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Chemical Composition and Structural Features of the Macromolecular Components of Plantation *Acacia mangium* Wood

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The wood of *Acacia mangium*, a prominent fast-growing plantation species used in the pulp-and-paper industry and, so far, poorly investigated for its chemical structure, was submitted to a detailed characterization of its main macromolecular components. Lignin (28% wood weight) isolated by mild acidolysis and characterized by permanganate oxidation, ^{1}H and ^{13}C NMR, and GPC, showed a very low content of syringylpropane-derived units (S:G:H of 48:49:3), a high degree of condensation, a low content of β-*O*-4 (∼0.40−0.43 per C6) structures, and a M_w of 2230. Glucuronoxylan (14% wood weight) isolated by alkaline (KOH) or by dimethyl sulfoxide extraction was characterized by methylation analysis, ^{1}H NMR, and GPC. About 10% of the xylopyranose (Xyl*p*) units constituting the linear backbone were substituted at *O*-2 with 4-*O*-methylglucuronic acid residues. Almost half of the Xyl*p* units (45%) were *O*-2 (18%), *O*-3 (24%) or *O*-2,3 (3%) acetylated. X-ray diffraction analysis of cellulose (46% wood weight), isolated according to the Kürschner−Hoffer method, showed a degree of crystallinity of 67.6%.

KEYWORDS: ***Acacia mangium*; wood; lignin; xylan; cellulose; analysis; permanganate oxidation; methylation analysis; NMR; XRD; GPC**

INTRODUCTION

Acacia mangium Willd. is a fast-growing dicotyledonous tree, native to northern Australia and Southeast Asia (*1*), where it has been planted because of its high silvicultural performance and its ability to grow in degraded soils (*2*). *A. mangium* is a well-known nitrogen-fixing tree, being used for land rehabilitation, particularly in eroded and nitrogen-deficient soils (*2*). In recent years, this hardwood species has been recognized as an excellent source of short cellulose fibers for papermaking (*3*−*6*). Extensive plantations are now growing in Southeast Asia, particularly Indonesia, supplying wood to the pulp-and-paper industry in that part of the globe (*7*). The interesting properties of *A. mangium* fibers together with its easy adaptation to tropical humid climates (*1*) suggest that extensive *A. mangium* plantations soon will spread to other regions of the world such as South America, competing seriously with other hardwood fiber sources.

Despite the increasing importance and use of short rotation plantation *A. mangium*, information on the detailed chemical and structural analysis of wood and pulp components is quite scarce and disperse, with a few exceptions related to the composition of extractives (*8*−*10*). To the best of our knowledge, no comprehensive work on the detailed characterization of the macromolecular components of *A. mangium* wood, lignin, cellulose, and hemicelluloses has been published so far. The composition and chemical structure of wood components, particularly lignin and polysaccharides, determine the wood's performance toward chemical processing, namely, the pulping and pulp-bleaching process as well as the quality of the final products.

This study was designed to quantify and structurally characterize plantation *A. mangium* wood lignin, xylan (the most abundant hemicellulose in this hardwood), and cellulose.

MATERIALS AND METHODS

Preparation of Plant Material. Standard industrial wood chips (average size = 30 × 20 × 3 mm) obtained from randomly harvested trees from one Indonesian *A. mangium* plantation (average rotation cycle of 7 years) were used. The chips were milled in an SK1 Retsch cross-beater mill (Haan, Germany), sieved to 40−60 mesh, and air-dried. The wood sawdust was submitted to Soxhlet extraction with ethanol/toluene (1:2, v/v) for 12 h, to remove extractives. In parallel, wood was submitted to Soxhlet sequential extraction with dichloromethane and methanol/water (1:2, v/v) (6 h each solvent). All of the chemical analyses were carried out using ethanol/toluene extractive-free sawdust.

Solvents and chemicals were pro-analysis grade products supplied by Aldrich and Sigma (Madrid).

Chemical Analysis of Wood. The ash content was determined following TAPPI T 211 om-93. Cellulose was quantified according to the Kürschner−Hoffer method (*11*). Acid insoluble (Klason) lignin and acid soluble lignin were quantified according to the methodology described elsewhere (*11*). Pentosans content was determined by treatment of wood with concentrated hydrochloric acid followed by the quantification of produced furfural by bromate−bromide titration

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(*12*). Holocellulose was isolated by peracetic acid method (*13*). Neutral and acid monosaccharides were released by Saeman hydrolysis (treatment with 72% H_2SO_4 at 20 °C followed by hydrolysis with 1 M H_2SO_4 at 100 °C). Neutral monosaccharides were quantified as alditol acetate derivatives by gas chromatography (*14*), whereas uronic acids were determined according to the colorimetric method using *m*-phenylphenol (*15*).

Isolation and Characterization of Lignin. Lignin was isolated from alkaline (0.3% aqueous NaOH) pre-extracted sawdust (in order to remove interfering polyphenols), by mild acidolysis [reflux with dioxane/water solution 9:1 (v/v) containing 1.5% HCl, in nitrogen atmosphere] according to a previously published procedure (*16*). The isolated lignin, containing 1.0% ash and 0.7% sugars, represents ~50% of the Klason lignin content in wood. The content of methoxyl groups was determined according to a modified Zeisel method (*17*), whereas the lignin elemental composition was analyzed using a CHNS-932 LECO instrument. The potassium permanganate oxidation of lignin and GC and GC-MS analysis of methylated aromatic carboxylic acids were carried out according to the previously published standard method (*16*, *18*, *19*).

The gel permeation chromatography (GPC) analysis of lignin was performed in a PL-GPC 110 system equipped with a 10 μm precolumn Plgel and a 300 × 7.5 mm, 10 μm MIXED D column Plgel and a refractive index detector. The precolumn, column, and injection system were maintained at 70 °C. The eluent (0.5% w/v LiCl in DMF) was pumped at a flow rate of 0.9 mL/min. The lignin solutions (0.5% w/v) were prepared just before analysis using DMF with 0.1 M LiCl (w/v) as solvent. The GPC columns were calibrated using lignin preparations previously characterized by ESI-MS (*20*).

The ^{1}H NMR analysis of acetylated lignin in deuterated chloroform ($CDCl_3$) solution (2% concentration) was carried out using a Bruker AMX 300 spectrometer (Strasbourg, France) operating at 300 MHz at room temperature. The acquisition parameters used were as follows: 12.2 μs pulse width (90°), 2 s relaxation delay, and 300 scans. The ^{13}C NMR spectra were recorded on the same spectrometer operating at 75.5 MHz. Lignin samples were dissolved in DMSO-d_6 (~23% concentration). The spectra were recorded in a 10 mm diameter tube at 318 K with TMS as internal reference. The inverse gated decoupling sequence, which allows quantitative analysis and comparison of signal intensities, was used with the following parameters: 90° pulse angle, 12 s relaxation delay, 16K data points, and 18000 scans.

Isolation and Characterization of Xylan. Xylans were isolated from wood holocellulose (prepared according to the peracetic acid method) by alkaline extraction (10% aqueous KOH) and precipitation in ethanol, after acidification to pH 3 with formic acid, or by dimethyl sulfoxide (DMSO) extraction and precipitation in ethanol, according to published methodologies (*12*, *20*). The ash content was quantified for each xylan sample obtained. The yields of the alkaline- and DMSO-extracted xylans were, respectively, 53 and 50% (wood xylan basis). The monosaccharides composition was determined according to the methodology used for wood. The xylan isolated by alkaline method was submitted to linkage (methylation) analysis, including carboxyl group reduction with $LiAlD_4$ in THF to identify the uronic acid moieties, as reported elsewhere (*21*). The GPC of DMSO-extracted xylan was carried out using *N*,*N*-dimethylacetamide, containing 0.1 M LiCl, as solvent and eluent, RI detection, and calibration with pullulan standards, according to methodology described in the literature (*21*).

The xylan isolated by DMSO extraction was analyzed by ^{1}H NMR after dissolution in D_2O. Spectra were recorded at 60 °C on a Bruker AMX 300 spectrometer operating at 300.13 MHz. Relaxation delay was 12 s, radio frequency 90° pulse, width of 10.2 μs, and ~400 scans. The chemical shifts are reported relative to sodium 3-(trimethylsilyl)-propionate-d_4 used as internal standard (δ 0.00).

Isolation and Characterization of Cellulose. Wood cellulose was isolated according to the Kürschner−Hoffer methodology (*11*) followed by treatment with diluted peracetic acid (5%) for 3−5 min at 80 °C, to obtain <1% of residual lignin. Cellulose was analyzed by X-ray diffraction as textured samples in pressed pellets of 1.3 cm diameter (*22*). The degree of crystallinity was corrected to the content of noncellulosic polysaccharides.

Table 1. Chemical Composition of *A. mangium* Wood

	rel abundance, % dry wood[a]
ashes	0.22
extractives	
ethanol/toluene	4.46
dichloromethane	1.32
methanol/water	4.05
lignin	
Klason lignin	27.1
acid soluble lignin	0.54
holocellulose	70.9
cellulose (Kürschner–Hoffer)	46.5[b]
pentosans	13.3
neutral monosaccharides[c]	
rhamnose	0.3
arabinose	0.2
xylose	10.9
mannose	1.0
galactose	0.6
glucose	48.0
uronic acids	7.6

[a] Extractives-free wood, except for extractives content. [b] Corrected for pentosans content. [c] Determined as anhydrous monosaccharides.

RESULTS AND DISCUSSION

Chemical Composition of Wood. The general chemical composition of *A. mangium* wood is summarized in **Table 1**. The extractives content [as determined by ethanol/toluene (1:2, v/v) extraction or by sequential extraction with dichloromethane and methanol/water (1:2, v/v)] was quite high when compared with that of other hardwoods (*23*). When the sequential extraction is performed, the main contribution to the extractives content came from the methanol/water extract (4.05%), in agreement with the high content of polyphenols in this wood (*10*). Lipophilic extractives were also quite abundant in *A. mangium*, as shown by the weight of dichloromethane extract (1.32%), being composed essentially of aliphatic acids and long-chain (>C20) aliphatic alcohols (*8*, *9*). The high content of extractives may constitute a negative point of this species when used as a fiber source, because extractives increase the chemical consumption during pulping and bleaching processes and may lead to pitch deposits in mill machinery and in pulp and paper, requiring high maintenance costs and decreasing the final product quality (*26*).

The lignin content of *A. mangium* wood was 27.6% (**Table 1**), a value above the range of lignin contents typically found in hardwoods, 20−26% (*24*). This can be explained, at least partially, by the presence of polyphenolic extractives that were not removed by ethanol/toluene extraction of wood. The high lignin/polyphenolic extractives content of *A. mangium* wood contributes to the high chemical consumption required to delignify wood and to the low pulp yield, when compared to those of other hardwoods (*25*). The polysaccharides fraction, comprising ~70% of the dry weight of extractives-free wood, was composed of ~47% cellulose, as determined by Kürschner−Hoffer method. This figure is in good agreement with the wood monosaccharides composition, which showed a glucose content of 48%. The mannose content (1.0%) suggests that ~1.0% of glucose belongs most likely to glucomannans. In hardwoods, typically, glucomannans possess a glucose/manose molar ratio of about 1:1 (*23*), thus representing ~2% of *A. mangium* extractive-free wood. *A. mangium* wood hemicelluloses were essentially of glucuronoxylan type, as suggested by xylose content (10.6%), the second monosaccharide in abundance after glucose, and by the high content of uronic acids. According to its structural features (degree of substitution with

acetyl and 4-*O*-methylglucuronic acid groups), the xylan content in extractive-free wood was estimated as ~14%, a value that agrees with the determination of pentosans content (**Table 1**) but that is lower than the xylan content typically observed in hardwoods (*23*). The presence of rhamnose, arabinose, and galactose, as well as the uronic acid content being much higher than that predicted in glucuronoxylan (~1.6%, wood weight basis), suggests the presence of pectins and other minor polysaccharides.

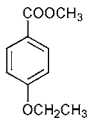
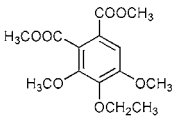
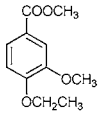
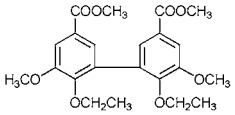
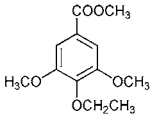
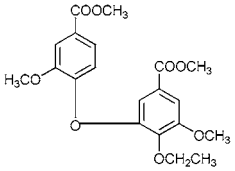
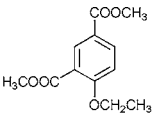
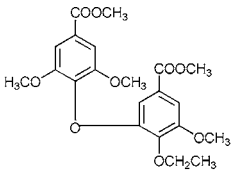
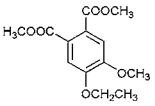
Structural Analysis of Lignin. Although some unavoidable side reactions may occur during the acidolytic lignin isolation, such as the partial cleavage of arylglycerol-β-aryl ethers and the formation Hibbert ketones, the occurrence of such reactions may be reduced by isolating lignin in several short-duration steps, instead of a single, long-duration extraction step (*16*). This allowed the isolation of a lignin with a rather high yield (50−60%), which was less degraded than analytes isolated by conventional acidolytic methodology.

The determination of methoxyl groups content (17.0%) and the elemental analysis (C, 59.5%; H, 5.9%; O, 34.6%) of *A. mangium* dioxane lignin allowed establishment of the empirical formula of the phenylpropane-derived unit (C_9 or ppu) as $C_9H_{8.70}O_{3.26}(OCH_3)_{1.12}$ (M_{ppu} = 203.6 u). The frequency of methoxyl group substitution in the aromatic ring was remarkably lower than that observed for other hardwood lignins (*23*), namely, *Eucalyptus globulus* lignin (*16*). To assess the structure of *A. mangium* lignin, this was submitted to permanganate oxidation (PO) and ^{1}H and ^{13}C NMR spectroscopy analyses. The molecular weight (M_w) was determined by GPC.

Permanganate Oxidation Analysis. The PO followed by GC-MS analysis of the resulting aromatic carboxylic acids provides relevant information on lignin structure. Although only the free phenolic aromatic units are accessible to this analysis (25−35% of total units), their degradation products reveal the main structural tendencies of lignin as a whole (*26*). The nine most prominent methylated degradation products formed in the oxidation of lignin with potassium permanganate and their relative abundances are presented in **Table 2**. *p*-Hydroxybenzoic acid (**1**), vanillic acid (**2**), and syringic acid (**3**) methyl esters are derived from uncondensed *p*-hydroxyphenyl, guaiacyl, and syringyl structural units, respectively (*18*). The isohemipinic acid methyl ester (**4**) is derived predominantly from phenylcoumaran-type structures, whereas the dicarboxylic acid methyl esters **5** and **6** can be assigned to dilignol structures linked by α-6 and β-β linkages (isotaxiresinol-type structures), as previously suggested (*27*). These structures may be also assigned to phenylisochroman-type structures linked by β-6 and γ-*O*-α linkages (*29*). The oxidation product **7** originates from biphenyl-type structures (5-5′), whereas products **8** and **9** are derived from diaryl ether substructures (4-*O*-5′).

The molar proportion of syringyl/guaiacyl/*p*-hydroxyphenyl-propane (S:G:H) units determined by PO technique was 35:62:3 (**Table 2**), corresponding to a hardwood lignin with a relatively low content of syringyl-type units (*25*), which is in agreement with data from methoxyl groups analysis. The proportion of uncondensed/condensed units (molar ratio of products **1**−**3**/products **4**−**9**) in lignin was 68:32, indicating a degree of condensation higher than that found in lignins of most industrial hardwoods (*25*). The significant contribution of diaryl ether type structures to the total amount of condensed structures was rather surprising. The high abundance of product **9** (7 mol %), similar to that found in other hardwoods such as *E. globulus* (*16*), was unexpected because of the low content of syringyl structural units in *A. mangium* lignin. This suggests that ~10% of syringyl-type phenolic units are involved in 4-*O*-5′ substructures. The low S:G ratio and the high degree of lignin condensation contributes to the lower reactivity of *A. mangium* lignin during pulping and bleaching processes and explains the more drastic process conditions, namely, higher alkalinity in kraft pulping and higher chlorine dioxide in bleaching, required by this species, when compared to other hardwoods (*25*).

Table 2. Relative Abundance of Aromatic Carboxylic Acids Issued from Permanganate Oxidation of *A. mangium* Wood Lignin (as Methyl Esters)

Oxidation product (carboxylic acid methyl ester)	Frequency of occurrence (mol. %)	Oxidation product (carboxylic acid methyl ester)	Frequency of occurrence (mol. %)
 **1**	3	 **6**	3
 **2**	37	 **7**	5
 **3**	28	 **8**	6
 **4**	5	 **9**	7
 **5**	6		

^{1}H NMR Analysis of Lignin. The ^{1}H NMR spectrum of acetylated lignin (not shown) allowed estimation of the relative abundance of several substructures. Signal assignments were made on the basis of known literature data (*29*, *30*). Calculations of all structural elements were made per one phenylpropane unit (C_9) using the resonance of methoxyl protons (3.6−4.0 ppm) as an internal standard. The most relevant results are summarized in **Table 3**.

The ^{1}H NMR spectrum showed several signals in the range of 0.7−1.5 ppm assigned to CH_2 and CH_3 groups in aliphatic chains, which account for 0.26/C_9. The signal integration in the ranges of 1.7−2.2 and 2.2−2.5 ppm allowed quantification of aliphatic hydroxyl groups and phenolic hydroxyl groups, respectively (*29*). Signals from phenolic acetate in 5-5′ biphenyl structures appeared at ca. δ 2.10 (*31*); however, the error inserted in the calculation of aliphatic and phenolic hydroxyl groups should be minimal because the percentage of biphenyl structures is usually quite low in hardwood lignins (*31*), as was previously confirmed by PO results (**Table 2**). Aliphatic hydroxyl groups of lignins include primary and secondary hydroxyl groups, mostly present in C-γ and C-α, respectively (*32*). The abundance

Table 3. Estimation of Frequency of Functional Groups and Major Interunit Linkages in *A. mangium* Lignin As Determined by ^{1}H NMR Spectroscopy

structural elements	number/C_9
$-CH_2-$ and $-CH_3$ (0.7–1.5 ppm)	0.26
H in acetylated aliphatic OH (1.7–2.2 ppm)	0.93
H in acetylated phenolic OH (2.2–2.5 ppm)	0.30
Hβ in β-β structures (3.0–3.2 ppm)	0.08
Hα in β-5 structures (phenylcoumaran) and Hα in noncyclic benzyl aryl ethers (α-*O*-4 structures) (5.3–5.7 ppm)	0.12
Hα in β-*O*-4 structures without Cα=O and Hα in β–1 structures (5.9–6.2 ppm)	0.40
aromatic H	2.03
formyl H in aldehyde groups (9.3–10.0 ppm)	0.03

Table 4. Frequency of Occurrence of Different Structural Elements in *A. mangium* Lignin As Determined by ^{13}C NMR Spectroscopy

structural elements	number/aromatic unit
β-β and β-5 structures	0.11
methoxyl groups	1.31
cinnamyl alcohol structures	0.03
β-*O*-4 structures without Cα=O	0.43
β-*O*-4 structures with Cα=O	0.02
Ar—O	2.1
Ar—C	1.7
Ar—H	2.2
CHO in benzaldehyde-type structures	0.01
CHO in cinnamaldehyde-type structures	0.02
ketone groups	0.15
S:G:H	48:49:3

of aliphatic hydroxyl groups (0.93/C_9) was slightly lower than the values published for other hardwoods (*29*, *32*). The amount of phenolic hydroxyl groups of *A. mangium* dioxane lignin (0.30/C_9) is within the range found for milled wood lignins (0.2−0.3 OH_{ph}/C_9) (*33*) and analogous to that reported for *E. globulus* dioxane lignin (0.30/C_9) (*16*).

The occurrence of β-β structures in hardwood lignins has been attributed primarily to the syringaresinol-type units (*33*), although β-β structures of dibenzyltetrahydrofuran could also be present (*32*). The estimation of β-β structures was obtained by integration of the signal centered at 3.1 ppm (Hβ) (*30*). Such estimation gives a value of 0.08/C_9. The signal in the range of δ 5.3−5.7 of the ^{1}H NMR spectrum of acetylated lignin is assigned essentially to Hα in cyclic β-5 structures (phenylcoumaran-type structures, 5.3−5.5 ppm) but also to Hα in noncyclic benzyl aryl ethers (α-*O*-4 structures, 5.5−5.7 ppm) (*29*, *34*). The number of units attached to an adjacent unit by β-5 and noncyclic benzyl aryl ethers in *A. mangium* lignin was ∼0.12/C_9.

The abundance of β-O-4 structures was estimated on the basis of the resonance of Hα in acetylated arylglycerol-β-aryl ethers at 5.9−6.2 ppm (*29*). The value obtained for the content of β-*O*-4 structures without C_α=O was 0.40/C_9, a quite low value when compared with other hardwood dioxane lignins [*E. globulus*, 0.52/C_9 (*16*, *36*)], *Betula verrucosa* MWLs (0.58−0.65/C_9), and even MWL softwood lignin [*Picea* abies 0.48/C_9 (*32*)]. The low β-*O*-4 content in *A. mangium* was shown to contribute also to its lower reactivity during wood kraft pulping and pulp bleaching with chlorine dioxide (*25*).

The spectral region of 6.25−7.90 ppm is assigned to aromatic protons (*29*). The estimated total aromatic hydrogens in *A. mangium* lignin was 2.03/C_9 units, which is a rather low figure considering the low S:G ratio. This result is in agreement with the high degree of condensation involving aromatic moieties, as was previously found by PO analysis.

The region of the spectrum at 9.30−10.00 ppm is assigned to signals of formyl protons in cinnamaldehyde-type units (9.55−9.75 ppm) and also to formyl protons in benzaldehyde-type units (9.75−10.00 ppm) (*29*, *30*). The resonances detected at 9.30−9.55 ppm can be assigned to formyl protons in nonconjugated aldehyde groups. Such groups may be formed at Cβ during lignin acidolysis as a result of partial degradation of β-*O*-4 linkages (*37*). The total amount of aldehyde groups was only 0.03/C_9, a value lower than that obtained for *E. globulus* dioxane lignin (*16*).

^{13}C NMR Analysis of Lignin. Additional structural information on *A. mangium* lignin was obtained by quantitative ^{13}C NMR spectroscopy, using methodology and carbon assignments reported previously (*38*−*40*). The most relevant results are summarized in **Table 4**.

The quantification of β-*O*-4 bonds in lignin structures without a C_α=O group in the propane chain per one aromatic group C_6 (integrated intensity of signals at 103−162 ppm) was done on the basis of Cγ resonances at 59.3−60.8 ppm (**Table 4**). The value obtained for the content of β-O-4 structures without a C_α=O group (0.43/C_6) was rather close to that determined by ^{1}H NMR spectroscopy per C_9 (0.40/C_9), indicating the similarity of results expressed on the basis of one aromatic ring (C_6) and one phenylpropane unit (C_9). The frequency of occurrence of β-*O*-4 structures with a C_α=O group should be rather low in *A. mangium* lignin (∼0.02/C_6), because only small intensity signals were detected at 195−197 ppm assigned to C_α in corresponding structures. The amount of β-β and β-5 bonds (0.11/C_6) was calculated by integration of the C_β resonance at 51.0−53.8 ppm.

The S:G:H ratio was obtained by integration of the respective spectral regions. The number of tertiary aromatic carbons of syringyl propane units (S) was estimated using the spectrum region of 103−110 ppm and those of guaiacyl propane units (G) using the region of 110−123 ppm. The contribution of tertiary aromatic carbons from *p*-hydroxyphenylpropane (H) units (namely, C-3 and C-5) in the 103−123 ppm range was negligible, as shown by PO analysis (only 3 mol % of H units were detected, **Table 2**). The number of H units was estimated from the resonance centered at 161 ppm, assigned to C-4 in the corresponding structures (*40*). The S:G:H ratio thus calculated was 48:49:3, a molar proportion different from that determined by PO technique (34:63:3). Such a difference suggests that the phenolic groups are not randomly distributed in syringyl propane and guaiacyl propane units, but predominate in the latter ones. Such uneven distribution leads to an overestimation of the proportion G units by PO analysis.

The ^{13}C NMR spectrum region at 123−137 ppm was integrated and attributed to quaternary carbons in aromatic nuclei linked with a carbon atom of another substructure (Ar−C). The total amount of Ar−C bonds was estimated as 1.7/C_6, which is a rather high value. Besides the highly condensed lignin structure, this result can be also explained by the presence of C-4 resonances from some syringyl structures at ∼134−135 ppm, thus increasing the amounts of calculated Ar−C substructures. It was impossible, unfortunately, to integrate these signals separately. For the same reason, the amount of calculated oxygenated aromatic carbons (Ar−O substructures) was lower than could be expected (2.1/C_6). The different carbonyl groups in lignin can be quantified by integration of the spectrum region at 191−210 ppm. The amounts of aldehyde groups were estimated by integrating the range at 191−192 ppm (CHO

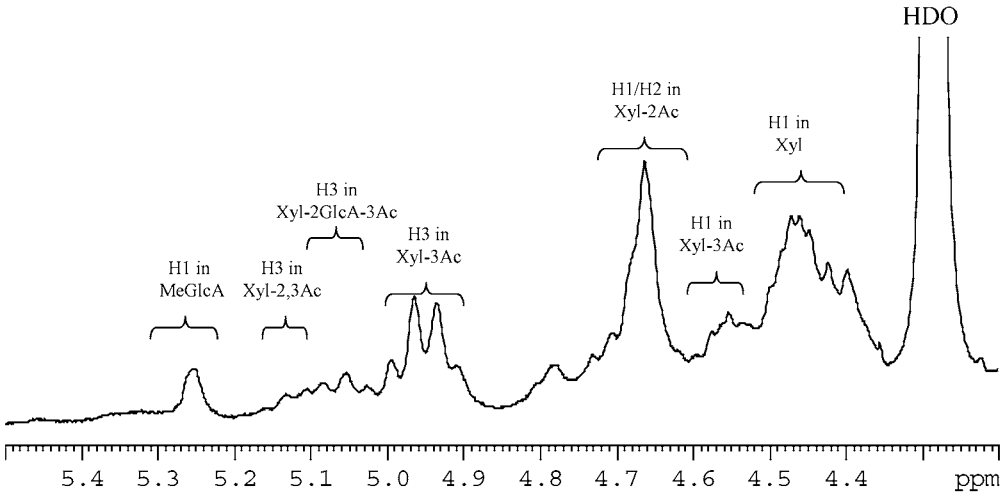


Figure 1. ^{1}H NMR spectrum (region of anomeric protons) of *O*-acetyl-(4-*O*-methylglucurono)xylan extracted from *A. mangium* wood by dimethyl sulfoxide (designations presented in **Table 6**).

groups in benzaldehyde-type structures, $<0.02/C_6$) and at 193.5−194.5 ppm (CHO groups in cinnamaldehyde-type structures, $0.02/C_6$). The ketone groups estimation ($0.15/C_6$) was based on the integral at 195−210 ppm. This integral includes ketone groups in both α- and *β*-positions of the propane chain. Part of these ketone structures belong certainly to Hibbert ketone structures formed during the isolation procedure (*16*). The methoxyl groups content estimated by ^{13}C NMR as $1.31/C_6$ was consistent with the relatively low syringyl content of lignin.

GPC Analysis of Lignin. The molecular weight of lignin in LiCl/DMF solution was assessed by GPC, using columns calibrated with ESI-MS-characterized *E. globulus* lignin fractions (*20*). The weight-average molecular weight (M_w) of *A. mangium* dioxane lignin was 2230, a value of the same order as that found for other hardwood lignins isolated by acidolysis (*25*).

Structural Analysis of Xylan. Xylans were extracted from peracetic acid holocellulose by 10% KOH aqueous solution or by DMSO, with isolation yields near 50%. The high purity of the isolated xylans was confirmed by the neutral monosaccharide analysis, which showed xylose contents of 94 and 95% in KOH- and DMSO-extracted xylans, respectively. The alkaline extraction promotes the hydrolysis of acetyl groups in xylans, yielding a suitable sample for linkage (methylation) analysis. On the other hand, DMSO extraction preserves as much as possible the original structure of the polysaccharide, allowing additional structural information to be obtained, namely, on the relative abundance and location of the original *O*-acetyl moieties in the xylan backbone.

Linkage (*Methylation*) *Analysis of Xylan.* The xylan extracted by aqueous KOH was submitted to methylation, followed by reduction with $LiAlD_4$ to preserve uronic acid moieties, acid hydrolysis, and GC-MS analysis of the partially methylated sugars as alditol acetates. The relative abundance of the most abundant partially methylated products identified in the linkage analysis of wood xylan is presented in **Table 5**.

The *A. mangium* xylan is essentially composed of a linear backbone constituted by (1→4)-linked *β*-D-xylopyranosyl units partially *O*-2 substituted with 4-*O*-methyl-α-D-glucuronosyl units. The frequency of this substitution was 1:10 (**Table 5**) as typically found in most hardwood glucuronoxylans (*23*).

The presence of a terminal fragment [→3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*A-(1→4)-D-Xyl*p*] previously suggested for birch xylan (*42*) and recently also reported for *E. globulus* wood xylan (*13*) was not confirmed for *A. mangium* xylan. Although the structural unit [→3)-Rha*p*-(1→] was found in a notable abundance (0.4/100 Xyl*p*), the [→2)-Gal*p*A-(1→] moiety was detected only in trace amounts. Probably, during the isolation of xylan under the strong alkaline conditions the mentioned terminal fragment suffered degradation, which did not allow the detection and balance of all the structural elements ([→3)-Rha*p*-(1→], [→2)-Gal*p*A-(1→], and [→4)-D-Xyl*p*]).

Table 5. Relative Abundance of Structural Units Deduced from Methylation Analysis of Xylan Extracted from *A. mangium* Wood by Aqueous KOH

structural units	rel abundance[a]
Xyl*p*-(1→)	0.7
→4)-Xyl*p*-(1→	86.2
→2,4)-Xyl*p*-(1→	12.8
→3,4)-Xyl*p*-(1→	0.3
→4)-Glc*p*-(1→	1.3
Glc*p*A-(1→	9.9
Gal*p*-(1→	0.2
→4)-Gal*p*-(1→	1.5
→2)-Gal*p*A-(1→	traces
→2,4)-Rha*p*-(1→	0.1
→3)-Rha*p*-(1→	0.4
Ara*f*-(1→)	0.4

[a] Relative abundance for 100 Xyl*p* units basis.

The results from linkage analysis (**Table 5**) show an apparent misbalance between the frequency of the [→2,4)-Xyl*p*-(1→] structure and of [Glc*p*A-(1→] units. Also, the presence of the [→3,4)-Xyl*p*-(1→] moiety was unexpected. Such a result could suggest the presence of substituents in the xylan backbone other than [Glc*p*A-(1→]. However, according to our previous results (*42*), this observation is more likely explained by the presence of acetyl groups in C_2 and C_3 of xylopyranosyl units, difficult to saponify (*42*) and resistant to the alkaline conditions during the xylan extraction and the methylation analysis. The nonassigned minor methylation products should belong to residual polysaccharides coprecipitating with xylan during its isolation.

^{1}H NMR Analysis of Xylan. The DMSO-extracted xylan was characterized by ^{1}H NMR using proton resonance assignments reported in the literature (*44*−*46*) and data from homonuclear (TOCSY) and heteronuclear (HSQC) correlation NMR experiments carried out in our laboratory (*13*). The quantification of different *β*-D-Xyl*p* units possessing *O*-acetyl groups was

Table 6. Relative Content of Acetyl Groups in Different Structural Units of Xylan Extracted from *A. mangium* Wood by Dimethyl Sulfoxide

structural fragment and short designation	rel abundance (per 100 Xyl*p* units)
→4)-*β*-D-Xyl*p*-(1→ (Xyl, nonacetylated Xyl units)	55
→4)[2-*O*-Ac]-*β*-D-Xyl*p*-(1→ (Xyl-2Ac)	18
→4)[3-*O*-Ac]-*β*-D-Xyl*p*-(1→ (Xyl-3Ac)	18
→4)[2-*O*-Ac][3-*O*-Ac]-*β*-D-Xyl*p*-(1→ (Xyl-2,3Ac)	3
→4)[4-*O*-Me-α-D-Glc*p*A-(1→2)][3-*O*-Ac]-*β*-D-Xyl*p*-(1→ (Xyl-2GlcA-3Ac)	6

performed on the basis of the methodology described elsewhere (*45*). **Figure 1** shows the anomeric region of the ^{1}H NMR spectrum (4.2−5.4 ppm) of *A. mangium* xylan with the assignment of proton resonances, whereas **Table 6** summarizes the results of proton signal integration in acetylated xylopyranose units.

^{1}H NMR results showed that ∼45% of xylopyranose units of *A. mangium* glucuronoxylan were acetylated at C_2, at C_3, or at both C_2 and C_3 atoms (**Table 6**), corresponding to a degree of acetylation (DS = 0.48) of the same order as that found in other hardwood xylans such as *E. globulus* (*13*) and birch (*45*). Among the acetylated xylopyranose units, about 53% contained one acetyl group at *O*-3, 40% are acetylated at *O*-2, and about 7% contained acetyl groups simultaneously at *O*-2 and *O*-3. ^{1}H NMR analysis showed also that the major part of Xyl*p* units substituted at *O*-2 with 4-*O*-methylglucuronic acid were acetylated at *O*-3, in agreement with results obtained for other hardwood xylans (*13*, *44*).

GPC Analysis of Xylan. The GPC chromatogram of the DMSO-extracted *A. mangium* xylan (not shown) presented a unimodal Gaussian molecular weight distribution, in agreement with the high purity of the isolated polysaccharide. The weight-average molecular weight (M_w) of xylan was 28000, a feature that fits well within the range of M_w values normally found for hardwood xylans (*25*).

Structural Analysis of Cellulose. X-ray diffraction (XRD) analysis allowed information to be obtained on the supramolecular structure of *A. mangium* wood cellulose. The unitary monoclinic cell dimensions of crystalline domains, according to the Meyer−Mark−Misch model (*46*), were, as expected, similar to those reported in the literature for cellulose I polymorph (*46*). The degree of crystallinity of *A. mangium* cellulose, as determined by XRD, was 67.6%, whereas the average crystallite width in the d_{002} plane was 4.74 nm. Such structural features are not significantly different from data reported for other hardwood celluloses (*25*).

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