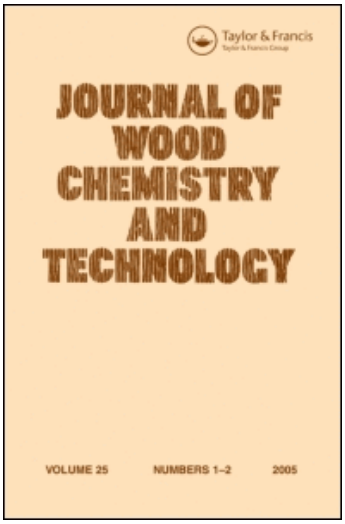

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

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To cite this Article Hromádková, Zdena , Ebringerová, Anna , Kačuráková, Marta and Alföldi, Juraj(1996) 'Interactions of the Beechvood Xylan Component with Other Cell Wall Polymers', Journal of Wood Chemistry and Technology, 16: 3, 221 — 234
To link to this Article: DOI: 10.1080/02773819608545805
URL: http://dx.doi.org/10.1080/02773819608545805

JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY, 16(3), 221-234 (1996)

INTERACTIONS OF THE BEECHWOOD XYLAN COMPONENT WITH OTHER CELL WALL POLYMERS

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ABSTRACT

Sodium chlorite holocellulose of beechwood was extracted in succession with aqueous ammonia solutions of increasing concentration (1-26% NH_4OH), and finally with 5% sodium hydroxide. The polymeric fractions obtained were composed mainly of 4-O-methylglucurono-xylan polymers which occur in two distinct molecular populations. The 1% NH_4OH-extract contained the most accessible polysaccharide fraction which represents a mixture of O-acetylated 4-O-methylglucuronoxylan, residual lignin, cellulose fragments and pectic polysaccharides of the rhamnogalacturonan type containing arabinan and galactan chains. A 4-O-methylglucuronoxylan-polygalacturonan complex with a minor proportion of neutral sugars and residual lignin was isolated from the 10% NH_4OH-extract. The results suggest that some of the residual lignin and pectic polysaccharides are bound by alkali-stable linkages to xylan and/or cellulose chains in the cell-wall complex.

INTRODUCTION

Interactions between the polymeric cell wall components of higher plants have attracted considerable interest[1]. They may play a significant role in proccessing of lignocellulose materials, like defibration, delignification, separation of cell wall

components, and bioconversion of cellulosic materials into chemicals and fuel. Chemical linkages between lignin, hemicelluloses, and recently also cellulose, have been reported by many authors[2-7]. Interactions between hemicelluloses and pectin were suggested for the xyloglucan in apple cell walls[8] and arabinoxylan in cereal tissues[9] and other plants[10]. Pectic substances which are primary cell wall components[1] occur in the case of hardwoods mainly in their bark or cambial tissues[11]. Their presence in wood consisting mostly of secondary cell walls was deduced from the occurence of galacturonic acid, arabinose, galactose, rhamnose, and these sugars containing aldobiouronic acids in the partial hydrolysate of hardwood and their xylan fractions[12-14]. However, rhamnose and galacturonic acid were also reported to be incorporated into xylan chains at their reducing end[15].

The aim of this study was to investigate the less abundant polysaccharides of beechwood and their interactions with xylan and the other cell wall components. For this purpose, sequential extractions of the chlorite holocellulose with aqueous solutions of ammonia and sodium hydroxide were used, and the structural and molecular properties of the isolated polysaccharide fractions were characterized.

RESULTS AND DISCUSSION

The extractibility of the carbohydrate polymers from the sodium chlorite-delignified beechwood during fractional extraction (Figure 1) indirectly reflects the interactions of these components in the cell walls. The yield and analytical characteristics of the fractions isolated with aqueous ammonia solutions of increasing concentration, and 5% NaOH are summarized in TABLE 1.

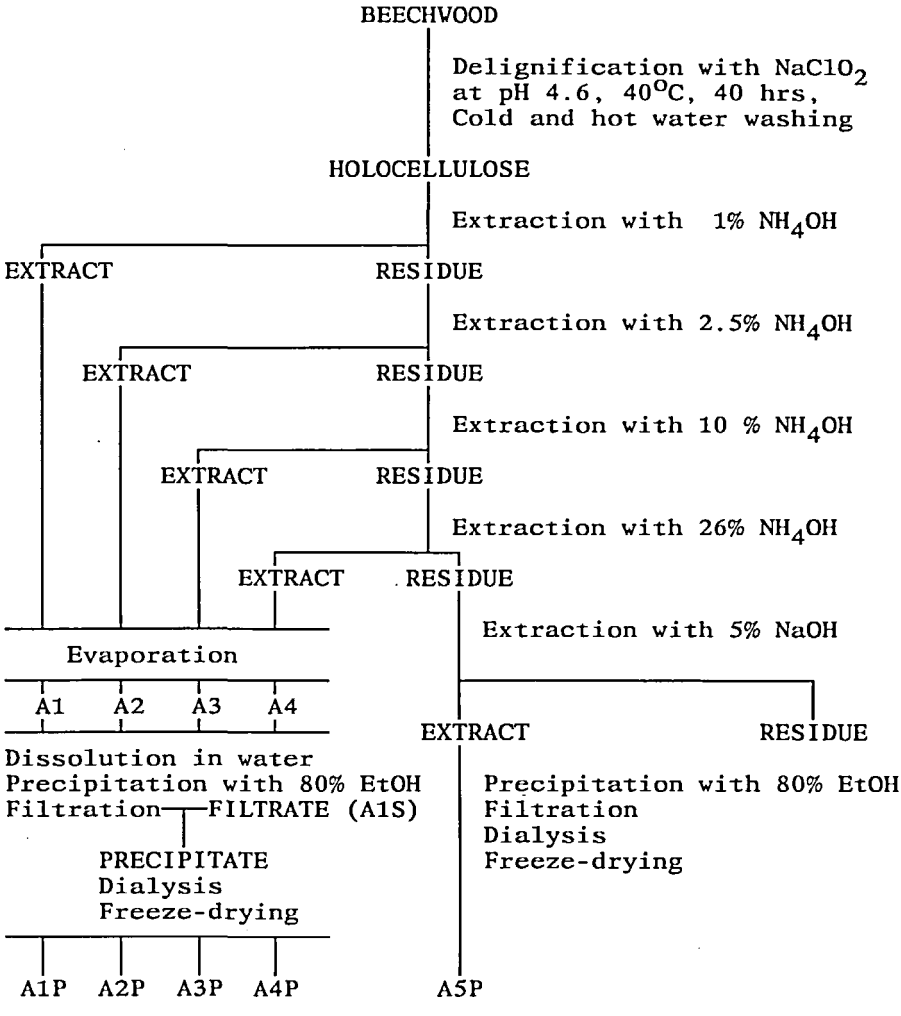

Figure 1. Fractional extraction scheme of beechwood holocellulose

The highest yield of polysaccharides was obtained even at the lowest concentration of aqueous ammonia. In the subsequent steps, the extractibility decreased to a minimum at 10 % NH_4OH and slightly increased at 26%

TABLE 1. Yield and characteristics of the hemicellulose fractions isolated from beechwood holocellulose

Fraction	Yield %[a]	Sugar composition (mole %)					UA %[b,c]	Xyl/MeGA[d]	DP[e]
		Ara	Xyl	Glc	Gal	Rha			
A1P	11.5	6.8	83.0	3.4	5.1	1.7[f]	12.5	8:1	89
A1S	7.1	12.9	78.9	2.5	5.7	T	nd	nd	nd
A2P	5.2	1.8	90.9	3.5	2.8	1.0	14.5	14:1	nd
A3P	0.9	1.9	91.7	3.2	2.3	0.9	29.5	9:1	110
A4P	4.2	0.8	94.7	2.4	2.1	T	12.8	13:1	nd
A5P[g]	14.6	T	95.5	2.7	1.3	0.2	12.2	12:1	120

[a]based on holocellulose; [b]determined by alkalimetric titration; [c]D-galacturonic and 4-O-methyl-D-glucuronic acids were estimated by p.c.; [d]calculated by integration of the ^{13}C NMR signal areas for C-1 of D-xylose and 4-O-methyl-D-glucuronic acid residues; [e]determined by viscometry in DMSO; [f]the fraction contains <0.1% of fucose; [g]the fraction contains <0.1% of mannose; T, traces.

NH_4OH, probably, due to the strong penetrating effect of ammonia vapours in the concentrated ammonia solution.

Fractions A1P-A4P released by aqueous ammonia amounted to about 66% of the totally extractable polysaccharides in this experiment. They are composed, similarly as the alkali-extracted fraction (A1P), mainly of 4-O-methylglucuronoxylan. This is seen in their IR spectra which exhibit absorption bands typical of hardwood xylans[16,17]. The spectra also show weak absorption bands at 3195, 1662, and 1610 cm^{-1} attributed to amide groups[18] which are formed by ammonolysis of ester groups.

Figure 2 shows the ^{13}C NMR spectra of the xylan fractions exhibited signals of both glucuronoxylan sugar constituents which were assigned in accord with published data[19,20]. As can be seen in TABLE 1, A1P-

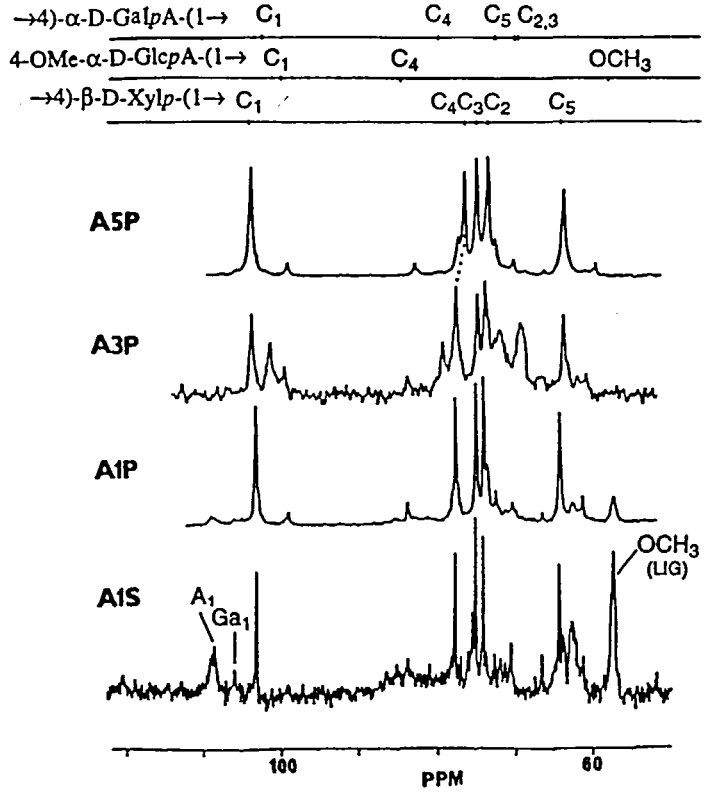

Figure 2. ^{13}C NMR spectra of beechwood holocellulose fractions A1S, A1P, A3P (in D_2O), and A5P (in DMSO-d_6)

A5P contain, in decreasing amounts, also minor proportions of arabinose, galactose, rhamnose, and galacturonic acid. The ^{13}C NMR spectrum of the most accessible xylan fraction (A1P) shows signals at δ 108-109 and 62.3 which are attributed to C-1 and C-5, respectively, of α-L-arabinofuranosyl residues[21,22]. The signals at δ 104-105.5 and 17.8 can be assigned to C-1 of β-D-galactopyranosyl and C-6 of rhamnopyranosyl residues, respectively[23,24]. All ammonia-extracted

fractions contain galacturonic acid, particularly A3P. After substraction of the IR spectrum of A3P from that of A5P, absorption bands at 1076, 1012, 951, and 855 cm^{-1} appeared, which are typical of IR spectrum of pectin[18]. The ^{13}C NMR spectrum of A3P exhibits well resolved signals at δ 100.60, 69.40, 69.68, 79.25, and 72.23 which are assigned[25,26] to C-1/C-5 of α-(1->4)-D-galacturonan chains.

The glycosyl linkage analysis of fractions A1P and A3P (TABLE 2) extended the ^{13}C NMR results. It shows that both fractions are mainly composed of terminal, 4-, and 2,4-linked xylopyranosyl residues which are derived from the xylan component. Reduction of the partially methylated acidic sugars of A1P yielded 2,3, 4-tri-O-methylglucose, 2,3,4-tri-O-methylgalactose, 2,3, 6-tri-O-methylgalactose, and 3,4-di-O-methylrhamnnose in molar ratios of 5:2:1: 0.8. In the case of A3P, 2,3, 4-tri-O-methylglucose and 2,3,4-tri-O-methylgalactose were estimated in the molar ratio of 3:1. The last mentioned sugars originate from galacturonic and glucuronic acid constituents, respectively. The presence of 3-linked rhamnosyl and fucosyl residues are indicative of rhamnogalacturonan RG II[27] in A1P. The results confirm that glucuronoxylan fractions are associated with pectic polysaccharides rich in arabinan and galactan. This prevalence of 4-linked glucosyl residues indicates the presence of cellulose and/or xyloglucan fragments. Due to their low abundance, no corresponding signals are distinguishable from the noise in the ^{13}C NMR spectra of the xylan fractions, except that of C-6 at δ 60.5.

As seen from the HPGPC chromatograms (Figure 3b), the xylan fractions contain two molecular populations. TABLE 3 gives the molecular weight average, polydispersity (M_w/M_n) and area % of the two peaks. The

TABLE 2. Methylation analysis* of fractions A1P and A3P

Glycosyl residue	Position of *OMe* groups	Deduced glycosyl linkage	Mole % A1P	Mole % A3P
Xyl*p*	2,3,4	terminal	2.4	1.3
	2,3	4-	76.1	83.5
	3	2,4-	7.0	8.9
Rha*p*	2,3,4	terminal	0.4	-
	3,4	2-	0.3	-
	2,4	3-	1.8	0.3
	2,3	4-	0.2	-
	3	2,4-	0.3	0.4
Fuc*p*	2,3,4	terminal	T	-
Ara	2,3,5	terminal (furanosyl)	1.6	0.3
	2,3,4	terminal (pyranosyl)	0.4	-
	3,5	2-	0.5	-
	2,3	5-	1.7	0.7
		2,3,5- (2,3,4-)	0.9	0.2
Gal*p*	2,3,4,6	terminal	0.8	0.3
	2,4,6	3-	0.3	-
	2,3,6	4-	1.4	1.1
	2,3,4	6-	0.9	-
	2,4	3,6-	0.6	-
	2,6	3,4-	0.1	0.2
Glc*p*	2,3,4,6	terminal	0.1	0.1
	2,3,6	4-	2.1	2.7
	2,3,4	6-	0.1	-

*Acidic sugars are not included;
T = traces.

TABLE 3. Molecular properties of fractions A1P-A5P derived from HPGPC

Fraction	Molecular weight peak (I) $M_w.10^{-3}$	M_w/M_n	Area %[a]	Molecular weight peak (II) $M_w.10^{-3}$	M_w/M_n	Area %[a]
A1P	104	1.01	23	31	1.24	77
A2P	95	1.01	45	26	1.18	55
A3P	96	1.01	43	30	1.20	57
A4P	105	1.02	47	29	1.29	53
A5P	103	1.01	52	30	1.30	48

[a]Calculated from the peak areas of the RI-detected chromatograms.

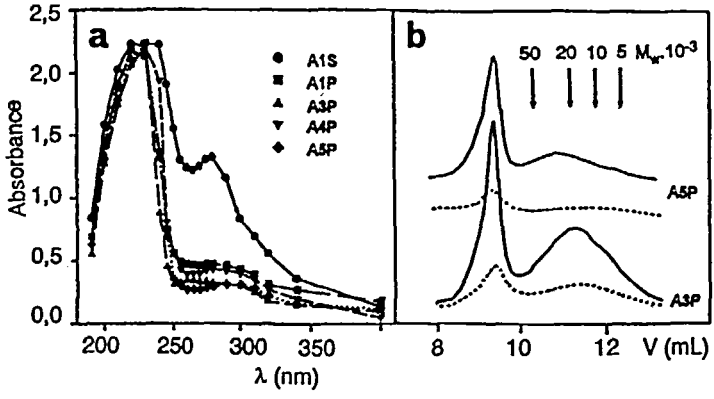

Figure 3. (a) UV-spectra of beechwood holocellulose fractions A1S (c=0.31 mg/ml), A1P (c=1.81 mg/ml), A3P (c=2.50 mg/ml), A4P (c=2.51 mg/ml), and A5P (c=3.37 mg/ml);
(b) HPGPC chromatograms of fractions A3P and A5P; (——) RI-detection, (...) UV_{254}-detection.

first peak (I) shows an apparent weight average molecular mass M_w 117-131 kD and the second one (II) M_w 26-31 kD. The ratio of both components varies in A1P-A5P. The most accessible xylan A1P has the highest proportion of the low molecular component. The average M_w values calculated from the proportion of peaks I and II are substantially higher than those calculated from viscosity data (TABLE 1). Recently, similar results were reported for wood polymers in birch kraft pulps[28].

Although A1P-A5P are free of Klason lignin (estimated as the acid-insoluble part after hydrolysis), all fractions exhibit an UV-absorption spectrum (Figure 3a) typical of phenolic substances[3]. By combining RI- and UV_{254}- detectors it was possible to distinguish the lignin component in the HPGPC chromatograms (Figure 3b). Lignin has a molecular weight distribution very

similar to that of carbohydrate components. Treatment of the fractions with dilute or strong sodium hydroxide solutions at elevated and room temperatures, respectively, caused an increase of the area % of peak II and its shift to somewhat lower sizes (24-29 kD), particularly after the hot alkaline treatment (results not shown). However, the similarity in the size distribution of the carbohydrate and lignin components remained unchanged.

The ethanol-soluble fraction A1S contains acetamide (^{13}C NMR: δ 178.46 and 22.47) originating from O-acetyl groups located in the most accessible xylan fraction of beechwood[29]. The weak absorption band at 1505 cm^{-1} in the IR spectrum as well as the absorption maximum at 280 nm indicate the presence of lignin structures. In accord, low signals at δ 121, 131, 142-144, 148-150, and 157 assigned to carbons of the aromatic nuclei of guaiacyl and syringyl units[30,31] as well as strong signals at δ 57 of the aromatic methoxyl groups are seen in the ^{13}C NMR spectrum of A1S. The pattern of carbohydrate signals in the ^{13}C NMR spectra of both A1P and A1S is essentially the same.

In conclusion, the presence of lignin and pectic polysaccharides in all xylan fractions, even after strong alkaline treatments implies that the release of the xylan component from the cell walls containing residual lignin is restricted by alkali-stable lignin-carbohydrate linkages. The pectic substances found in the beechwood might be a component of the lignin-carbohydrate complex of cell walls which are deposited simultaneously with the cellulose and hemicelluloses during cell wall biogenesis[32,33]. The high proportion of 3-linked rhamnosyl residues in comparison to the fucosyl content indicates that

rhamnose integrated according to Johansson and Samuelson[15] into the reducing end of some xylan chains might represent the suggested[10] native connection of xylan to pectin. In addition, the galacturonan chains substituted with single xylosyl residues which were reported[8,27] to be present in primary cell walls might be the counterpart of such connections.

EXPERIMENTAL

Material

Chips of particle size 0.3-1 mm were prepared from beechwood (*Fagus sylvatica* L.) free of bark and delignified by acidic sodium chlorite treatment under mild conditions[34]. The holocellulose obtained in the yield 83.5% on wood contained 1.5% Klason lignin and neutral sugars (in rel.% w/w): galactose (0.5), glucose (56.7), mannose (2.7), arabinose (2.6), xylose (36.5) and rhamnose (1.0).

Methods

The methods for sugar analysis by paper chromatography (p.c.) and GLC of alditol trifluoroacetates, determination of the DP by viscometry in DMSO, and uronic acid content by alkalimetric titration were described in previous papers[14,20,22,25]. IR spectra were obtained with a Perkin-Elmer G 983 spectrophotometer operating at 4 cm^{-1} resolution and equipped with a data station DS 3700 by using the KBr pellet technique. UV spectra were measured with a Pye-Unicam 1700 UV spectrophotometer. ^{13}C NMR spectra (75.4 MHz) were recorded with a FT-NMR spectrometer (Bruker AM-300) at 40 ^{o}C for solutions in D_2O (internal methanol, 50.15 ppm relative to Me_4Si) and DMSO-d_6.

Glycosyl linkage analysis of the hemicelluloses was performed by methylation with the DMSO-solid NaOH-CH_3I reagent[35] as previously described[24]. Acidic sugars separated from the hydrolysate of the permethylated product by anion exchange chromatography were reduced with $LiAlD_4$. The partially methylated alditol acetates were analysed by GLC and GLC-MS[22]. HPGPC of the polysaccharide fractions was performed on Separon HEMA-BIO S-100 and S-1000 columns calibrated with pullulan standards[36].

Fractional Extraction

The holocellulose (50 g) was ectracted in succession with 1, 2.5, 10, and 26% NH_4OH, respectively, and finally with 5% NaOH. In each step, the material/liquid ratio was 1:20 (g/mL) and the extraction was performed at room temperature for 24 hrs under stirring in nitrogen atmosphere. The insoluble residue from each extraction step was filtered off and washed once with the same amount of the corresponding extractant, then it was washed with distilled water and dried on air. The extract and first washing were combined and in the case of ammonia extracts evaporated to dryness under reduced pressure at 40°C. The solids obtained were dissolved in water, and hemicelluloses were precipitated into 4 vol of ethanol and separated by filtration, dialyzed and lyophilized (A1P-A4P). The filtrate after separation of A1P was evaporated to dryness (A1S). The hemicellulose from the last (alkaline) step was precipitated from the extract with 4 vol of ethanol and separated by filtration. The precipitate was suspended in water, acidified with 5 % acetic acid, dialyzed and recovered after neutralization to pH 7 by freeze-drying (A5P).

20 mg of fractions A1, A3P and A5P were dissolved in 0.5% NaOH (2 mL) and the solution was heated at 90°C under reflux and nitrogen for 6 h. After cooling, the solution was poured into 4 vol of ethanol and the formed precipitate was separated by centrifugation, washed with 80% ethanol until free of UV_{254}- absorbing material, dialyzed and freeze-dried. A further part of these fractions (20 mg) was treated with 5% NaOH at room temperature for 24 hrs in nitrogen atmosphere. The polysaccharidic material was recovered as described above.

ACKNOVLEDGEMENT. This work was suppported, in part, by the Grant agency for science, Slovakia (grant No. 2/1236).

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