glc analyses used to monitor polysaccharide purity and the proportion of sugars in the fractions required that the hemicelluloses first be

hydrolyzed. The oven-dried sample (0.5 g) was dissolved in ice-cold 72% H_2SO_4 (5 mL). After four hours at room temperature, the hydrolysis mixture was diluted to 0.3% acid and refluxed for 6 hours. The supernatant was prepared for analysis at a concentration of 10 $\mu g/\mu L$, after filtration neutralization with $Ba(OH)_2$ and concentration.

Quantitative Analysis by TLC

Analyses were performed on manually prepared plates with a 0.25 mm layer of silica gel H (grain size 10-40 micrometer), a buffering agent consisting of equal volumes of 1 M H_3PO_4 and 0.1 M Na_2HPO_4 at pH 5, and a CMC binder. The chromatograms were developed in the ascending mode using butyl acetate, 95% ethanol, pyridine, water (8:2:2:1) for about 7 hours. A solution consisting of 1% each of D-glucose, D-galactose, D-mannose, L-Arabinose, and D-xylose was used as a reference.

A CS-910 dual wavelength thin-layer scanner employing a reflecting sawtooth method was used for quantitative analysis of thin-layer chromatograms. A tungsten lamp and a scanning speed of 40 mm/min were employed. Measurements were made at λ_s = 390 nm with the reference λ_r = 700 nm. The R_f values of the sugars were: glucose, 0.414; galactose, 0.343; mannose, 0.486; xylose, 0.650; and arabinose, 0.564.

Preparation of the Aldononitrile Peracetates and GLC Analysis

The aldononitrile peracetates of sugars were prepared by concentrating and drying 10 mL of the final hydrolysate solution on a water bath at 60°C. A small amount of the dried sample was dissolved in anhydrous pyridine (2 mL), after which NH_2OH-HCl (0.1 g) was added. The solution was heated on a water bath for 1 hour at 90°C, diluted with distilled water and extracted with chloroform. The chloroform extract was washed with water, dried with Na_2SO_4, filtered, and analyzed by gas chromatography.

Gas chromatographic analyses utilized a Model GC-7AG instrument equipped with a flame ionization detector, an R-112 recorder, and a C-E1b microprocessor. A 1.5 m x 3 mm glass column packed with 3% ECNSS-M on 80-100 mesh acid-washed, DMCS treated Gas Chrom W was used. Analysis conditions were: carrier gas (N_2), 50 mL/min; column, 185°C; and detector, 230°C.

RESULTS AND DISCUSSION

Chemical Composition

Apart from its greater lignin content, the chemical composition of *Metasequoia glyptostroboides* is characteristic of many other coniferous species (e.g., see Table 1). The residual lignin content of a traditional chlorite holocellulose of the fossil wood was 5.41% while that of modified chlorite holocellulose used to isolate the hemicelluloses was only 2.58%. Thus, the isolation of the pure hemicelluloses was facilitated by the lower lignin content which did not unduly hinder the fractionation processes.[3]

Measurement of Composition

Yield data and the results of tlc and glc analyses of the holocellulose and the various polysaccharide fractions are summarized Table 2. The data obtained for the sugar composition of the materials by the two analytical methods were very similar.

Based on the data in Table 2, *Metasequoia glyptostroboides* contains about 22% hemicelloses; about 10.5% arabinoxylan, 6.3% glucomannan, and 4.8% galactoglucomannan.

TABLE 1

Comparison of the fossil wood with *Picea jejoensis* and *Abies nephrolepis*

Component[a]	Fossil Tree yield, %	*Picea j.*[8] yield, %	*Abies n.*[8] yield, %
ash	0.39	0.31	0.58
pentosan	10.53	11.45	11.57
cellulose	47.60	48.45	49.92
lignin			
Klason	31.17	29.12	30.85
acid-soluble	0.50		
holocellulose[b]	77.30		
holocellulose Klason lignin	5.41		

[a]Percentage of extractive-free wood.

[b]Traditional chlorited method.

TABLE 2

Yield, Sugar Composition, and Purity Data

	yield[a] %	AM[b]	Sugar Composition, %					Purity, %
			ga	gl	ma	ar	xy	
Holocellulose	67.03	T	4	65	13	4	14	
		G	3	63	16	4	14	
KOH Extract	18.36	T	6	14	26	10	44	
		G	5	12	27	9	43	
$NaOH-H_3BO_3$ Extract	7.46	T	5	27	63	2	3	
		G	4	27	62	3	4	
Residual	40.90	T	1.5	91.9	5.2	0.6	0.6	
		G	1.0	92	6	0.5	0.5	
GGM-I	4.01	T	10	31	51	4	4	92
		G	10	29	53	5	3	92
GGM-II	0.81	T	9	25	61	0	5	95
		G	9	26	55	5	5	90
GM	6.26	T	1	37	60	2	0	98
		G	1	38	56	4	1	95
AX-II	7.23	T	4	4	3	15	74	89
		G	3	5	2	21	69	90
AX-IV	3.30	T	3	4	1	10	82	92
		G	3	4	2	12	79	91

[a]Extractive-free wood basis.

[b]Analytical method; T-tlc and G-glc.

Extraction with Alkali

It was desired to extract as much hemicellulose as possible while minimizing degradation. The initial extraction with KOH was employed to maximize xylan removal.[6] The subsequent extraction with NaOH-H_3BO_3 preferentially removed the glucomannan component.[6,7] Exclusion of air during the extractions minimized oxidative degradation.[7]

The yield of the residual extracted holocellulose (mainly cellulose) was 40.90%. The residual, analyzed by quantitative tlc and glc, consisted of 92% glucose, 6% mannose and about 2% other sugars. This suggests that the residual hemicellulose is closely linked to the cellulose (see Table 2).

Isolation and Purification

The KOH extract of the holocellulose was a mixture of arabinoxylan and galactoglucomannan while secondary NaOH-H_3BO_3 extract was predominantly glucomannan contaminated with a small amount of the other components. Chemically homogeneous representatives of the major constituents were obtained by repeated fractionation.

The results of further purification of galactoglucomannan GGM-I by the technique described by Timell[7] are reported in Table 3. Little change occurred in the hexose composition, suggesting that the polysaccharides from the isolation are chemically homogeneous. The galactoglucomannan had a galactose:glucose:mannose ratio of approximately 0.3:1:1.7, in contrast to a ratio of 1:1:3 commonly found in coniferous woods. The glucomannan GM had a glucose:mannose ratio of approximately 1:1.6, in contrast to a ratio of 1:3 commonly found in coniferous woods. Thus, both polysaccharides have less mannose in the molecule main chain than other coniferous glucomannans.

The sugar compositions of the polysaccharides obtained by the current method are compared in Table 4 with the data obtained by Timell for *Tsuga canadensis*[3] and Dutton for *Pinus ponderosa*.[4] The purity of the fractions ob-

TABLE 3

Galactoglucomannan Purification

	Yield[a]	Sugar Composition, %					Ratio	Purity
	%	ga	gl	ma	ar	xy	ga:gl:ma	%
GGM-I[b]	4.01	10	31	51	4	4	0.3:1:1.6	92
GGM-I[c]	3.21	9	32	54	2	3	0.3:1:1.7	95

[a]Extractive-free wood basis.

[b]See isolation scheme in Figure 1.

[c]After refractionation.

TABLE 4

Comparison of the Fossil Tree with *Tsuga canadensis* and *Pinus ponderosa*

	Sugar Composition, %					Purity
	ga	gl	ma	ar	xy	%
GGM-I	10	30	51	4	4	92
GGM-I[a]	19	19	57	2	3	95
GGM-II	9	25	61	0	5	95
GGM-II[a]	12	21	59	2	6	92
GM	1	37	60	2	0	98
GM[a]	4	24	72	0	0	99
AX-II	4	4	3	15	74	89
PPX_4[b]	5	2	2	15	77	92
AX-IV	3	4	1	10	82	92
PPX_5[b]	1	1	1	24	73	97

[a]Hexosan fractions from *Tsuga canadensis*[3]

[b]Pentosan fractions from *Pinus ponderosa*[4]

tained by the different methods is quite close, indicating that the extraction and fractionation process adopted in this study is as effective, if not more so, than the traditional techniques.

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