ARTICLES

Determination of Arylglycerol-β-aryl Ethers and Other Linkages in Lignins Using DFRC/^{31}P NMR

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An analytical method for lignins has been developed that involves derivatization followed by reductive cleavage (DFRC), depolymerization, and quantitative ^{31}P NMR spectroscopy. This technique detects and quantifies the various ether linkages present in softwood residual kraft lignins (RKL) and milled wood lignins (MWL). In addition, the technique supplies new quantitative information about β-aryl ethers linked to condensed and noncondensed aromatic moieties, including dibenzodioxocins. Within RKL, β-aryl ether bonds connected to condensed phenolic moieties predominated over those connected to noncondensed phenolic moieties. In addition, the amount of DFRC monomers determined by gas chromatography was minute in the RKL but large in the MWL. This indicates that almost all noncondensed β-aryl ether linkages were cleaved during kraft pulping. The method offers new avenues for the detailed investigation of the bonding patterns of native and technical lignins.

Keywords: *Aryl ether bonds; kraft; lignins; milled wood lignin (MWL); ^{31}P nuclear magnetic resonance (NMR); phosphorus; pulping*

INTRODUCTION

Native lignins are built from phenylpropane units linked together by bonding patterns of still impreciesly known frequency and occurrence. Most of the conclusions regarding the bonding patterns of native lignins have been derived from chemical degradation techniques, such as hydrogenolysis (*1*), acidolysis (*2*, *3*), and thioacidolysis (*4*). Recent degradative methods include ether cleavage protocols using trimethylsilyl iodide (*5*) and dry hydrogen iodide (*6*, *7*). In 1997, a new selective α,β-aryl ether cleavage protocol, termed derivatization followed by reductive cleavage (DFRC), was proposed by Lu and Ralph (*8*−*10*). The DFRC method uses a considerably milder depolymerizing environment and a much simplified procedure and yields a simpler mixture of monomers in higher yields than other procedures. Although DFRC provides a clean and selective protocol for ether scission, the primary DFRC monomers detected and quantified by gas chromatography (GC) (*8*−*10*) are confined to phenylpropane units connected to β-aryl ether bonds on both sides of the phenyl propanoid units or terminal phenylpropane units connected to the polymer via a β-aryl ether bond. Consequently, by using GC alone for detecting the DFRC monomers, the total amount of β-aryl ether linkages cannot be revealed. For example, if a β-aryl ether connects two macromolecules or oligomers that themselves are interlinked via structures other than β-aryl ethers, the size of the fragments will preclude them from being detected by GC as DFRC monomers. Quantitative ^{31}P nuclear magnetic resonance (NMR) can overcome this limitation, and its applicability is explored in this paper.

Quantitative ^{31}P NMR spectroscopy is a nondestructive technique that allows the structural elucidation of all hydroxyl bearing moieties of lignin (*12*−*15*) including stereochemical details of its β-aryl ether constituents (*16*). Although the technique has contributed significantly to our understanding of the hydroxyl-bearing functional groups, it is seriously limited because it cannot provide information about the etherified or carbon−carbon-linked bonding pattern of lignins. However, when the aryl ether linkages are cleaved, the corresponding phenolic hydroxyl moieties that are released can readily be detected and determined by quantitative ^{31}P NMR. Therefore, the combination of DFRC with quantitative ^{31}P NMR offers significant potential for the detailed investigation of the bonding patterns that occur within lignin involving β-aryl ether linkages.

For the present study, softwood milled wood lignin (MWL) was used as a sample of an isolated lignin. In addition, the same wood was subjected to kraft pulping and a sample of residual kraft lignin (RKL) was isolated from it (*17*), representing a technical lignin. Both lignins were then subjected to DFRC degradation, and the resulting product mixtures, together with the original lignins, were analyzed by quantitative ^{31}P NMR and GC.

MATERIALS AND METHODS

Materials. Black spruce (*Picea mariana*) MWL was isolated using the classical procedure of Björkman (*18*). A conventional

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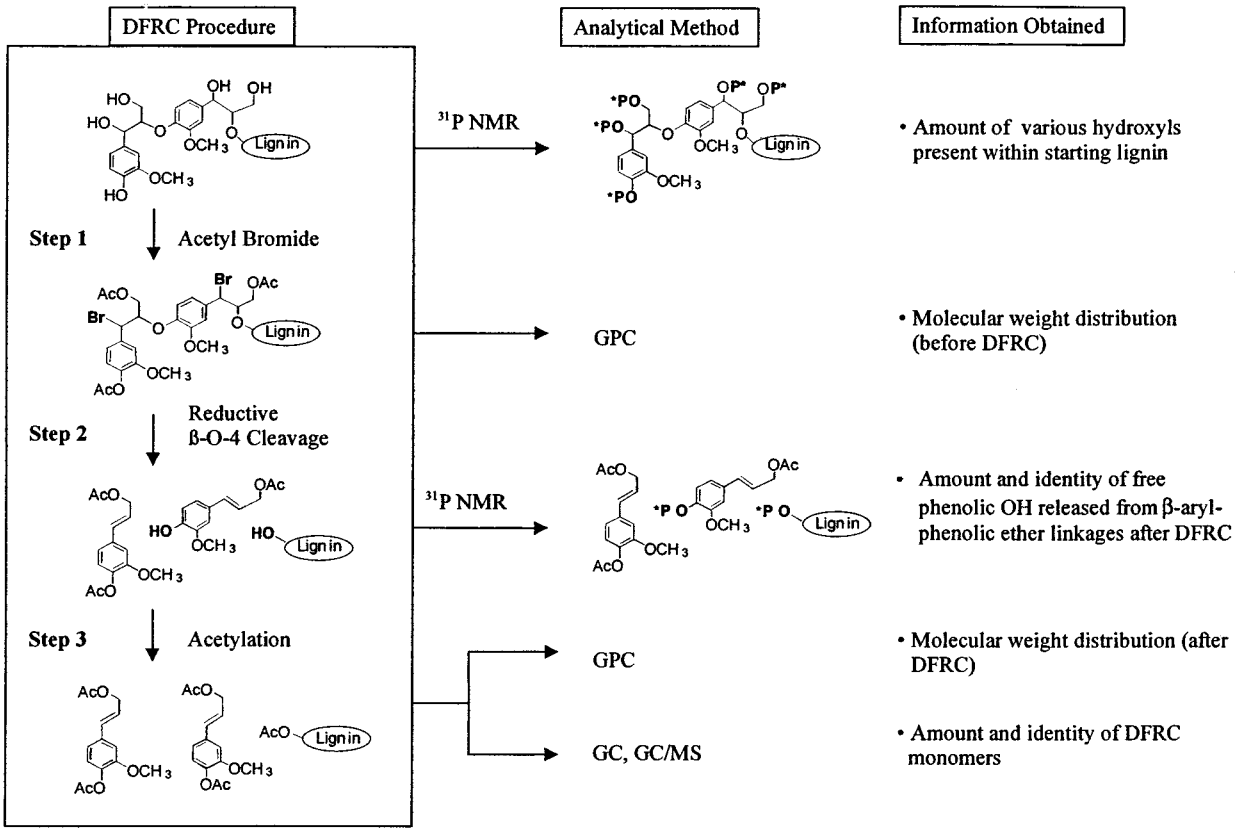
Scheme 1. DFRC Method and Ensuing Analytical Techniques Applied



kraft pulp having a kappa number of ~30 was also prepared using the same wood in a batch laboratory digester. The RKL was isolated from this pulp using acidolysis in a dioxane/water (85:15 v/v) medium in the presence of 0.1 M HCl (*16*, *17*). The Klason plus UV lignin content of the resulting preparation was 93.1%. Both lignins were then washed three times at room temperature with dichloromethane to remove most extractives.

DFRC Procedure. The DFRC procedure was nearly identical to that developed by Lu and Ralph (*8*). However, the precise amounts of the lignins and the reagents used as well as the material flow for subsequent analyses were different (see Scheme 1). Furthermore, due to the subsequent ^{31}P NMR analyses, a number of precautionary steps have been added, which must be adhered to in order to ensure quantitative reliability.

Step 1. Acetyl Bromide Derivatization. Acetyl bromide in acetic acid (1:9 v/v; 25 mL) was added to a lignin sample (100 mg) in a 100 mL round-bottom flask. Care must be taken that no zinc dust residues are present in the flask from a previous workup. This may cause negative errors. The flask was sealed and placed in a water bath, with accurate temperature control, at 50 °C for 3 h with magnetic stirring. The solvent was then rapidly evaporated to dryness under reduced pressure, using a rotary evaporator connected to a vacuum pump and a cold trap. The residue was dissolved in an acidic solution (dioxane/acetic acid/water 5:4:1) and transferred into a 25 mL volumetric flask. One portion of the solution (2.5 mL) was withdrawn and stored at −10 °C for subsequent GPC analyses.

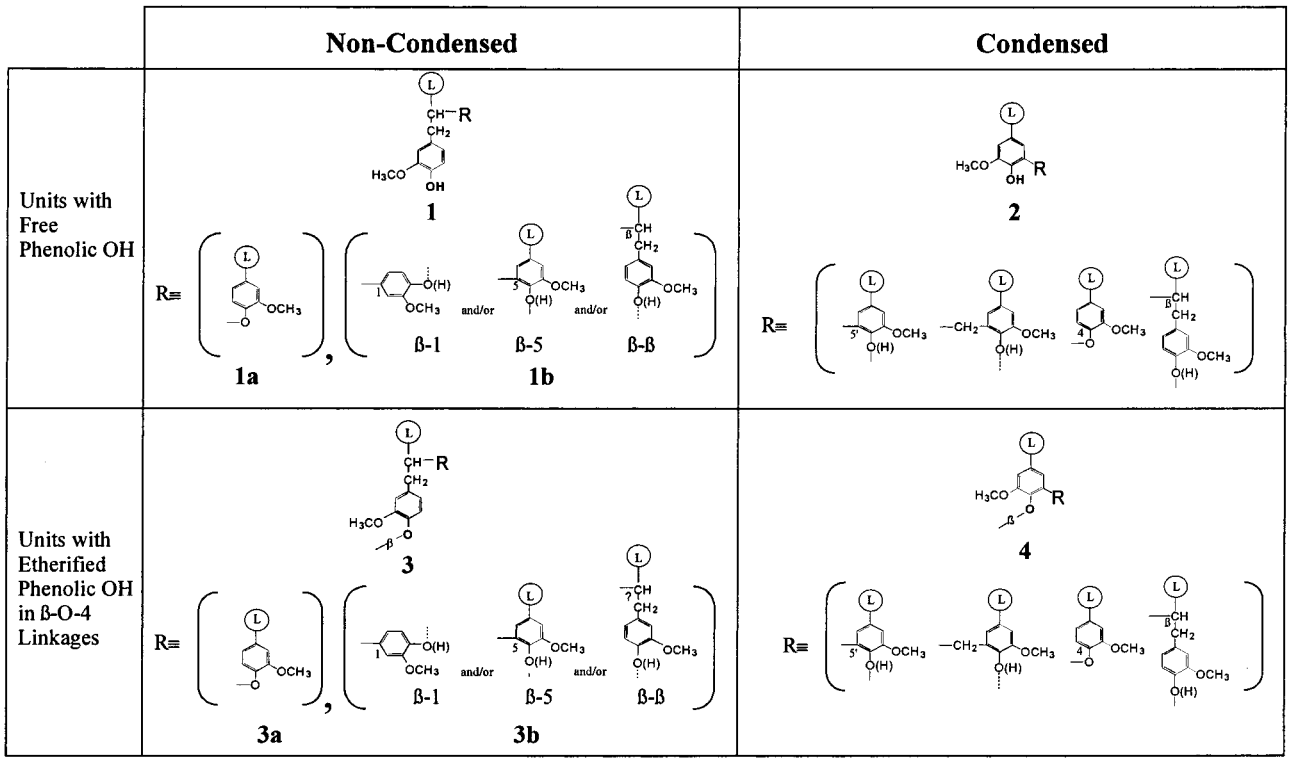
Step 2. Reductive Cleavage. The remaining solution from step 1 was transferred into a 100 mL round-bottom flask. Zinc dust (450 mg) was added, and the mixture was stirred at room temperature for 30 min. The reaction mixture was then quantitatively transferred to a saturated ammonium chloride solution (15 mL) in a separating funnel using high-purity unstabilized (HPLC grade) dichloromethane (15 mL). A solution of internal standards, cholesterol (193.9 mg) and tetracosane (2.7 mg) in high-purity dichloromethane (2 mL), was added for subsequent quantitative ^{31}P NMR and GC analyses, respectively. The aqueous layer was then extracted with dichloromethane (2 × 15 mL). The combined extracts were dried with anhydrous sodium sulfate and then were evaporated to dryness using a rotary evaporator connected to a vacuum pump equipped with a cold trap. Finally, to facilitate the evaporation of acetic acid a dioxane/water (85:15) solution was added, and then the sample was freeze-dried. The dry powder was then dissolved in 25 mL of high-purity dichloromethane in a volumetric flask. A portion of this solution (5 mL) was withdrawn for subsequent acetylation while the remaining solution was completely dried and stored at −10 °C for subsequent quantitative ^{31}P NMR analyses.

Step 3. Acetylation. This step was carried out in accordance with the procedure of Wallis et al. (*19*). The solution withdrawn from step 2 was evaporated and dissolved with acetic anhydride (5.0 mL). *N*-Methylimidazole (0.1 mL) was then added to the stirred solution. After stirring for 10 min at room temperature, the solution was quantitatively transferred into a separating funnel together with water (15 mL) and dichloromethane (20 mL). The dichloromethane layer was separated and dried. It was then used for subsequent gel permeration chromatography (GPC) and GC analyses.

Gel Permeation Chromatography. GPC was carried out on a Waters system at ambient temperature using three Ultrastyragel columns connected in series. Chloroform was the eluent (1 mL/min), and fractions were monitored at 280 nm using a Hewlett-Packard refractive index detector (1047A) and a Waters photodiode array (model 996). All lignins were chromatographed after excess acetyl bromide had been removed by evaporation. The derivatized lignins and the DFRC product mixtures were dissolved in chloroform (~1 mg/mL), and 20 μL of these solutions was injected. The elution volume versus molecular weight calibration curve was constructed using a series of monodisperse polystyrene standards.

Gas Chromatography. The acetylated DFRC product mixture was dissolved in dichloromethane (0.5 mL). An aliquot of this solution (2 μL) was then injected (splitless mode) into a Hewlett-Packard 5979 gas chromatograph equipped with a DB-5 30 m × 0.25 mm silica capillary column. The injection port temperature was 270 °C, and the oven temperature was

Scheme 2. Classification of the Various Phenylpropanoid Units Containing Free and Etherified Phenolic OH and *β-O*-4 Linkages That May Be Present in Softwood MWL and RKL[a]



[a] Moieties 1−4 can be detected by DFRC/^{31}P NMR, whereas the sum of **1a** and **3a** can be determined by GC analysis as the DFRC monomers.

raised from 100 to 280 °C with a gradient of 8 °C/min. A Hewlett-Packard 5972 mass spectrometer was interfaced to the gas chromatograph, and the products were identified by comparing their fragmentation patterns with those of commercially available samples or literature fragmentation patterns (*10*). The evaluation of product ratios was carried out by using tetracosane as the internal GC standard. The GC response factors of the independently synthesized authentic compounds to the internal standard (tetracosane) were determined to be 3.44 for coniferyl diacetate and 2.03 for 4-acetoxycinnamyl acetate.

Quantitative ^{31}P Nuclear Magnetic Resonance. Quantitative ^{31}P NMR spectra of the lignins were obtained using published procedures (*12*−*17*). About 40 mg of dry lignin or dried material after DFRC (already containing the internal standard) was accurately weighed into a 5 mm NMR tube, and the sample was then dissolved in 800 μL of pyridine and deuterated chloroform (1.6:1, v/v). The relaxation reagent, chromium acetylacetonate (27.9 mg), was dissolved in 5 mL of pyridine/chloroform (1.6:1 v/v). An aliquot of this solution (100 μL) was added into the tube. The mixture was then phosphitylated with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (120 μL). The spectra were acquired using a JEOL 270 MHz spectrometer with inverse gated decoupling and a delay time of 25 s between acquisitions. After the DFRC procedure (step 2), an internal standard (cholesterol) was added prior to the final workup. As such, the ratio of internal standard to the amount of the starting lignin sample was known. This was an essential step because the incorporation of bromine in the lignin would alter the initial weight of the sample. Consequently, the integral value of the internal standard (^{31}P signal at 144.8 ppm) was used for the calculation of absolute amount of each functional group (*12*).

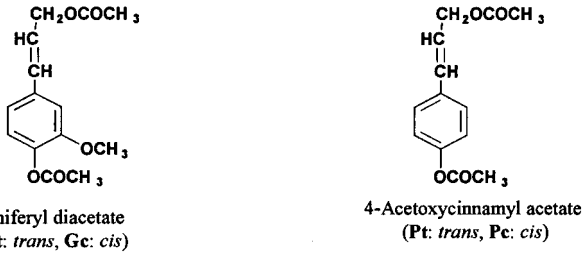
RESULTS AND DISCUSSION

Principles of the Method. An experimental flow diagram of the method used in this study is shown in Scheme 1. The DFRC procedure involving depolymerization of the lignin comprises three steps: (i) acetyl bromide derivatization; (ii) reductive ether linkage cleavage (Zn in acetic acid); and, (iii) acetylation. Lu and Ralph (*21*) have shown that during the acetyl bromide derivatizing step, primary alcohols and phenolic hydroxyls are acetylated, whereas benzylic α-hydroxyls are displaced by bromide and benzyl aryl ethers are quantitatively cleaved to yield aryl acetates and acetylated α-bromide products. During the ensuing reductive cleavage step, α- and *β*-aryl ether linkages are selectively cleaved, quantitatively releasing DFRC monomers (*8*) and various free phenolic hydroxyl groups depending on the linking patterns present in the starting lignins (Scheme 1). The identities of the various phenolic moieties released after the reductive cleavage were revealed by quantitative ^{31}P NMR measurements.

Because quantitative ^{31}P NMR determines the amount of the various hydroxyl groups (aliphatic, phenolic, and carboxyl moieties), such spectra "before DFRC" allowed the amounts of moieties **1** and **2** (Scheme 2) to be determined, whereas the ^{31}P NMR spectra of the sample "after DFRC" provided quantitative information about the free phenolic hydroxyl groups released from the *β*-aryl ether linkages that is, moieties **3** and **4** in Scheme 2. Because all free phenolic OH groups present originally in lignin were acetylated during the acetyl bromide derivatization, all peaks of the phenolic OH region in the ^{31}P NMR spectra after DFRC derived from the units bearing free phenolic OH groups released from *β-O*-4 linkages during the reductive cleavage stage.

The final acetylation step is usually carried out to facilitate subsequent GC-MS and GPC analyses, causing all free phenolic hydroxyl groups to be acetylated. Consequently, the main DFRC products, coniferyl di-

Scheme 3. Main Monomeric Products Quantified after DFRC for the MWL and RKL Samples



acetate and 4-acetoxycinnamyl acetate (Scheme 3), were quantitatively determined using a flame ionization detector (FID); all other minor monomers were identified using a mass spectral detector.

In theory, the main DFRC monomers (Scheme 3) should be produced from the following two structures:

-propyl-G-(4-*O*-β)-**propyl-G-(4-OH)**

(a terminal noncondensed guaiacyl unit with a β-aryl ether bond on one side), or

-propyl-G-(4-*O*-β)-**propyl-G-(4-*O*-β)**-propyl-G-

(an internal noncondensed guaiacyl unit linked via aryl ether bonds to adjacent aryl units), where ≡G is guaiacyl, propyl is a propane side chain, and 4 and β are the linkage sites, respectively.

In the above two structures the units in boldface type are eventually detected as DFRC monomers. Therefore, it is possible to determine the sum of the terminal phenolic moieties by GC analysis as the DFRC monomers, which possess a β-aryl ether on one side (structure **1a**, Scheme 2), and internal etherified phenolic moieties, which possess two β-aryl ethers on both sides (structure **3a**, Scheme 2).

Effect of DFRC on the Molecular Weight Distribution. Figure 1 shows the GPC chromatograms of lignin samples of MWL and RKL before and after DFRC. Prior to DFRC, the molecular weight of the RKL was found to be somewhat higher to that of MWL. This was most likely caused by a small amount of high molecular weight fraction (~10^5 g/mol) that was present in the RKL.

As anticipated, after DFRC the sharp peak responsible for the low molecular weight moieties (at elution volume = 29−30 mL) significantly increased in the MWL and RKL, the effect being more pronounced for MWL. These observations imply that the DFRC procedure indeed caused significant depolymerization of the lignins and that significant amounts of β-*O*-4 linkages are present within RKL. More specifically, after DFRC the amount of monomer present in the sample (determined as the ratio of the monomer ultraviolet signal to the total area of the chromatogram) was 34.5% for MWL, whereas the RKL sample contained ~16.9% of monomer. One should note that these are relative amounts of monomeric products; the monomers, having double bonds, can be assumed to have a stronger absorbance than the higher molecular weight fractions.

Determination of Units Bearing Free Phenolic Hydroxyl Groups (Structures 1 and 2, Scheme 2). The amounts of the various hydroxyl groups determined before DFRC (Table 1) using the described techniques are in reasonable agreement with the values previously obtained for black spruce MWL. Notably, softwood MWL contained at least 50% more phenolic hydroxyl groups belonging to uncondensed guaiacyl structures **1**. The amount of similar moieties linked to condensed structures **2** was 50% less. RKL, however, contained at least 20% more phenolic units belonging to condensed phenolic moieties **2** than noncondensed phenolic guaiacyl units **1**. This corroborates the occurrence of condensation reactions during kraft pulping described earlier (*14*, *20*, *22*).

Determination of Units Bearing Etherified Phenolic Hydroxyl Groups in β-*O*-4 Linkages (Structures 3 and 4, Scheme 2). Phenylpropanoids containing etherified phenolic OH and β-*O*-4 linkages can be a part of noncondensed units, **3**, and of condensed units, **4** (Scheme 2). The precise amounts of these two moieties can be distinguished and determined using the proposed DFRC/^{31}P NMR methodology. The amount of units bearing etherified phenolic OH in β-*O*-4 linkages, linked to noncondensed moieties **3**, was determined from the "after DFRC" ^{31}P NMR spectra (Figure 2) by integrating the appropriate region shown in Table 1. Furthermore, the amount of a phenylpropanoid units containing etherified phenolic OH with β-*O*-4 units linked to condensed moieties **4** was determined by integrating the region 144.5−140.5 ppm, in the "after DFRC" ^{31}P NMR spectra.

As described earlier, DFRC cleaved β-*O*-4 linkages, resulting in the formation of an equimolar amount of "new" phenolic hydroxyl groups that can be determined by quantitative ^{31}P NMR. Consequently, the amount of

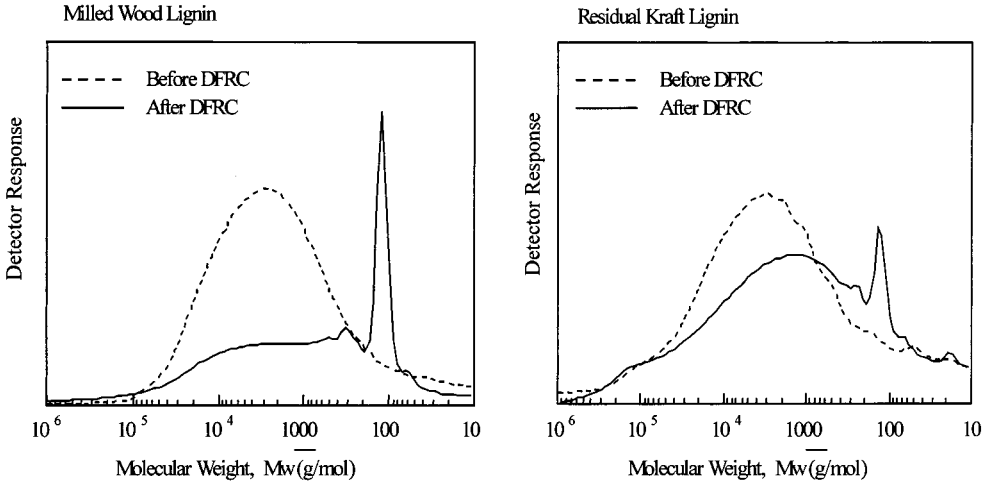


Figure 1. GPC chromatograms of lignin samples before and after DFRC.

Table 1. Values of Hydroxyl Moieties Determined by DFRC/^{31}P NMR Analysis (Millimoles per Gram)

		phenolic OH				
			noncondensed phenolic OH			
	aliphatic OH	condensed phenolic OH[a]	guaiacyl OH[a]	*p*-hydroxy-phenyl OH	total phenolic OH	−COOH
integrated chemical shift range (ppm):	149−146	144.5−140.5	140.5−139	137.5−138.5		135.5−134.5
MWL						
before DFRC	4.21	0.50(2)	0.76(1)	0.08	1.34	0.15
after DFRC	0.94	0.53(4)	1.09(3)	0.00	1.62	0.17
RKL						
before DFRC	1.82	1.23(2)	0.99(1)	0.21	2.43	0.29
after DFRC	0.92	0.33(4)	0.28(3)	0.00	0.61	0.34

[a] Superscripts (1), (2), (3), and (4) correspond to the structures **1**, **2**, **3**, and **4** shown in Scheme 2, respectively.

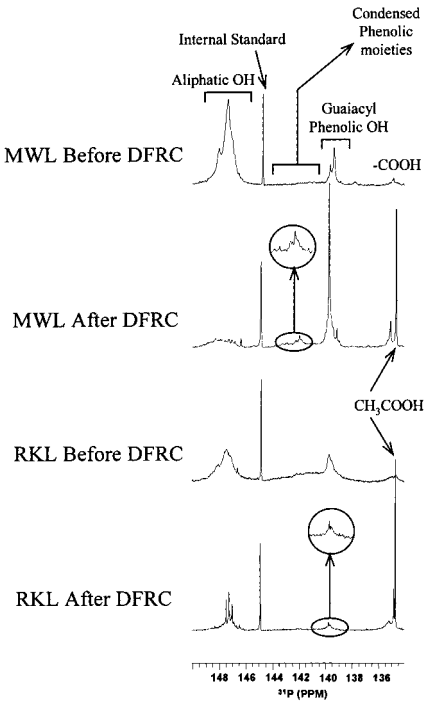


Figure 2. Quantitative ^{31}P NMR spectra of MWL and RKL before and after DFRC.

phenolic hydroxyl groups determined after DFRC (Table 1) is equivalent to the amount of β-aryl ether structures present in a given sample. Furthermore, the combination of DFRC with ^{31}P NMR distinguishes condensed phenolics from noncondensed β-*O*-4 linkages and the *p*-hydroxyl phenyl moieties.

As anticipated, after DFRC the amount of phenolic groups increased significantly for all samples examined (Figure 2). For MWL, the total amount of β-aryl ether linkages that the liberated phenolic −OH groups represents (sum of noncondensed and condensed) was 1.62 mmol/g. This figure is equivalent to 30.6 per 100 C9 units, based on an assumed C9 molecular weight of 187 g/mol for native softwood lignin. The amount of noncondensed β-aryl ether linkages, **3**, was nearly double that of condensed moieties, **4**, indicating that about two-thirds of the etherified phenolic moieties in β-aryl ether structures present in MWL are noncondensed units connected to another phenylpropane unit bearing β-*O*-4, β-1, β-5, and β-β linkages. One-third of the etherified phenolic moieties in the β-aryl ether structures of softwood MWL contained a substituent group ortho to the phenolic hydroxyl (i.e., the C5 position was substituted), possibly 5-5′, 5-*O*-4, and/or 5-β linkages, with the majority being dibenzodioxocins. Dibenzodioxocins can also be determined using DFRC/^{31}P NMR by integrating the region responsible for 5-5′ liberated phenols (141.2−142.5 ppm). More details of this determination are to be found in subsequent publications.

It was originally thought that within RKL, most β-aryl ether linkages were cleaved during the kraft pulping process. However, in recent years a number of workers have reported that a significant amount of such structures remains unaltered within isolated RKL (*16*, *17*, *23*, *24*). The proposed DFRC/^{31}P NMR method revealed that the total amount of β-aryl ethers present in the RKL isolated from the end of a conventional kraft cook was 0.61 mmol/g, or ∼11 per 100 C9 units. It is also likely that this value may be an underestimate, because the pulp sample was subjected to acidolysis for the purpose of isolating the residual lignin, thus cleaving some β-*O*-4 structures (*4*, *25*).

After DFRC, a ^{31}P NMR signal centered at ∼140.7 ppm appeared for both RKL and MWL (Figure 2). With appropriate corrections for the substituent effects of *o*-OCH_3 substituted phenols (*15*), we attribute this signal to biphenyl-type phenylpropane dimeric phenols bearing various side-chain substituents. Such moieties are calculated to give rise to ^{31}P NMR chemical shifts at ∼140.5−142.0 ppm. The precise identification of these moieties has been the subject of detailed studies in our laboratory, and it has been confirmed that the liberated phenolic OH after DFRC bears a 5-5′ biphenyl carbon−carbon linking pattern connected with the presence of dibenzodioxocins (*26*). This information provided an estimate for dibenzodioxocins in softwood MWL ∼12−13 per 100 C9 units.

Determination of the Primary Degradation Product Monomers (Structures 1a and 3a, Scheme 2). After DFRC, the primary degradation monomers (Scheme 3) were detected and quantified using GC-MS (coniferyl diacetate) and GC-FID (4-acetoxycinnamyl acetate). These monomers are derived from the following structures:

-propyl-G-(4-*O*- β)-**propyl-G-(4-OH)**
(**1a**)

and

-propyl-G-(4-*O*-β)-**propyl-G-(4-*O*-β)**-propyl-G-
(**3a**)

Figure 3 shows the GC-FID chromatogram of the DFRC products of MWL and RKL. The chromatogram obtained from the DFRC degradation of MWL was very similar to that obtained by Lu and Ralph (*10*), and the main monomers, **Gc**, **Gt**, **Pc**, and **Pt**, were determined

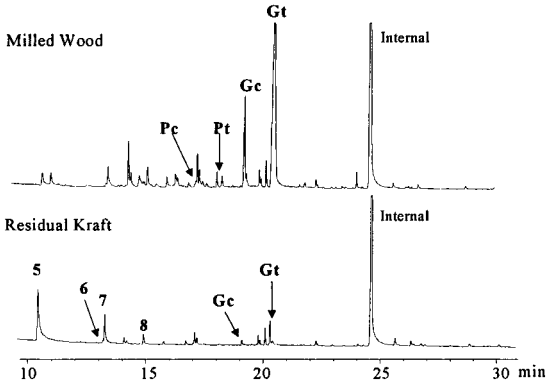


Figure 3. GC-FID chromatograms of DFRC monomers derived from black spruce MWL and RKL samples (for signal identity see Scheme 3). Unit names of **5**−**8** originate from their respective end-group (*10*).

Table 2. Yields (Micromoles per Gram) of the Main DFRC Monomers by GC Analysis[a]

sample	G monomer[b]	P monomer[c]	total
MWL	619	18	637
RKL	21	0	21

[a] Error ±2%. [b] G monomer, coniferyl diacetate. [c] P monomer, 4-acetyoxycinnamyl acetate.

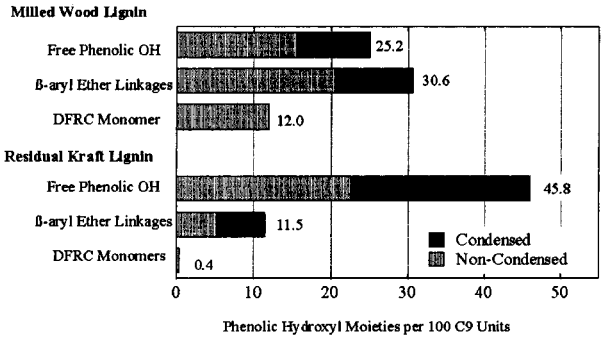
by integrating the corresponding peak areas. Individual yields (millimoles per gram) were then calculated from peak integrals, taking into account the presence of the internal standard and the GC-FID response factor for each compound.

The yield of the main DFRC monomer (**G**) resulting from RKL was 21 mmol/g (Table 2). This is considerably smaller than that obtained from the corresponding MWL, 637 mmol/g, which is in good agreement with the value of 651 μmol/g obtained by Lu and Ralph (*11*) for pine MWL (This demonstrates that almost all end groups of MWL connected to β-aryl ethers and all etherified phenolic OH groups connected via β-aryl ethers were cleaved during kraft pulping.) Despite the significantly reduced amount of β-aryl ethers in the RKL sample, phenylpropanoid structures such as **1a** and/or **3a** survive kraft pulping and are enriched in the RKL.

In addition to the main monomers, >20 minor monomers derived from a variety of end groups, including those containing α-carbonyl groups, were also identified in the MWL after DFRC in agreement with Lu and Ralph (*10*). In contrast, the RKL yielded only four of these minor compounds: peaks 5, 6, and 7 (Figure 3), originating from benzyl alcohol, benzaldehyde, and eugenol end groups, respectively, and peak 8, originating from guaiacyl α-carbonyl β-ether units (*10*). Although the presence of such minor moieties in RKL is not surprising, the data imply that kraft pulping cannot effectively cleave all β-*O*-4 linkages. This is shown by the small signals in the chromatogram of the DFRC products of the RKL (Figure 3). The two weak signals that appear between 25 and 30 min are most likely due to dimeric products. Their fragmentation patterns were characteristic of 5-5′ biphenolic moieties that could be correlated to the 5-5′ biphenyl structures observed in the ^{31}P NMR spectra after DFRC. Work to elucidate these structures is underway.

Relative Quantities of Free Phenolic Hydroxyl Moieties. Scheme 4 compares the data obtained for the various moieties determined by ^{31}P NMR, DFRC/^{31}P NMR, and DFRC/GC analyses. The number of units (expressed per 100 C9 units) bearing free phenolic OH groups (sum of structures **1** and **2)** was found to be 25.2 for MWL and 45.8 for RKL. It is also of interest to note that the frequency of units bearing free phenolic OH groups attached to noncondensed moieties **1** is higher in RKL than in MWL. Similarly, the amount of units bearing free phenolic OH groups attached to condensed structures **2** was 2.5 times higher in the RKL compared to MWL.

Scheme 4. Values of Phenolic Hydroxyl Moieties Determined by DFRC/^{31}P NMR[a]



[a] The amount of coniferyl diacetate monomer (Scheme 3) determined by GC resulted in an estimate of structures **1a** and **3a**.

It is remarkable that a significant amount (11.5 per 100 C9 units) of β-aryl ether linkages survive kraft pulping. Our data indicate that β-aryl ether linkages that participate in condensed structures (**3b** and **4**) are more stable under kraft pulping conditions than those connected to noncondensed moieties. The DFRC/GC-MS analytical data also support this notion, because the sum of β-etherified phenolic moieties connected to another β-aryl ether and the free phenolic OH (DFRC monomers: **1a** and **3a**) was much more abundant in the MWL than in the RKL.

The combination of DFRC and quantitative ^{31}P NMR is a useful procedure for the determination of the absolute amounts of β-aryl ether linkages in milled wood and technical lignins. In addition, the method can distinguish the amount of β-aryl ether linkages connected to condensed and noncondensed moieties present in lignins. β-Aryl ether linkages connected to condensed structures were significantly more abbundant within RKL than in MWL (Scheme 4). Most of the β-aryl ethers connected to noncondensed units and/or other terminal units were not observed in the DFRC/GC analyses of the RKL. The connectivity patterns within RKL are being further investigated using DFRC followed by LC-MS techniques.

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