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Quantitative Characterization of a Hardwood Milled Wood Lignin by Nuclear Magnetic Resonance Spectroscopy

EWELLYN A. CAPANEMA,*,† MIKHAIL YU. BALAKSHIN,† AND JOHN F. KADLA‡

Department of Wood and Paper Science, North Carolina State University, Raleigh, North Carolina, 27695-8005, and Department of Wood Science, University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z4

The structure of *Eucalyptus grandis* milled wood lignin (MWL) was investigated by 2D $^{1}H-^{13}C$ HSQC, HMQC, and $^{1}H-^{1}H$ TOCSY correlation NMR techniques and by quantitative ^{13}C NMR as well as by the permanganate oxidation degradation technique. The combination of 2D NMR and quantitative ^{13}C NMR spectroscopy of nonacetylated and acetylated lignin preparations allowed reliable identification and calculation of the amount of different lignin structures. About 85% of side-chain moieties were estimated on the structural level. This information was substantiated by data on the quantity of various functional groups and interunit linkages as a whole. A modified method for calculation of the ***h:g:s*** ratio has been suggested and compared with previously suggested approaches. *E. grandis* MWL has been determined to have an ***h:g:s*** ratio of 2:36:62. The amounts of various phenolic/etherified noncondensed/condensed guaiacyl and syringyl moieties were approximately estimated. *E. grandis* MWL contained ∼0.60/Ar of β-*O*-4 moieties along with small amounts of other structural units such as pino/syringyresinol (0.03/Ar), phenylcoumaran (0.03/Ar), and spirodienone (0.05/Ar). The degree of condensation was estimated at ∼21%; the main condensed structures are 4-*O*-5 moieties (∼0.09/Ar). The structure of *E. grandis* MWL was compared with those of other lignin preparations isolated from various hardwoods.

KEYWORDS: Correlation 2D NMR; hardwood; lignin; milled wood lignin (MWL); quantitative ^{13}C NMR

INTRODUCTION

Lignin is a very complex irregular polymer formed by random dehydrogenative polymerization of lignin precursors of the cinnamyl type. Most softwood lignins consist predominantly of guaiacyl (***g***) units, whereas the structure of hardwood lignins is more complex due to the presence of both guaiacyl (***g***) and syringyl (***s***) units (**Figure 1**). There are a few comprehensive models on the structure of hardwood lignins (**Table 1**). Nimz (*1*) suggested the structure of beech lignin on the basis of thioacidolysis and nonquantitative ^{13}C NMR studies, whereas Adler (*2*) has drawn a scheme of birch lignin based predominantly on the results of permanganate oxidation. However, despite extensive investigations during the past five decades, the structure of lignin, especially that of hardwood lignins, is not well understood. Therefore, the development of methodologies for lignin investigation is of primary importance to enable further progress in the elucidation of lignin structure.

NMR spectroscopy is one of the most powerful tools in lignin chemistry. Some of the first works on the quantification of birch milled wood lignin (MWL) with ^{13}C NMR were published more than 20 years ago (*3*, *4*). Later, Chen and Robert (*5*, *6*) and Kanitskaya et al. (*7*, *8*) reported methods for the quantification of hardwood lignins by ^{13}C and ^{1}H NMR. In the 1990s, various hardwood lignins were analyzed by multidimensional correlation NMR techniques (*9*−*13*). Most recently, a combination of quantitative ^{13}C NMR of nonmodified lignin with ^{1}H NMR of the acetylated preparation has been suggested (*14*).

In our previous study (*15*) we reported a comprehensive approach for the quantification of lignin substructures using a combination of correlation 2D NMR methods and quantitative ^{13}C NMR of nonacetylated and acetylated lignin preparations. Using this approach we obtained information on lignin structure comparable to that reported from other wet chemistry techniques, but requiring only rather short experimental times and small amounts of sample. The results obtained for a spruce MWL were in good agreement with the vast databases for this lignin preparation and showed some of the advantages of the NMR method. However, the calculation algorithm should be modified for the quantification of hardwood lignins.

For further development of the NMR analysis of hardwood lignins we used a MWL isolated from *Eucalyptus grandis* plantation wood. The importance of plantation *Eucalyptus* species for the industry, particularly for the pulp and paper industry, is increasing, especially in southern Europe and South America. However, information on the structures of eucalypt

* Corresponding author [telephone (919) 515-5797; fax (919) 515-6302; e-mail capanema@unity.ncsu.edu].
† North Carolina State University.
‡ University of British Columbia.

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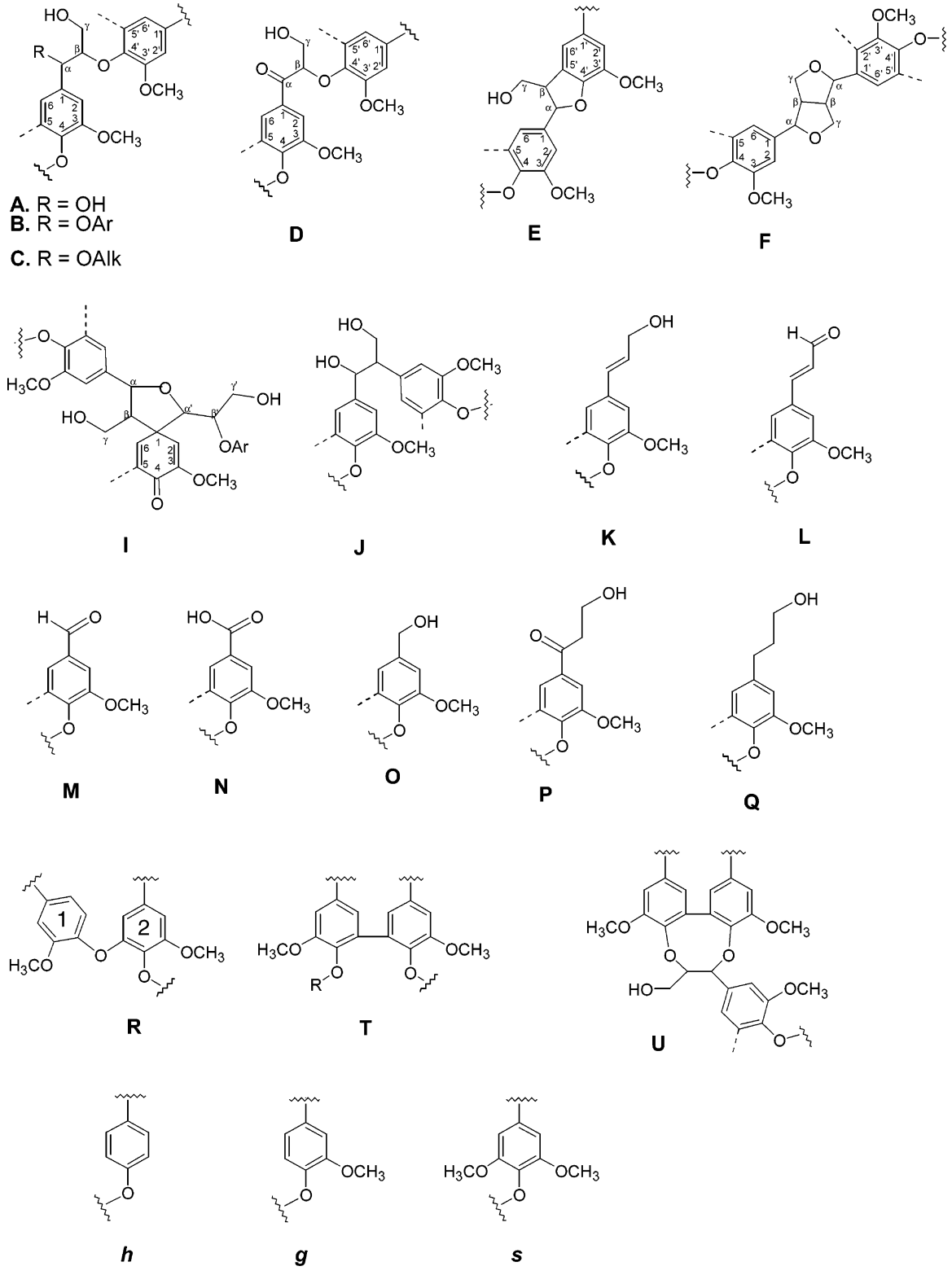

Figure 1. Lignin substructures.

lignins is limited. Recently, the structure of *Eucalyptus globulus* lignins has been comprehensively studied at the University of Aveiro (*14*, *16*−*18*). In the early 1990s Piló-Veloso et al. (*19*, *20*) summarized their studies on the structure of a *E. grandis* lignin using various wet chemistry methods as well as quantitative FTIR and ^{1}H NMR spectroscopic techniques. ^{13}C NMR was used, however, only in a semiquantitative mode. Therefore, the utilization of modern correlation and quantitative NMR techniques can further provide valuable information into the structure of *E. grandis* lignin.

MATERIALS AND METHODS

Samples. Wood meal prepared from *E. grandis* plantation wood was extracted with ethanol/toluene 1:2 (v/v). The wood meal (20 g) was then refluxed in a N_2 atmosphere with 1 L of 0.3% NaOH solution during 1 h to remove tannins. The yield of the solid residue after the alkaline extraction was 84%. The Klason lignin content of the alkali-extracted sawdust was slightly lower than that before the treatment (26.4 versus 28.4%). The resulting wood residue was subjected to ball milling in toluene (48 h) for isolation of the MWL preparation (*21*). The yield of the MWL was 24.8% of the alkali-extracted wood.

Table 1. Amounts of Various Moieties in Hardwood Lignins

unit	beech (*1*)	birch (*2*)	*E. globulus* (*14*)	*E. grandis* (*22*)	*E. grandis* (present work)
Structures					
β-*O*-4/α-OH					55
β-*O*-4/α-CO			4		2
phenylcoumaran	6	6	3	10	3
pino/syringyresinol	5	3	5	4	3
β-1/α-OH	15	7			2
spirodienone: guaiacyl					1
syringyl					4
Ar-CH=CHCH$_2$OH			2	3	<1
Ar-CH$_2$CH$_2$CH$_2$OH					<1
Ar-COCH$_2$CH$_2$OH					<1
Ar-CH=CHCHO			4	3	1
Ar-CHO			3		3
Ar-COOH					2
Ar-CH$_2$OH					2
Interunit Linkages					
β-*O*-4 total	65	60	56	47	61
α-*O*-4 noncyclic		6–8	20	18	nd
γ-*O*-alkyl total[a]					23
5-5′	2	4.5	3[c]	6[e]	3
4-*O*-5′: guaiacyl	1.5	1	1.5[c]	6[e]	3
syringyl		5.5	10[c]		6
6(2)-condensed: guaiacyl		1	4[c]		3
syringyl		1	10[c]		3
Functional Groups					
OMe	136	164[b]	164	145	160
total OH		186[b]	117–121	138	144
aliphatic OH		166[b]	88–91	110	125
primary		86[b]	68	75	70
secondary		80[b]	20 (48[d])	35	55
benzylic			16	35	54
phenolic OH		20[b]	29–30	28	19
total carbonyl			24	19	17
aldehyde			9		4
ketone			15		8
α-CO			10	8	8
nonconjugated			10	8	3
COOH			4		5
degree of condensation			18	50	21
s:g:h		50:50	84:14:2	50:50	62:36:2

[a] Amount C_9 units involved; in the case of symmetric moieties (γ-*O*-γ), the amount of these structures will be half of the present value. [b] Calculated from ref *4*. [c] Calculated from the yield of the products of permanganate oxidation (*14*) using correction coefficient according to ref *24*. [d] From ref *17*. [e] Assumed from literature data for hardwood lignins.

Acetylation of the MWL preparations was carried out according to a published procedure (*22*). The acetylated lignin was recovered by evaporation of the acetylation mixture (pyridine/Ac_2O) with ethanol (*22*), in contrast to typical precipitation in ice water (*5*), which can result in the loss of material and therefore lignin fractionation. To further avoid material loss, no purification of the acetylated preparation was performed.

The analysis of sugars in the MWL preparations was performed according to the method of Coimbra et al. (*23*). Permanganate oxidation was performed using a standard method (*24*).

NMR Analysis. The NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer at 300 K using DMSO-d_6 as the solvent. Chemical shifts were referenced to TMS (0.0 ppm). The heteronuclear multiple quantum coherence (HMQC) analysis was performed with ~5% solution of lignin by applying a 90° pulse width, a 0.12 s acquisition time, a 1.0 s pulse delay, and a $^1J_{C-H}$ of 147 Hz. Conditions for the HSQC analysis were as follows: a 90° pulse width of 15 μs, 1.2 s pulse delay (d_1), and acquisition of 192 data points. The conditions for the TOCSY analysis were 65 ms spin lock, 90° pulse on decoupler channel, and 512 scans per increments.

The quantitative ^{1}H NMR spectrum of acetylated MWL was recorded at a lignin concentration of ~2% in DMSO, a 90° pulse width, and a 1.3 s acquisition time. A relatively high relaxation delay of 7 s was applied to ensure complete relaxation of aldehyde protons, and a total of 128 scans were collected. For the quantitative ^{13}C NMR, 60−70 mg of lignin in 0.25 mL of DMSO-d_6 was placed in a Shigemi microtube; a 90° pulse width, a 1.4 s acquisition time, and 1.7 s relaxation delay were used. Chromium(III) acetylacetonate (0.01 M) was added to the lignin solution to provide complete relaxation of all nuclei. As has been shown in our previous work (*15*), these experimental conditions do not affect the quality of the spectra, but do allow a 4-fold decrease in the experimental time. A total of 20000 scans were collected.

RESULTS AND DISCUSSION

Characterization of the *E. grandis* Lignin by 2D Correlation NMR. The first step of our research was a detailed elucidation of the various substructures of the *E. grandis* lignin with HSQC, HMQC, and TOCSY 2D NMR correlation techniques. The assignment of the signals was made on the basis of the vast lignin NMR databases (*5*, *6*, *12*, *25*−*30*). Signals of typical lignin units (**Figure 1**) such as β-*O*-4 moieties with α-OH (**A**), phenylcoumaran (**E**), and pino/syringyresinol (**F**) structures can be easily seen in **Figures 2** and **3**. In the aliphatic region, some minor structures, such as dihydrocinnamyl alcohol type units (**Q**) and Ar-COCH$_2$CH$_2$OH (**P**) type moieties have been identified (**Figure 2**) on the basis of the reported chemical shifts (*25*). The structure **P** was also confirmed by a TOCSY technique (**Figure 3**). Weak signals of cinnamyl aldehyde structures (**L**) have been detected in the MWL (**Figure 2B**); however, their intensity was significantly lower as compared to that previously observed in spruce MWL (*15*). The amount of cinnamyl alcohol type derivatives (**K**) is close to the detection limit of the HMQC technique (<0.01 unit/Ar) as their signals were detected only in the spectrum of the acetylated milled wood lignin (MWL-Ac) preparation (**Figure 2B**) but not in the nonacetylated lignin.

In contrast to softwood MWLs, the amount of dibenzodioxocin moieties (**U**) in the *E. globulus* MWL was below the detection limit; the characteristic signals of CH-α and CH-β at about 84/4.85 and 82.5/4.14 ppm, respectively (*12*), were not observed in the MWL-Ac spectrum (**Figure 2B**). Small amounts of dibenzodioxocin structures have been previously reported in poplar ^{13}C-enriched MWL (*12*) as well as in α-^{13}C selectively enriched *E. globulus* MWL (*16*). However, it is important to note that these lignins were isolated from juvenile wood, which might have higher amounts of dibenzodioxocin structures than matured hardwood lignins, according to our recent paper (*31*).

Signals of spirodienone structures (**I**) were assigned (**Figure 2**) on the basis of reported chemical shifts (*30*). However, this work (*30*) deals with spectra of acetylated lignins, and therefore the signals of the nonacetylated spirodienone structures should be discussed. A cross-signal of moderate intensity at 81/5.13 ppm in the spectrum of the nonacetylated lignin and MWL-Ac was assigned to CH-α in spirodienone structures (**I**). Considering the effect of the solvent, the signal at ~83/5.2 ppm observed in acetone (*30*) could shift to 81.3/5.13 ppm in DMSO. This suggestion has been confirmed by the TOCSY spectrum of the nonacetylated MWL, which shows correlation between the proton at 5.13 ppm and the proton at 2.75 ppm (**Figure 3**). The latter shows a one-bond correlation with carbon at 59.7 ppm in the HMQC spectrum of the nonacetylated MWL, where the signal at 59.7/2.75 ppm was assigned to CH-β in the spirodienone structure (**Figure 2A**). The assignment of CH-α and CH-β signals is in agreement with a shift of the signal at 59.7/2.75 ppm to a higher field after lignin acetylation, whereas the signal at 81.3/5.13 ppm, which would not shift after acetylation, can still be observed in the MWL-Ac spectrum (**Figure 2B**). The

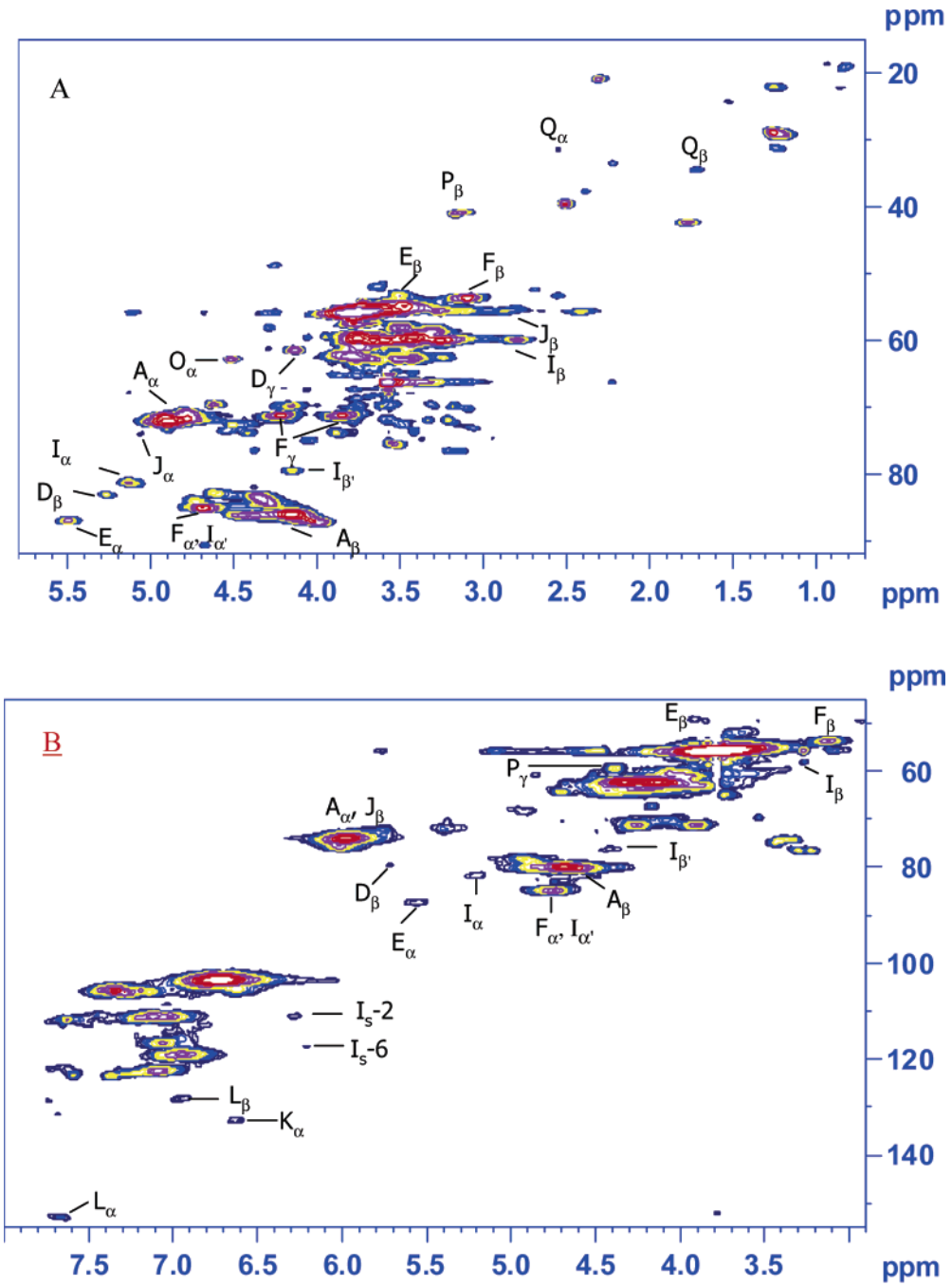

Figure 2. HMQC spectra of *E. grandis* milled wood lignin samples: (**A**) nonacetylated; (**B**) acetylated.

signal at 79.5/4.14 ppm in the HSQC spectrum of MWL was tentatively assigned to CH-β' in the second side chain of the spirodienone moieties. This assignment is confirmed by the shift in this signal to 76.1/4.42 ppm in the MWL-Ac spectrum.

The assignment of the signals of spirodienone moieties is consistent with their disappearance after selective reduction of carbonyl groups in the *E. grandis* MWL with $NaBH_4$ (*32*). It is noteworthy that the intensity of spirodienone moiety signals in the spectra of juvenile hardwood lignins (*12*, *16*) is much lower than can be observed in the spectrum of a birch MWL isolated from mature wood (*11*, *30*) and in our spectra (**Figure 2**).

The cross-peak at 83.5/5.28 ppm was assigned to CH-β in β-*O*-4 moieties of ***s***-type with α-carbonyl groups (**D**). This assignment was confirmed by a TOCSY experiment showing the correlation of the corresponding proton at 5.28 ppm with the proton at 4.12 ppm, the correlation of $\beta-\gamma$ protons. The corresponding signals of the CH-γ in the HMQC spectrum were observed at 61.5/4.12 ppm. Thus, the chemical shifts for the β-*O*-4/α-CO moieties of ***s***-type were slightly different from those reported for model compounds (*25*), but consistent with results obtained from lignin reduction with $NaBH_4$ (*32*). The assignment of CH-β was confirmed by a shift of its signal to 79.8/5.65 ppm in the spectrum of MWL-Ac, in agreement with previous publications (*11*, *12*).

The spectrum of the *E. grandis* MWL shows a cross-peak at 62.8/4.51 ppm (**Figure 2**), which was tentatively assigned to benzyl alcohol (Ar-CH_2OH) units on the basis of the close chemical shift reported for the corresponding model compounds (*25*). This assignment was supported by HMBC techniques, which showed a signal at 62.8/6.71 ppm attributed to the three-bond correlation between C-α/$H_{2,6}$. This correlation is not possible if the signals at 62.8/4.51 ppm in the HMQC spectra belong to CH-γ. These moieties have been previously detected

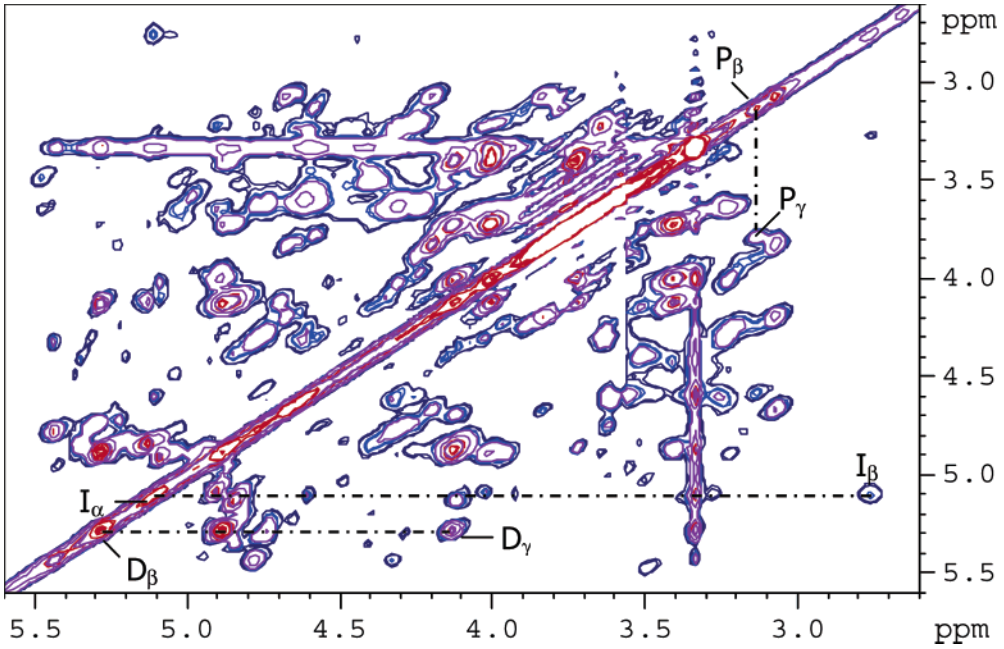

Figure 3. TOCSY spectrum of *E. grandis* milled wood lignin.

in aspen kraft lignin (*33*) but have not been seen in the spectra of other lignins (*9*−*12*, *15*).

Analysis of the Structure of the *E. grandis* MWL with Quantitative ^{13}C NMR. After detailed characterization of the *E. grandis* MWL with correlation 2D NMR techniques, the lignin structure was quantified using ^{13}C NMR. The integral at 160−102 ppm was set as the reference, assuming that it includes six aromatic carbons. It follows that the integral values for other structural moieties are expressed per one aromatic ring (Ar). According to the HMQC studies, the amount of vinyl carbon atoms in cinnamyl alcohol and cinnamyl aldehyde structures in the eucalypt MWL preparation is very little, and correction for their contribution to the integral at 160−102 ppm, usually used for softwood MWL (*5*, *6*, *15*), was not made.

Importantly, the resolution of signals, particularly in the oxygenated aliphatic region, is much better in the spectra of *E. grandis* MWL than that observed previously for softwood lignin (*5*−*9*, *15*), resulting in more accurate quantification. The very close values for the integrals in the main clusters of signals in the spectra of the nonacetylated MWL and acetylated MWL (MWL-Ac) (**Tables 2** and **3**) indicate that no lignin fractionation occurred during its acetylation. Although quantitative ^{1}H spectra were also acquired, they are used only for quantification of specific moieties because the accuracy of calculation based on ^{1}H NMR spectra of lignin is much lower than that for ^{13}C NMR spectra (*5*).

The 2D and ^{13}C spectra indicate that there are no tannin contaminants in the MWL preparations. The amount of sugars in the MWL was quite low for hardwood MWL, 1.31 ± 0.04%. This value is consistent with the very small signals observed for sugar moieties in the HMQC and ^{13}C spectra (**Figure 2B**; *4*). The relatively intense sugar signals in the HSQC spectrum are due to the very high sensitivity of this technique to even minute amounts of carbohydrates (*33*).

Quantification of Side-Chain Moieties on Structural Level. Similarly to our previous work on the NMR analysis of spruce MWL, great effort was made to quantify the various lignin moieties on the structural level.

Carbonyl Structures. Signals at 195−193 ppm belong to carbon atoms of carbonyl groups in cinnamyl aldehyde (**L**) and α-CO/*β*-*O*-4 (**D**) moieties. The amount of cinnamyl aldehyde structures was estimated from the ^{1}H spectrum of the MWL-Ac, the signal at 9.65−9.55 ppm. The resonance of the aromatic protons at 7.5−6.2 ppm was used as the reference. The amount of aromatic H was calculated from the Ar_{C-H} of the ^{13}C spectra (**Table 3**). This approach gives the value for the cinnamyl aldehyde structure of ∼0.01/Ar. Correspondingly, the frequency of α-CO/*β*-*O*-4 (**D**) is 0.02/Ar. The amount of α-aldehyde moieties (**M**) (resonance of C-α at 193−191 ppm) is ∼0.03/Ar.

Table 2. Signal Assignment in the Spectrum of Nonacetylated MWL

no.	spectroscopic range	assignment	amount
1	200–196	α-CO except **D**	0.03
2	196–193	$\mathbf{D}_{\alpha}$, $\mathbf{L}_{\gamma}$	0.03
3	193–191	$\mathbf{M}_{\alpha}$	0.03
4	182–180	$\mathbf{I}_{g}$-4	0.01
5	177–175	$\mathbf{I}_{s}$-4	0.04
6	175–168	aliphatic COOR	0.03
7	168–166	conjugated COOR	0.02
8	158–156	***h***-4	0.02
9	157–151	$\boldsymbol{s}_{et}$-3,5, $\mathbf{R}_{et}$-3,5, $\mathbf{T}_{et}$-3, **L**-α, ***g***-4 in conjugated CO/COOR, $\mathbf{I}_{g}$-3,6, $\mathbf{I}_{s}$-3,5	1.30
10	150–149	$\boldsymbol{g}_{et}$-3 noncondensed	0.12
11	113–110	***g***-2	0.28
12	110–101	***s***-2,6; **R**-2,6	1.28
13	90–82.5	C-*β* in *β*-*O*-4 (C-α′ in **I** with *β*-*O*-4), $\mathbf{E}_{\alpha}$, $\mathbf{F}_{\alpha}$	0.70
14	77–71	$\mathbf{A}_{\alpha}$, $\mathbf{J}_{\alpha}$, $\mathbf{F}_{\gamma}$, carbohydrates	0.67
15	64–62	$\mathbf{I}_{\gamma}$, $\mathbf{J}_{\gamma}$, $\mathbf{E}_{\gamma}$, $\mathbf{O}_{\alpha}$	0.12
16	58–54	OMe	1.59
17	54–53	$\mathbf{E}_{\beta}$, $\mathbf{F}_{\beta}$	0.09
18	35–34	$\mathbf{Q}_{\beta}$	<0.01
		Clusters	
	210–200	nonconjugated CO	0.03
	200–190	conjugated CO	0.09
	162–142	***h***-4, ***s***-3,5, ***g***-3,4 (except **R**), **R**-3,5, $\mathbf{I}_{g}$-3,6, $\mathbf{L}_{\alpha}$	2.00
	125–102	C_{Ar-H}	2.14
	90–58	Alk-*O*-, I_{β}	2.41
	90–77	Alk-*O*-Ar, α-*O*-Alk	0.83
	77–65	γ-*O*-Alk, OH_{sec}	0.81
	65–58	OH_{prim} + I_{β}	0.77

Spirodienone Structures. The peaks at ∼182 ppm (∼ 0.01/Ar) and 176 ppm (∼0.04/Ar) in the spectra of acetylated and

Table 3. Signal Assignment in the Spectrum of Acetylated MWL

no.	spectroscopic range	assignment	amount
1a	200–196	α-CO except **D**	0.02
2a	196–193	$\mathbf{D}_\alpha$, $\mathbf{L}_\gamma$	0.03
3a	193–191	$\mathbf{M}_\alpha$	0.03
4a	182–180	$\mathbf{I}_g$-4	0.01
5a	177–175	$\mathbf{I}_s$-4	0.04
6a	172–169.6	primary aliphatic OH	0.71
7a	169.6–168.6	secondary aliphatic OH	0.59
8a	168.6–166	phenolic OH, conjugated COOR	0.21
9a	158–156	$\boldsymbol{h}_{et}$-4	0.015
10a	148–144.5	$\boldsymbol{g}_{et}$-4 except **R** and conjugated ***g***	0.17
11a	142.5–144.5	**E**-3, conjugated $\mathbf{s}_{et}$-4 and conjugated **R**-4	0.08
12a	114–110	***g***-2	0.32
13a	110–101	***s***-2,6; **R**-2,6	1.28
14a	88–86	$\mathbf{E}_\alpha$	0.03
15a	60–59	$\mathbf{P}_\gamma$	<0.01
16a	58–54	OMe	1.60
17a	54–53	$\mathbf{F}_\beta$	0.06
18a	50–48	$\mathbf{J}_\beta$, $\mathbf{E}_\beta$	0.05
		Clusters	
	210–200	nonconjugated CO	0.03
	200–190	conjugated CO	0.08
	162–142	***h***-4, ***s***-3,5, $\boldsymbol{g}_{et}$-3,4 (except **R**), **R**-3,5, $\mathbf{I}_g$-3,6, $\mathbf{I}_s$-3,5, $\mathbf{L}_\alpha$	1.90
	125–101	C_{Ar-H}	2.15
	90–58	Alk-*O*-	2.34
	90–77	Alk-*O*-Ar, α-*O*-Alk	0.83
	77–65	γ-*O*-Alk, OH_{sec}	0.82
	65–58	OH_{prim}	0.70

Table 4. Calculation of Various Lignin Moieties by ^{13}C NMR

structure	calculation	value
α-CO/β-O-4 (**D**)	$(I_{196-193})_{na} - \mathbf{L}^a$	0.02
	$(I_{196-193})_{ac} - \mathbf{L}^a$	0.02
pino/syringyresinol (**F**)	$((I_{54-52})_{na} - \mathbf{E})/2$	0.03
	$(I_{54-52})_{ac}/2$	0.03
phenylcoumaran (**E**)	$(I_{88-86})_{ac}$	0.03
β-1 (**J**)	$(I_{50-48})_{ac} - \mathbf{E}$	0.02
β-O-4/α-OH (**A**)	$(I_{77-70.5})_{na} - \mathbf{J} - \mathbf{F} - 3$ carbohydrates	0.55
β-O-4 total	$(I_{90-82.5})_{na} - \mathbf{E} - \mathbf{F}$	0.61
γ-*O*-Alk total	$I_{77-65} - OH_{sec}$	0.23
phenolic OH	$(I_{168.6-166})_{ac} - COOR_{conj}$	0.19
side chain	$I_{100-45} - OMe - 5.5carb\% + 2 \times (\mathbf{Q} + \mathbf{K} + \mathbf{L}) + \mathbf{R} + (I_{175-165})_{na} + I_{210-190}$	2.67
s	eq 1: $OMe - 1 + \boldsymbol{h}$	0.62
	eq 5_{na}: $(I_{110-103})_{na}:2 - \mathbf{R} + \mathbf{I}_s$	0.60
	eq 5_{ac}: $(I_{110-103})_{ac}:2 - \mathbf{R} + \mathbf{I}_s$	0.60
g	eq 1′: $1-\boldsymbol{h}-\boldsymbol{s}$	0.36
	eq $5'_{na}$: $(I_{113-110})_{na} + \mathbf{R} - \mathbf{I}_s$	0.34
	eq $5'_{ac}$: $(I_{114-110})_{ac} + \mathbf{R} - \mathbf{I}_s$	0.36
h:g:s (100% base)	from eq 1	2:36:62
	from eq 5_{na}	2:34:64
	from eq 5_{ac}	2:36:62
degree of condensation	$(2\boldsymbol{s} + 3\boldsymbol{g} + 2\boldsymbol{h}) - (I_{125-103})$	0.21
etherified total	$1.00 - OH_{ph} - \mathbf{I}$	0.76
s-etherified	total $\boldsymbol{s} - PhOH_{\boldsymbol{s}} - \mathbf{I}_s$	0.49
g-etherified	total $\boldsymbol{g} - PhOH_{\boldsymbol{g}} - \mathbf{I}_g$	0.25
g-etherified noncondensed	$(I_{150-149})_{na}$	0.12
4-*O*-5′$_{et}$ ($\mathbf{R}_{et}$)	$\boldsymbol{g}_{et} - (I_{148-145.5})_{ac}$	0.08

[a] **L** = 0.01/Ar as estimated from ^{1}H NMR. $(I_{i-j})_{na}$ and $(I_{i-j})_{ac}$ are integral values in the region of $(i - j)$ ppm in the spectra of nonacetylated and acetylated lignins, correspondingly.

nonacetylated MWL have been assigned, on the basis of recent publications (*30*, *32*), to C-4 in spirodienone structures of the guaiacyl ($\mathbf{I}_g$) and syringyl type ($\mathbf{I}_s$), correspondingly. In addition to an assortment of CH signals detected by 2D NMR, the resonance at ∼52.5 ppm in the ^{13}C spectra (**Figure 4**) was attributed to the quaternary C-1 in $\mathbf{I}_s$ moieties on the basis of literature assignment (*30*) and considering solvent shift.

Phenylcoumaran, Pino/syringyresinol, and β-1 Structures. The amount of the phenylcoumaran structures (**E**), 0.03/Ar, was estimated from the resonance at ∼87 ppm in the spectrum of the MWL-Ac. The integral at 50−48 ppm in the spectrum of MWL-Ac (0.05/Ar) embodies the phenylcoumaran and β-1/α-OH (**J**) structures. It follows that the amount of the β-1 (**J**) structures is about 0.05 − 0.03 = 0.02/Ar. Rather good resolution of the signals of the C-β in pino/syringyresinol structures (**F**) from the methoxyl (OMe) signals, in contrast to the spectra of spruce MWL, enables their amount (0.06/Ar) to be directly calculated from the integral at 54−52.5 ppm in the spectrum of the MWL-Ac (**Table 3**) or by subtracting the amount of the phenylcoumaran structures from the integral at 54−52 ppm in the spectrum of the nonacetylated MWL (**Table 4**). In the ^{13}C spectra of MWLs acquired in acetone-d_6, the OMe signals of spirodienone moieties (**I**) would be overlapped with the signals of pino/syringyresinol (**F**) structures (*30*). However, there are no extra signals at 54−53 ppm in the HSQC and HMQC spectra obtained in DMSO (**Figure 2**). Therefore, the OMe signals of **I** structures are likely overlapped with other OMe signals at a slightly lower field and do not interfere with the resonance of **F** and **E** moieties. The difference in the location of the OMe signals in **I** structures is consistent with their strong solvent dependence (*30*).

***β*-*O*-4/α-*OH* Structures**. The amount of β-O-4/α-OH (**A**) moieties can be estimated by the resonance of the C-α at 77−71 ppm in the spectrum of nonacetylated MWL. This region also includes the C-γ in **F** moieties as well as small amounts of C-α in β-1 (**J**) structures and carbohydrates. The amounts of the β-β and β-1 moieties have been estimated above as 0.06/Ar and 0.02/Ar, respectively. The amount of carbohydrate determined by wet chemistry is ∼1.3%. As there are three carbohydrate atoms in this region, the contribution of carbohydrates to the integral of 77−71 ppm is ∼0.04/Ar. Therefore, the amount of β-O-4/α-OH moieties is

$$0.67 - 0.06 - 0.02 - 0.04 = 0.55/\mathrm{Ar}$$

It should be noted that this calculation is strongly dependent on the amount of sugars in lignin preparations and therefore is not recommended for lignins with significant carbohydrate content.

Ar-CH$_2$OH Structures. The integral at 64−62 ppm in the spectrum of the nonacetylated MWL is 0.12/Ar. It embodies the C-γ in phenylcoumaran and β-1 and supposedly C-γ in spirodienone moieties as well as C-α in Ar-CH$_2$OH (**O**) structures. According to the carbohydrate analysis the contribution of the C-5 in xylose is very low, below 0.005/Ar. Therefore, the sum of these moieties identified separately is 0.03 + 0.02 + 0.05 = 0.10/A, and the amount of Ar-CH$_2$OH structures is ∼0.02/Ar.

Aliphatic Moieties. The signals of dehydrocinnamyl alcohol type (**Q**) structures were detected in the HMQC spectra (**Figure 2**). However, as indicated by the HMQC spectrum, the C-α signal of the DHCA structures will be overlapped in the ^{13}C spectrum with a rather strong signal from other aliphatic moieties. In contrast, the C-β signal is nonobscured; signals of other moieties, such as secoisolariciresinol, have not been detected in this region. Therefore, the amount of dehydrocinnamyl alcohol structures was estimated from the integral at 35−34 ppm as <0.01/Ar (**Table 2**). From the resonance at 60−59

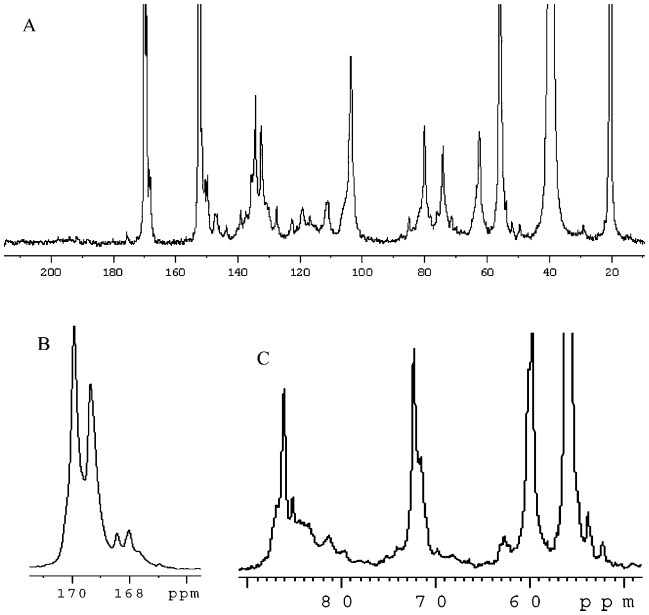

Figure 4. ^{13}C NMR spectra of *E. grandis* milled wood lignin samples: (A) acetylated; (**B**) expanded acetyl region, acetylated; (**C**) expanded oxygenated aliphatic, nonacetylated.

ppm in the spectrum of MWL-Ac the amount of **P** moieties was estimated to be ~0.01/Ar (**Table 3**).

Functional Group Analysis. Structural level information of the lignin side chain can be obtained through analysis of the various types of interunit linkages and functional groups present.

Methoxyl Groups. The amount of methoxyl groups (OMe) was estimated from the integral at 58−54 ppm as ~1.60/Ar. Other minor moieties, which can be included in this integral (*15*), were not considered because their amounts were in the range of the integration error, which is ~3%.

Carbonyl Groups. The amounts of α-aldehyde, cinnamyl aldehyde, and α-CO/*β-O*-4 were estimated above. The integral at 200−196 ppm was attributed to α-ketone moieties with the exception of those of type **D**. The total amount of conjugated carbonyl moieties (integral at 200−190 ppm) was ~0.08−0.09/Ar. Although the integration of the resonance of nonconjugated CO groups (210−200 ppm) gave the value of ~0.03/Ar (**Tables 2** and **3**), there was no noticeable peak in the region (**Figure 4**), and the contribution of the experimental error due to baseline deviation at the rather large (10 ppm) interval to the value obtained might be significant.

Carboxyl Groups. The amounts of aliphatic and conjugated COOH groups were estimated from the spectrum of the nonacetylated MWL to be about 0.03/Ar and 0.02/Ar, respectively (**Table 2**). The HMQC spectra do not show any signals at δ_C/δ_H 150−140/7−8 corresponding to CH-α of cinnamic acid (*25*). Therefore, the conjugated carboxyl groups were attributed to aromatic acids Ar-COOH (**N**).

Hydroxyl Groups. The amount of hydroxyl groups was estimated in the acetylated MWL from the region of 172−165 ppm (**Table 3**). The amount of primary OH (0.71/Ar) is close to that in spruce MWL (*15*), whereas the value for secondary OH (0.59/Ar) is significantly higher. After correction for the amount of Ar-COOH (**Table 4**), the quantity of phenolic OH (PhOH) was calculated to be ~0.19/Ar. In the area of phenolic OH there are two signals corresponding to guaiacyl (***g***) and syringyl (***s***) PhOH (*29*). However, the former embodies only $PhOH_{\boldsymbol{g}}$ of noncondensed type (0.07/Ar), whereas the signal of the $PhOH_{\boldsymbol{s}}$ (~0.12/Ar) also includes condensed $PhOH_{\boldsymbol{g}}$. Permanganate oxidation estimates the ***g*:*s*** ratio of the phenolic moieties as 54:44 (**Figure 5**). It then follows that the total amount of $PhOH_g$ is 0.54 × 0.19 ~ 0.10/Ar, meaning the amount of condensed units is ~0.03/Ar and thus the amount of $PhOH_{\boldsymbol{s}}$ is ~0.09/Ar.

The amount of primary OH groups (0.71/Ar) is slightly lower than the integral value at 65−58 ppm (0.76/Ar) in the spectrum of nonacetylated MWL (**Table 2**). This is due to the contribution of the C-*β* in the spirodienone moieties in this area. However, there is very good correlation in the amount of primary OH groups estimated from the integrals at 172−169.6 and 65−58 ppm in the MWL-Ac spectrum (**Table 3**).

Alk-O-Ar Moieties. The maximum amount of Alk-*O*-Ar moieties can be estimated from the region of 90−77 ppm. The integral value is 0.83/Ar and includes also α-atoms of pino/syringyresinol (0.06 C-α/Ar) and spirodienone moieties (2 × 0.05 = 0.10 C-α/Ar). The remaining, 0.83 − 0.06 − 0.10 = 0.67/Ar is the maximum amount of Alk-*O*-Ar moieties.

***β*-*O*-4 *Structures*.** As the resolution of the eucalypt MWL in the area of 90−77 ppm is much better than that in the spectrum of spruce MWL, it is possible to estimate the total amount of *β-O*-4 structures from the integral at 88−82.5 ppm (0.70/Ar) in the spectrum of the nonacetylated MWL, which also includes *β*-5 and *β-β* structures. The quantity of the latter has been calculated above from the integral at 54−53 ppm and can be subtracted from the integral at 88−82.5 to get the value for the *β-O*-4 moieties:

$$\beta\text{-}O\text{-}4 \text{ total} = 0.70 - 0.09 = 0.61/\text{Ar}$$

The difference between the amounts of the identified *β-O*-4 moieties (**A** + **D**) and total *β-O*-4, ~0.04/Ar, can be predominantly attributed to the *β-O*-4 linkages in the side chain II of the spirodienone moieties.

***γ*-*O*-*Alk Moieties*.** The cluster at 77−65 ppm embodies moieties with secondary OH groups and *γ-O*-Alk ethers. Subtracting the amount of secondary OH groups (**Table 4**) from the integral value of this region gives the amount of *γ-O*-Alk ethers as ~0.23/Ar. From these moieties, only pinoresinol structures have been identified (~0.06/Ar). The structures of other *γ-O*-Alk ethers (~0.17/Ar, which include small amounts of carbohydrate atoms without OH such as Xyl-4 and Glc-4,5) are unknown. Some weak signals at δ_C/δ_H 70−65/3.5−4.5, which can be attributed to *γ*-ether moieties, were detected in the HMQC spectrum (**Figure 2**).

Total Side-Chain Carbons. The sum of all carbon atoms in the side chain was estimated as 2.67/Ar (**Table 4**). The theoretical value for the phenylpropane unit is 3.00. The deficit of 0.33/Ar arises from moieties with short side chains detected in the *E. grandis* MWL, such as Ar-CHO (0.03/Ar), Ar-COOH (~0.02/Ar), and $ArCH_2OH$ (~0.02/Ar). They have only one carbon atom in the side chain; therefore, the deficit of carbon atoms in the side chain from these structures is (3 − 1) × (0.03 + 0.02 + 0.02) = 0.14/Ar. The rest, 0.33 − 0.14 = 0.19 C/Ar, might belong to some minor unidentified structures in the aliphatic region and Ar-CH_2-*O*-Alk type structure, which give resonance in the same area with *γ*-ethers (77−65 ppm). As they have only one carbon in the side chain, the amount will be 0.19/2 = 0.10/Ar. However, this suggestion needs further examination, and the accuracy of measurement needs also to be taken into account.

h:g:s* *Ratio. The elucidation of the ***h:g:s*** ratio is an important point in the analysis of hardwood lignin structures. There are a few different degradation methods for the estimation of the ***h:g:s*** ratio; however, each of them gives a number only for specific

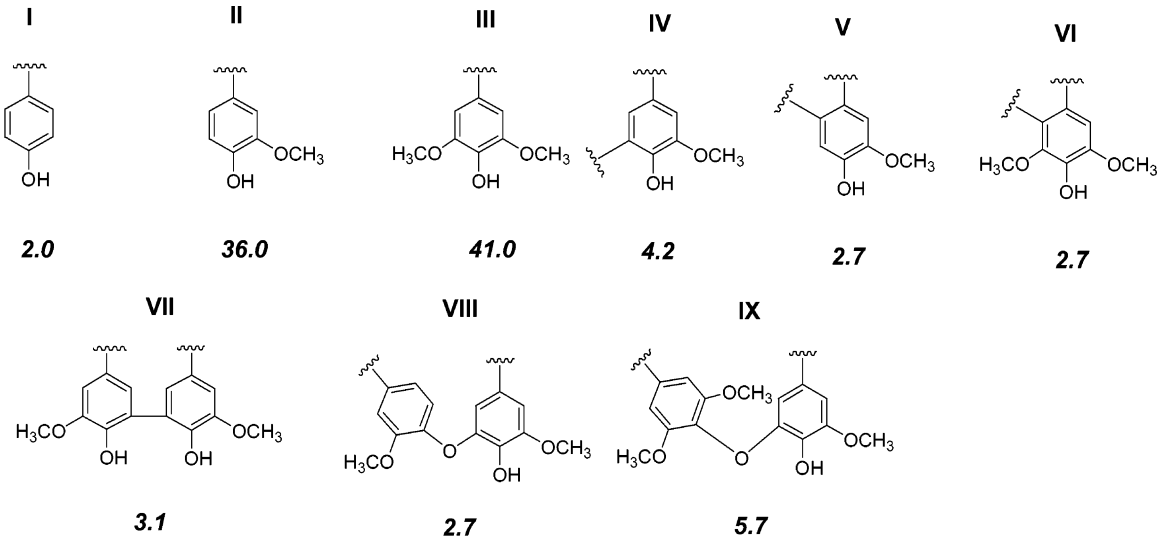

Figure 5. Relative frequency of phenolic units calculated from results of permanganate oxidation assuming the yield of the products of 60%/COOH according to ref *25*. ***h:g:s*** = I:(II + IV + V + 2VII + VIII + IX):(III + VI) = 2:54:44. Degree of condensation (DC) = IV + V + VI + 2VII + VIII + IX = 24%.

lignin fractions and does not provide the correct value for the whole lignin. The advantage of ^{13}C NMR over the degradation techniques for the estimation of ***h:g:s*** ratios is the analysis of the whole lignin structure. A few ways for the estimation of the amounts of ***g*** and ***s*** units have been suggested. Landucci showed (*34*) that the most accurate way to estimate the ***h:g:s*** ratio in native lignins is from calculation of the amount of OMe/Ar using the following equations:

$$\mathrm{OMe} = 2\boldsymbol{s} + \boldsymbol{g};\ \boldsymbol{s} + \boldsymbol{g} + \boldsymbol{h} = 1$$

The amount of ***h*** units in the *E. grandis* MWL was estimated from the resonance at 162−157 ppm as 0.02/Ar. It then follows that

$$\boldsymbol{s} = \mathrm{OMe} - 1 + \boldsymbol{h};\ g = 1 - \boldsymbol{s} - \boldsymbol{h} \qquad (1)$$

Therefore, from the amount of OMe groups in the lignin (1.60/Ar), the ***h:g:s*** ratio comes as 2:36:62. However, this method cannot be used for technical lignins when demethylation/demethoxylation occurs. Therefore, evaluation of alternative methods of ***g:s*** ratio calculation from aromatic signal is useful.

Lappierre et al. suggested estimating the ratio of only noncondensed ***g*** and ***s*** units as follows (*35*):

$$\boldsymbol{g{:}s} = (\boldsymbol{g}\text{-}6 + \boldsymbol{g}\text{-}2)/(\boldsymbol{s}\text{-}2 + \boldsymbol{s}\text{-}6) = (I_{119} + I_{112})/(I_{110-102}) \qquad (2)$$

Indeed, signals of ***g***-6 in noncondensed units are located at 119 ppm, except those conjugated with CO/COOR groups. Substitution at ***g***-5 shifts the resonance of ***g***-6 to a lower (5-5′ units) or higher (β-5, 4-*O*-5′ moieties) field (*25*−*29*). However, the signal of ***g***-2 at 112 ppm involves practically all ***g***-2 carbons in noncondensed and condensed at ***g***-5 and ***g***-6 moieties except those in 4-*O*-5′ structures. Thus, this approach gives neither the ***g:s*** ratio for noncondensed units nor the ***g:s*** ratio for the whole preparation.

Another approach (*14*, *36*) suggested using the ratio of the resonance of the ***g*** signals at 123−110 ppm to that of the ***s*** signals at 110−103 ppm as follows:

$$(I_{125-110}/3)/(I_{110-103}/2) \qquad (3)$$

The advantage of this approach is the integration of the clusters corresponding to the whole ***g*** and ***s*** regions. This decreases the experimental error from incomplete resolution of signals. However, eq 3 suggests an equal proportion of noncondensed moieties in ***g*** and ***s*** units. In reality, the degree of condensation is usually higher in ***g*** units than that in ***s*** units, and this equation would thus result in overestimation of the ***s***-unit content. Indeed, the use of this formula for the *E. grandis* MWL gives the ***h:g:s*** ratio of 2:28:70 (on the 100% basis), implying the OMe content of 1.68/Ar, which is somewhat higher than the experimental value.

Alternatively, Chen (*5*, *6*) estimates the amount of ***s*** units as

$$\boldsymbol{s} = (I_{103-110}/2)/\mathrm{Ar} \qquad (4)$$

The amount of ***g*** units calculates as

$$\boldsymbol{g} = 100 - \boldsymbol{s} - \boldsymbol{h} \qquad (4')$$

This approach does not suffer from unknown amounts of condensed ***g*** units. However, an error in estimation of ***s*** units consequently affects the accuracy of the ***g***-unit calculation. To eliminate this shortcoming, we suggest estimating the amount of ***g*** units from the integral of ***g***-2 at 113−110 ppm. The amount of ***g***-2 condensed moieties is negligible, and the substitution at ***g***-5 and ***g***-6 does not affect the chemical shift of ***g***-2 significantly. The percentage of ***s***-condensed carbons is also rather low as compared to the total amounts of ***s***-2,6 carbons, and it is in the range of the accuracy of the NMR experiment. Therefore, this approach should be more accurate for the estimation of the entire ***g:s*** ratio than eqs 2−4.

In addition, correction for the 4-*O*-5′ units is needed. Earlier, Chen (*5*, *6*) postulated that ***g***-2 and ***g***-6 in 4-*O*-5′ units (ring II) are located at δ_C <110 that is in the area of syringyl C-2 and C-6. This suggestion was recently experimentally confirmed (*37*, *38*). The amount of 4-*O*-5′ structures in the *E. grandis* MWL was assumed to be similar to the proportion of phenolic 4-*O*-5′ units revealed by the permanganate oxidation degradation technique (**Figure 5**) as 0.08−0.09/Ar. Besides, corrections for spirodienone moieties of ***s*** type, C-2 and C-6 atoms, which resonate at about 111−114 and 116−117 ppm (*30*, *32*), respectively, are also necessary. After the correction for the content of 4-*O*-5′ and spirodienone moieties, the ***h:g:s*** ratio was in the range from 2:32:60 to 2:36:60 or from 2:34:64 to 2:36:62 on a 100% basis (**Table 4**). This was in good agreement

Table 5. Approximate Amounts of Various Aromatic Moieties

aromatic moiety	amount (per 100 Ar)	% of total *g* or *s* units
h units total	2	
g units total	36	
phenolic	10	28
noncondensed	7	
condensed	3	
etherified	25	70
noncondensed + 6-condensed	12	
5-condensed	13	36
phenylcoumaran	3	
4-*O*-5′	8	
s units total	62	
phenolic	9	15
etherified	49	79
spirodienone	4	

with the OMe group content. Permanganate oxidation of lignin preparations gives a higher proportion ***g:s*** ratio (**Figure 5**), indicating that ***s*** units are more etherified than ***g*** moieties.

Degree of Condensation (DC). The amount of tertiary aromatic carbons (C_{Ar-H}) in the lignin preparation was obtained from the integral at 125−102 ppm to be 2.15/Ar (**Tables 2** and **3**). The theoretical amount of C_{Ar-H} atoms can be calculated from the ***h:g:s*** ratio (eq 1) considering the contribution of two carbons of ***s*** and ***h*** units and three carbons of ***g*** units in this region. Specifically

$$\text{theor } C_{Ar-H} = (2\boldsymbol{s} + 3\boldsymbol{g} + 2\boldsymbol{h}) = [3 \times 0.36 + 2(0.62 + 0.02)] = 2.36/\text{Ar}$$

The difference between the theoretical and experimental values gives the amount of condensed moieties:

$$\text{DC} = 2.36 - 2.15 = 0.21/\text{Ar} = 21\%$$

This value is slightly lower than the number (24%) obtained from the permanganate oxidation technique for phenolic lignin moieties (**Figure 5**) because of the higher proportion of ***g*** units in the phenolic structures than in the whole lignin.

Oxygenated Aromatic Region. The amounts of C_{Ar-O} and C_{Ar-C} cannot be estimated the same way as used for softwood lignins because the signals of ***s***-4 are in the area of 138−134 ppm and overlap with C_{Ar-C} (*25*). The integral at 162−142 ppm (2.00/Ar) in the spectra of the nonacetylated lignin includes predominantly C-3,4 in ***g*** units except 4-*O*-5′ moieties and C-3,5 in ***s*** units and 4-*O*-5′ structures. The value of the integral decreased in the spectrum of the MWL-Ac to 1.90/Ar due to a shift of the C-4 in the acetylated ***g***-OH to a δ_C of ∼138. The difference in the integral value at 162−142 ppm in the spectra of the nonacetylated MWL and MWL-Ac, 0.10/Ar, corresponds well to the amount of ***g***-OH calculated above (0.10/Ar).

The amount of etherified ***s*** units ($\boldsymbol{s}_{et}$), predominantly in β-*O*-4 and 4-*O*-5′ units (ring 1), can be approximately calculated as follows:

$$\boldsymbol{s}_{et} = \text{total } \boldsymbol{s} - \text{PhOH}_s - \mathbf{I}_s = 0.62 - 0.09 - 0.04 = 0.49/\text{Ar}$$

or ∼80% of the total ***s***-unit content (**Table 5**). Although $\boldsymbol{s}_{et}$ moieties give the major contribution to the integral at 156−150 ppm (1.30/Ar), the assignment of this resonance exclusively to $\boldsymbol{s}_{et}$ moieties (*7*, *39*) results in their overestimation by >30%, the contribution of the other moieties is still significant. This is even more dangerous if an erroneous value, obtained by this way, is used in further calculations (*7*).

The integral at 142−144 ppm (0.08/Ar) embodies ***g***-4 in phenylcoumaran (0.03/Ar) and conjugated etherified ***s*** and 4-*O*-5′ moieties. The amount of the latter is 0.08 − 0.03 = 0.05/Ar.

The total amount of guaiacyl etherified units ($\boldsymbol{g}_{et}$) is

$$\boldsymbol{g}_{et} = \text{total } \boldsymbol{g} - \text{PhOH}_g - \mathbf{I}_g = 0.25/\text{Ar}$$

The amount of ***g***-noncondensed etherified moieties was estimated from the resonance of C-3 at ∼149 ppm in the spectrum of the nonacetylated lignin (*5*, *6*) to be ∼0.12/Ar. This constitutes one-third of the total ***g*** units. It likely also includes a small amount of ***g***-6 condensed moieties, as the substitution at ***g***-6 does not affect significantly the chemical shift of the ***g***-3 atom (*40*).

The integral at 148−144.5 ppm in the spectrum of the MWL-Ac preparation (0.17/Ar) gives the amount of $\boldsymbol{g}_{et}$ moieties excluding 4-*O*-5′$_{et}$ (ring II). It follows that

$$4\text{-}O\text{-}5'_{et} = \boldsymbol{g}_{et} - I_{148-144.5} = 0.25 - 0.17 = 0.08/\text{Ar}$$

The total amount of C-4 etherified moieties is 1 − PhOH − **I** = 1 − 0.19 − 0.05 = 0.76/Ar (**Table 4**). The maximum amount of Alk-*O*-Ar moieties was estimated above as 0.67/Ar. The rest of etherified moieties 0.76 − 0.67 = 0.09/Ar should belong to the Ar-*O*-Ar moieties, predominantly of the 4-*O*-5′ type. This value is very close to the one obtained from permanganate oxidation and used in our calculations.

Comparison of the *E. grandis* MWL with Other Hardwood Lignins. Comparison of the structure of various hardwood lignins is of interest to estimate differences between hardwood lignins. However, it should be done in connection with an isolation procedure and analytical methods used. This discussion is valuable, allowing evaluation not only of differences in lignin structure but also of the effect of experimental conditions and calculation methods on the results obtained.

Comparison of the Structure of E. grandis Lignin with Previous Reports. Our results are rather different from previously published information on the structure of *E. grandis* lignin (*19*, *20*) (**Table 1**). One of the major reasons for these discrepancies is probably due to the differences in methods used for the lignin isolation. Piló-Veloso et al. (*19*, *20*) used the extraction of wood with NaOH not **before**, but **after**, the milling. This resulted in the elimination of a high amount of MWL during the alkaline extraction of the milled wood. Therefore, a lignin preparation obtained by subsequent extraction of the residue with 90% acetone has a very low yield, ∼1% of wood, and might not be representative.

Comparison of E. grandis MWL with the Structure of E. globulus Dioxane Lignin. **Table 1** shows substantial differences between the *E. globulus* dioxane lignin and our *E. grandis* MWL. The *E. globulus* dioxane lignin has a lower β-*O*-4 content and a higher amount of phenolic OH and various carbonyl groups. Another remarkable difference is the rather high content of ***s***-2 (***s***-6) condensed moieties in the *E. globulus* dioxane lignin, whereas their amounts in the corresponding *E. globulus* MWL are much lower (*18*). The *E. grandis* MWL has a higher amount of aliphatic OH groups than the *E. globulus* dioxane lignin. The amount of secondary and benzylic OH groups in the *E. globulus* dioxane lignin determined by wet chemistry methods was quite low (*14*); however, ^{13}C NMR of the acetylated lignin gave a much higher value (*17*) (**Table 1**). These substantial differences are probably due to, at least in part, modification of the lignin during the acidolysis isolation procedure.

The *E. globulus* lignin has a higher ***s***-unit content than the *E. grandis* MWL. However, the difference in methoxyl content

is much lower than the difference in the calculated ***s:g*** ratio, likely due to the difference in the calculation methods discussed. A very large amount of noncyclic α-*O*-4 moieties has been reported in the *E. globulus* dioxane lignin using ^{1}H NMR (**Table 1**), in contrast to our estimations for the *E. grandis* MWL. This is also surprising considering the strongly acidic conditions of the dioxane lignin isolation procedure.

Comparison of E. grandis MWL with the Structure of Birch MWL. Among other hardwood lignins, birch MWL has been the most investigated. The birch MWL, analyzed by wet chemistry methods (*2*), has a lower ***s:g*** ratio than the *E. grandis* MWL, whereas ^{13}C NMR studies (*4*) report OMe contents rather similar to ours (**Table 1**). The amounts of *β*-*O*-4 and pino/syringyresinol moieties in the birch and *E. grandis* MWL are very close, but the amount of phenylcoumaran structures reported for the birch MWL (*2*) is twice that of *E. grandis* MWL. Similarly, the amount of *β*-1/α-OH moieties, assigned on the basis of acidolysis of the birch MWL, is significantly higher than our value. Thus, the results obtained from acidolysis appear to include spirodienone moieties in the total amount of *β*-1 structures (*30*). Although the total degree of condensation of the birch and *E. grandis* MWL is rather close, there are differences in the amounts of various condensed moieties. In part, they can be caused by the fact that the values for the birch MWL represent the whole lignin, as permanganate oxidation was performed after cleavage of alkyl ether bonds in the lignin (*2*), whereas the values for the permanganate oxidation of the eucalypt lignins were obtained without pretreatment with CuO and represent only phenolic moieties.

About 6−8% of the units in the birch MWL were attributed to noncyclic α-*O*-4 moieties, on the basis of the liberation of phenolic-OH during mild acid treatment of the lignin. However, the amount of α-*O*-4/*β*-*O*-4 moieties in a birch MWL was below the detection limit of the HMQC NMR technique (*11*, *12*), similar to our results for *E. grandis*. The increase in phenolic OH observed after mild hydrolysis (*2*) could be caused by degradation of spirodienone moieties.

Finally, we can conclude that the approach suggested allows detailed identification and quantification of various lignin moieties of a hardwood lignin. More than 80% of side-chain moieties of *E. grandis* MWL were estimated on the structural level. This information is substantiated by the data on the quantity of various functional groups and interunit linkages as a whole. A modified method for the calculation of the ***h:g:s*** ratio has been suggested and compared with previously suggested approaches. The amounts of various phenolic/etherified noncondensed/condensed guaiacyl and syringyl moieties were approximately estimated. Comparison data obtained for the structure of *E. grandis* MWL with other hardwood lignins indicates that the results obtained reflect not only difference in the lignin structures but also the dependence of lignin structure on the isolation method, experimental techniques, and calculation methods.

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