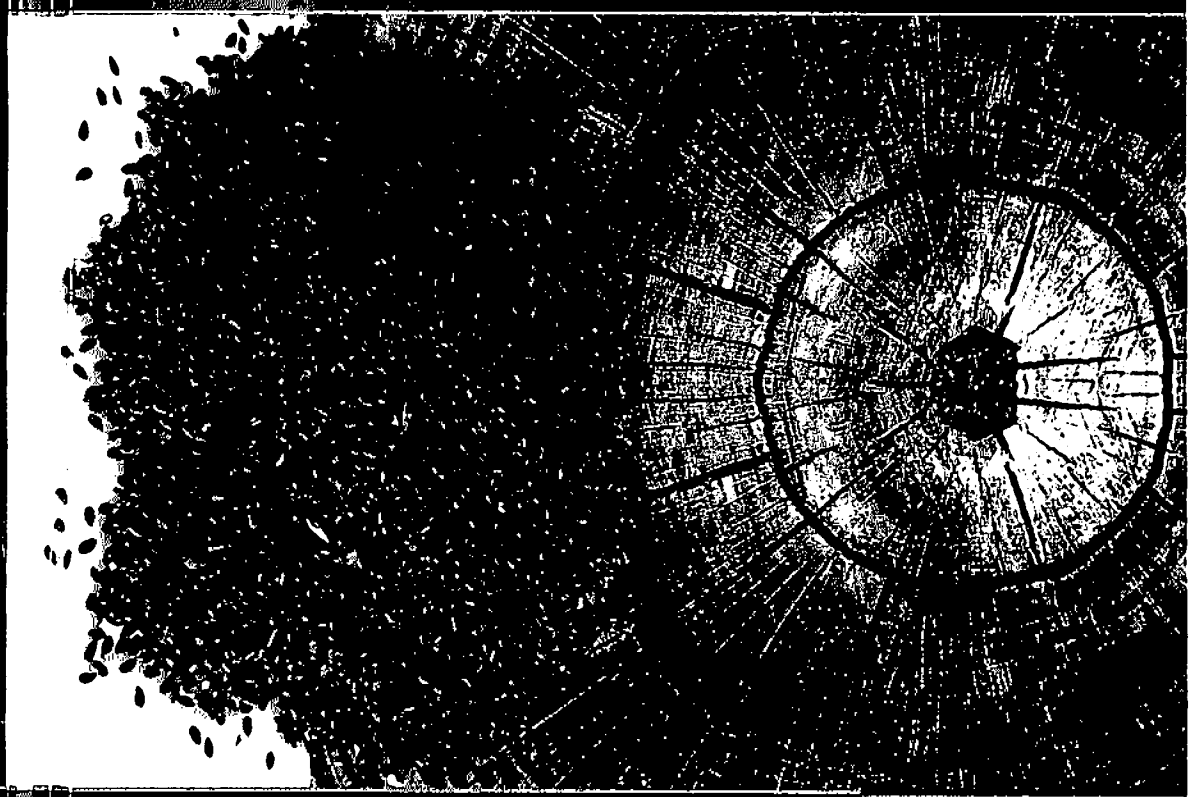
Lignin and Lignans

Advances in Chemistry



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6 Heteronuclear NMR Spectroscopy of Lignins

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CONTENTS

INTRODUCTION

Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for examining lignin structure. Earlier chapters covered NMR techniques that involve viewing proton (H) and carbon-13 (C-13) nuclei, which are naturally present in lignin. Additional structural information can be gained by applying derivatization procedures that covalently link other NMR active nuclei to lignin and observing the resulting NMR spectrum of that nuclei.

Important considerations in selecting NMR-active nuclei for labeling functional groups in lignins are the sensitivity of the nuclei in an NMR experiment, the availability of suitable derivatizing reagents, and the ease of obtaining quantitative derivatization under mild conditions. Several heteronuclear NMR cases are discussed in this chapter, with primary emphasis given to the most informative one, phosphorus-31 NMR.

SILICON-29 NMR

Trimethylsilylation of hydroxyl groups has long been used to facilitate gas chromatographic separation. Its use to characterize the labile protons in lignin was not explored until the late eighties.

The application of silicon-29 (^{29}Si) NMR spectra to trimethylsilylated lignin allows aromatic, aliphatic and carboxylic acid hydroxyl groups to be distinguished

[1,2]. Silylation can be used after methylation with diazomethane (to eliminate signal overlap with phenolic groups) to detect different alcoholic groups in kraft lignin and humic acids [3]. In theory, the observation of the ^{29}Si nuclei (natural abundance of 4.7%) is twice as sensitive as a comparable ^{13}C experiment; the negative gyromagnetic ratio and rather long spin-lattice relaxation times of this technique, however, cause signal reduction when proton decoupled spectra are acquired. Consequently, the acquisition of ^{29}Si NMR spectra requires high sample concentrations and long delay times. Alternatively, the ^{29}Si NMR signals can be considerably enhanced using the INEPT (Insensitive Nuclei Enhanced by Polarization Transfer) sequence, but this poses major limitations in quantitatively interpreting the spectra [4].

NITROGEN-15 NMR

Nitrogen-15 (^{15}N) NMR spectroscopy has found only limited utility in lignin chemistry due to difficulties with recording ^{15}N NMR spectra.

The low natural abundance (0.365%) of the ^{15}N isotope dictates a low sensitivity for the method. The negative magnetic moment of the ^{15}N nucleus results in a negative Nuclear Overhauser Effect (nOe), which can lead to complete disappearance of the ^{15}N NMR signal under incorrectly chosen acquisition conditions. The significant variation in the relaxation time of ^{15}N atoms with different chemical environments may also result in the elimination of certain signals in ^{15}N NMR spectra. These factors limit the applicability of this technique to the analysis of nitrogen-incorporated lignins when functional group detection of the nitrogen-containing moieties is required. ^{15}N NMR spectroscopy in solution has been applied to the study N-containing products after oxidative ammonolysis of lignin model compounds, DHP, and Organocell lignin [5]. ^{15}N-enriched NH_3 was used in the ammonolysis reaction to ensure sensitive and reliable detection of ^{15}N NMR signals. The formation of formamide, acetamide, substituted benzamides, and urea was detected by ^{15}N NMR spectroscopy.

A possible application of ^{15}N NMR spectroscopy to lignin analysis is the study of lignin biodegradation products. The incorporation of nitrogen-containing functional groups can be detected and identified by this technique. Kniker et al. analyzed sulphonated lignin and organosolv lignin incubated with ^{15}N-enriched ammonium sulfate for 600 days to demonstrate the influence of ammonia fertilizer on pathways of microbial decomposition of lignin [6]. The introduction of amide-peptide structures into lignin was detected. Kögel-Knabner et al. [7] provided more examples of using ^{15}N NMR spectroscopy for the structural analysis of lignin-related humic substances.

MERCURY-199 AND TIN-119 NMR

The selective mono-mercuration of aromatic rings with mercury acetate followed by ^{199}Hg NMR spectroscopic analysis has been used to elucidate the aromatic substitution patterns in lignins [8]. Direct and indirect (HMQC 2D ^{199}Hg-^{1}H spectra) ^{199}Hg NMR spectra of the formed derivatives were recorded; $J(^{199}Hg, ^{1}H)$ coupling constants provided information about the position of the mercury

substitution on the aromatic ring. Mercury acetate was found to introduce Hg at the C-5 position of guaiacyl lignin model compounds and C-2 position of syringyl compounds.

Tin-119 (^{119}Sn) NMR spectroscopy has been used to characterize bis(tri-butyltin) oxide derivatives of various lignin-related phenols and lignin-containing material isolated from spent bleach liquors [9]. Signals of the model derivatives were well resolved, but the broad signals displayed by actual spent liquors limited the utility of the spectra.

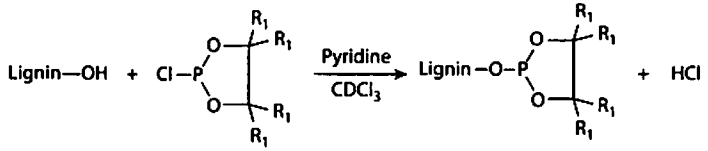
FLUORINE-19 NMR

Florine-19 (^{19}F) NMR spectroscopy has also been explored for determining various hydroxyl groups in lignin; the OH groups are first converted into the corresponding fluorobenzoic acid alkyl and aryl esters and/or flurobenzyl ethers [10] under phase transfer catalysis conditions. While relatively small amounts of lignin sample (100 mg) and short acquisition protocols (one hour) are required for this technique, the method suffers from a somewhat cumbersome workup procedure that could introduce complexities in quantification and an inability to distinguish between primary and secondary hydroxyl groups. Despite these limitations, this approach has been applied successfully for determining the total phenolic and carboxylic acid groups in oxidized [11] and residual [12] lignins using 2-fluorobenzoic acid as an internal standard.

Another promising application of ^{19}F NMR spectroscopy, reported by Sevillano et al. [13], is the use of trifluoromethyl and trifluoromethoxyphenylhydrazine for the quantitative detection of carbonyl groups in lignin. The reliability of the technique, however, depends on the efficiency and the quality of the purification steps required after derivatization. A new method, capable of quantitatively detecting different classes of carbonyl groups for a series of model compounds [14] and lignins [15], employs a selective fluoride-induced trifluoromethylation of carbonyl groups (using trifluorotrimethylsilane), followed by ^{19}F NMR spectral analysis of the derivatives. These studies have shown that the trifluoromethyl ^{19}F-NMR chemical shifts vary significantly and consistently for derivatives of various aldehydes, ketones and quinones that may be present in complex lignocellulosic materials.

PHOSPHORUS-31 NMR

The use of phosphorus-containing derivatizing reagents for lignin analysis has grown in importance. Phosphorus-31 is a nucleus that is 100% naturally abundant. The sensitivity of a ^{31}P NMR experiment is about 15 times less than that of a proton NMR experiment. The range of ^{31}P chemical shifts is more than 1000 ppm for a variety of phosphorus compounds and the average line width is about 0.7 Hz [16]. Various types of organophosphorus compounds give signals within narrow ranges, characteristic of the oxidation state of the phosphorus nuclei. Furthermore, relationships have been identified between phosphorus chemical shifts and structure that in some instances even reveal stereochemical information [17,18]. All of these factors make phosphorus an ideal reporter group for NMR studies of labile



SCHEME 6.1 The phosphitylation reaction of labile protons in model compounds and lignins with reagents I (R_1 = H, 2-chloro-1,3,2-dioxaphospholane) and II (R_1 = CH_3, 2-chloro-4,4,5,5-tetra-methyl-1,3,2-dioxaphospholane) (Based on Lucas, H. J., Mitchell, F. W., and Scully, C. N., *J. Am. Chem. Soc.*, 72, 5491, 1950.)

groups in lignin. The reactions of various phospholane chlorides with labile centers present in coal samples were investigated by Verkade's group in the mid 1980's [19–23].

Early work focused on the phospholanes produced after the derivatization of OH groups with 1,3,2-dioxaphospolanyl chloride (I), Scheme 6.1 [24,25,27,28]:

A detailed review of the advantages and limitations of this form of spectroscopy has been published [24]. In part I of series of papers *^{31}P NMR in Wood Chemistry*, we evaluated this technique's potential by investigating a large variety of model compounds with structures likely to occur in lignins [25]. This research showed that this technique could distinguish not only most forms of phenolic hydroxyls (ArOH) but also primary and secondary aliphatic hydroxyls (R^1-OH and R^2-OH) and *erythro-* and *threo-*forms of β–O–4-structures [25]. Supporting evidence for the latter assignment was sought by using the lignin alkylation studies of Adler et al. [26]. Methylation of black spruce milled wood lignin resulted in the complete elimination of the two broad signals at 135.0 and 134.2 ppm attributed to the *erythro-* and *threo-*forms, respectively, of the phosphitylated *alpha-*hydroxyl groups in β–O–4 structures [27]. Early observations revealed a slight concentration-dependence of the ^{31}P NMR chemical shifts among a variety of phosphorus derivatives. The effect was explored to better understand the behavior of the signals from derivatized lignins [28]. Since chloroform and pyridine are used as solvents for the derivatization reaction, their role was examined separately. The resulting knowledge permitted spectra with greater resolution to be obtained and facilitated the assignment of ^{31}P signals in lignins (Table 6.1).

The derivatization of lignins with reagent I allowed visualization of the overall distribution of hydroxyl groups. However, signal overlap between the syringyl phenolic structures and those belonging to condensed phenolic groups limited its capacity for distinction and accurate determination of these moieties. Another phosphitylation reagent, 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (II, Scheme 6.1), was developed; it was found to be particularly good at resolving this region at the expense of fine resolution between the primary and secondary hydroxyls [29]. The different patterns of ^{31}P NMR spectra of ball milled cottonwood lignin phosphitylated with reagents I and II are illustrated in Figure 6.1.

The ^{31}P NMR signals, after use of reagent II, were well resolved for the free phenolic hydroxyls belonging to guaiacyl, syringyl, *p*-hydroxyphenyl units and most C5 and C6 related condensed phenolic forms. In addition, signals due to carboxylic

TABLE 6.1
^{31}P NMR Chemical Shifts Ranges of Various Functionalities in Lignins after Derivatization with Reagent I

Chemical Shift, ppm	Lignin Functionality
136.5–135.8	OH group in xylan
136.8–135.2	*Erythro* α-OH in β-O-4 syringyl units
135.2–135.4	*Erythro* α-OH in β-O-4 guaiacyl units
134.5–133.7	*Threo* α-OH in β-O-4 syringyl and guaiacyl units
133.7–133.2	γ-OH in α-carbonyl containing units, cinnamyl alcohols
133.2–132.7	γ-OH in β-O-4 units
132.7–132.1	Primary OH (probably phenylcoumaran type)
132.1–131.6	Phenolic OH in syringyl units
131.6–131.0	Phenolic OH in biphenyl units, cinnamyl aldehydes
130.4–129.7	Phenolic OH in guaiacyl units
129.7–129.3	Phenolic OH in guaiacyl units and catechol structures
127.1–126.5	COOH groups in aliphatic acids and cinnamic acids

Source: Based on Argyropoulos, D. S., Bolker, H. I., Heitner, C., and Archipov, Y., *J. Wood Chem. Technol.*, 13(2), 187–212, 1993.

acids were well separated from all other signals, allowing direct access to this important information related to the fundamental changes occurring within lignins under oxidative conditions [30]. The signal assignments for reagent II with lignin are given in Table 6.2. The structure/chemical shift relations of phosphitylated phenols of more than sixty lignin model compounds were explored using Hammett principles. This provided a set of empirical parameters that permits the accurate prediction of ^{31}P NMR chemical shifts of lignin-related phenolic compounds derivatized with II [31].

The ^{31}P spin-lattice relaxation behavior of phosphitylated lignins has also been investigated [28]. Further work, using two-dimensional ^{31}P NMR spectroscopic techniques, clarified the assignments of one-dimensional ^{31}P NMR spectra of lignins [32]. This research confirmed the absence of through-bond and through-space ^{31}P-^{31}P and ^{31}P-^{1}H couplings from within a ^{31}P NMR spectrum of phosphitylated lignins. Detailed measurements of the phosphorus spin-lattice and spin-spin relaxation times at various static magnetic fields and temperatures found that the predominant spin relaxation mechanism of phosphorus in phosphitylated lignins was due to chemical shift anisotropy [32]. This background information was then used to design an experimental protocol for obtaining quantitative ^{31}P NMR spectra of phosphitylated lignins [33]. The quantitative reliability of this methodology was verified by an international round-robin effort designed to validate analytical techniques; ^{31}P NMR data were compared to amminolysis [36] and permanganate oxidation [36,37] of lignin standards [34,35].

Recently, cellulose was dissolved in a variety of imidazolium chloride-based ionic liquids (IL's) bearing a series of substituents, which imparted varying degrees

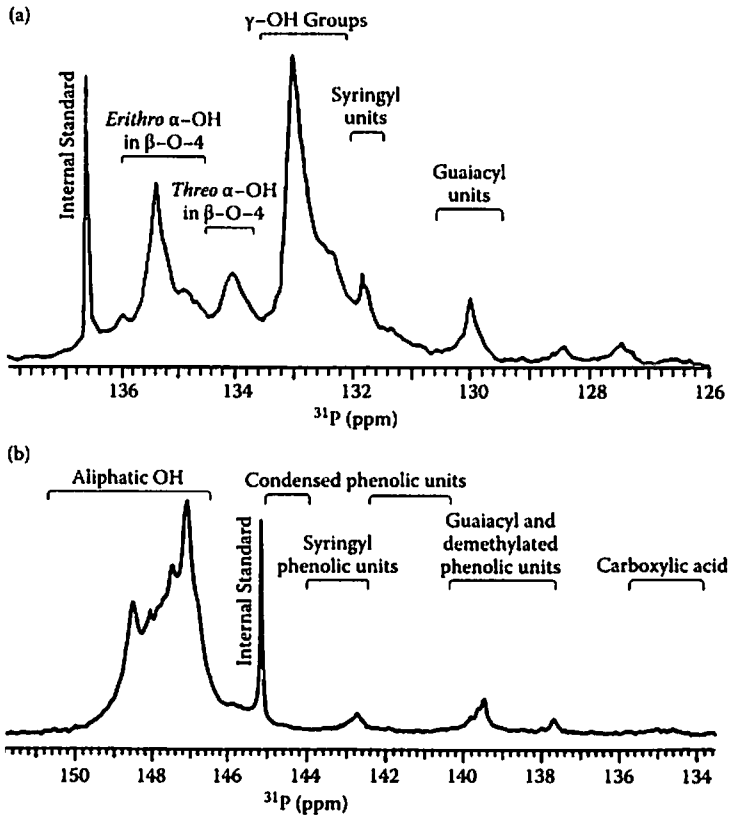


FIGURE 6.1 ^{31}P NMR spectra and assignments for ball milled cottonwood lignin phosphitylated with reagent I (a) and reagent II (b) ((a) Based on Argyropoulos, D. S., Bolker, H. I., Heitner, C., and Archipov, Y., *J. Wood Chem. Technol.*, 13(2), 187–212, 1993; Argyropoulos, D. S., *J. Wood Chem. Technol.*, 14(1), 65–82, 1994. (b) Based on Granata, A. and Argyropoulos, D. S., *J. Agric. Food Chem.*, 43(6), 1538–1544, 1995.)

of hydrophobicity [75]. While in solution, the cellulose was esterified with the hydrophobic phosphitylation reagent 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (II), inducing phase separation. Ionic liquids bearing hydrophobic substituents were found to create environments that reduced the phase separation. A similar, but less pronounced phase separation, was also observed during the phosphitylation of isolated enzymatic mild acidolysis lignin (EMAL) (74a–c), which is the major non-polysaccharide constituent of wood. A careful choice of IL and cosolvent addition resulted in homogenization and the advancement of quantitative ^{31}P NMR analysis procedures on lignocellulosics. Ultimately, this methodology provided a means of probing the structure of lignin on the fiber, without its prior isolation; the whole wood cell wall is made soluble in ionic liquids [76], allowing for the acquisition of detailed quantitative ^{31}P NMR spectra [75].

TABLE 6.2
^{31}P NMR Chemical Shifts Ranges of Various Functionalities in Lignins after Derivatization with Reagent II

Chemical Shift, ppm	Lignin Functionality
150.8–146.3	Aliphatic OH group
144.3–142.8	Condensed phenolic units: diphenylmethane type
143.7–142.2	Syringyl phenolic units
142.8–141.7	Condensed phenolic units: 4-O-5' type
141.7–140.2	Condensed phenolic units: 5-5' type
140.2–138.4	Guaiacyl and demethylated phenolic units
138.6–136.9	*p*-Hydroxyphenolic units
135.6–133.7	Carboxylic acids

Source: Argyropoulos, D. S., *Research on Chemical Intermediates*, 21(3–5), 373–395, 1995; Argyropoulos, D. S., Bolker, H. I., Heitner, C., and Archipov, Y., *J. Wood Chem. Technol.*, 13(2), 187–212, 1993.

APPLICATIONS OF QUANTITATIVE ^{31}P NMR

Early efforts to apply quantitative ^{31}P NMR were centered around quantitatively determining ArOH groups and the two diastereomeric forms of the α–OH in β–O–4 linkages in lignins [38]. The technique was applied to fractionated guaiacyl (G) and guaiacyl/syringyl (GS) DHPs prepared by continuous ("Zutropf," ZT) and discontinuous ("Zulauf," ZL) dehydrogenation schemes. Comparison of the data to that of milled wood lignin samples from softwood and hardwood species revealed that GS-DHPs resemble GS milled wood lignins to a greater extent than G milled wood lignins. The total phenolic OH contents of ZT-DHPs were always lower than those of ZL-DHPs, which is in agreement with the theory that predicts more β–O–4-linkages in the ZT-DHPs. Secondary aliphatic-OH groups in G-DHPs were extremely low. This underlies again the principal differences of G-DHPs compared to GS-DHPs and MWLs. The *erythro:threo* ratios of the G-type samples were found to vary between 1 and 1.5, indicating only minor dependence on molar mass or mode of preparation. The *erythro:threo* ratio in GS-type samples was found to vary from 1.6 to 4.3, showing the highest value for low molar mass ZL-DHPs and lowest values for the cherry tree MWL. A significant amount of the quantitative functional group data collected for both types of synthetic lignins was later found to correlate well with the results of a "Lignin" computer simulation model developed by Jurasek [39].

Early spectroscopic evidence indicated that quantitative solution-state ^{31}P NMR could be used to follow and quantify the formation of stable carbon-carbon and carbon–oxygen bonds during kraft pulping. The use of reagent II, with four bulky methyl groups on the glycol bridge of the dioxaphospholane (Scheme 6.1), dramatically increased the resolution of the ^{31}P spectra in the region responsible for the uncondensed and condensed phenolic structures (Figure 6.2a).

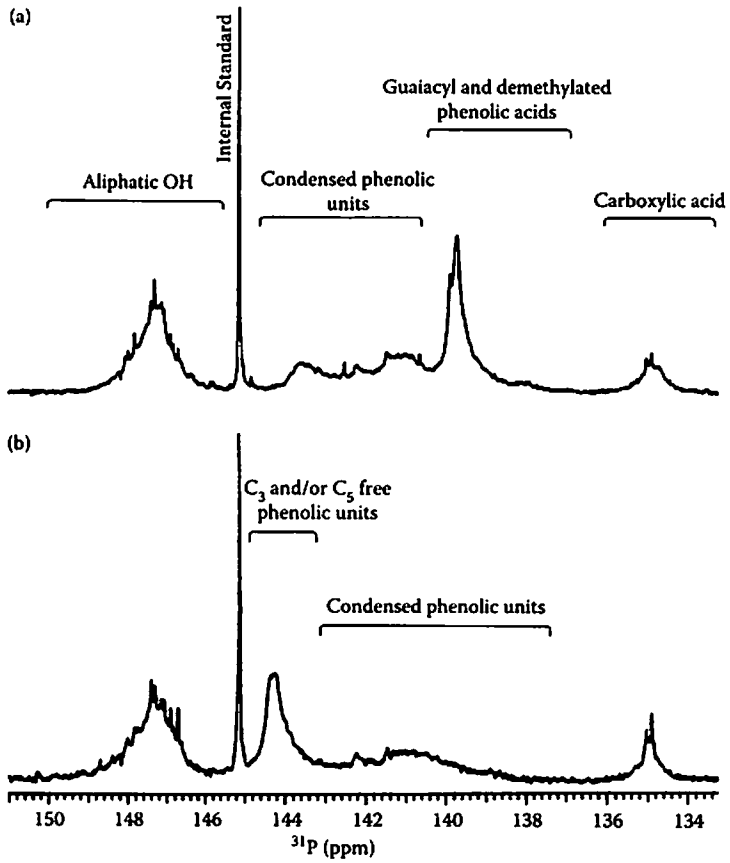


FIGURE 6.2 Quantitative ^{31}P NMR spectra and region assignments of (a) a kraft solubilized black spruce (*Picea mariana*) lignin sample and (b) its Mannich product (Jiang, Z. and Argyropoulos, D. S., *Can J. Chem.*, 76(5), 612–622, 1998.)

The phenolic hydroxyl groups belonging to condensed structures could be quantified by integrating the region between 140.3–144.4 ppm in the spectrum of Figure 6.2a. However, no information about condensed units lacking free phenolics can be obtained by this method. A significant amount of condensed structures was found to accumulate within solubilized kraft lignins at about 16% delignification. These species were found to further accumulate at subsequent levels of delignification [29].

To better understand the nature of the condensed structures in lignins, researchers examined the application of the Mannich reaction to probe these structures [40]. The Mannich reaction was applied to a large variety of lignin-model compounds to selectively and quantitatively block the available aromatic C3 and C5 positions [40]. Quantitative ^{31}P NMR provided a determination of the units that bear no substituents at the aromatic C3 and/or C5 positions.

Smit et al. combined thioacidolysis and phosphitylation with reagent II, followed by ^{31}P NMR spectroscopy, to measure the total amount of condensed and uncondensed units in wood and lignin [41]. Application of the method to *Picea radiata* wood and milled wood lignin showed that approximately 77% of the C9 units in the milled wood lignin and 71% of the C9 units in wood could be quantified. The amount of condensed structures/C9 units in MWL was determined to be: as high as 8% β–5, 5% 4–O–5, and 16% 5–5 structures.

Tohmura and Argyropoulos have developed an analytical method for lignins that involves the combination of DFRC with quantitative ^{31}P NMR [45a]. More specifically the methodology involves: (a) derivatization followed by reductive cleavage (DFRC) [42–44], (b) depolymerization, and (c) quantitative ^{31}P NMR spectroscopy [45]. This technique was shown to detect and quantify the various ether linkages present in softwood residual kraft and milled wood lignins. In addition, the technique supplied new quantitative information about β-aryl ethers linked to condensed and noncondensed aromatic moieties, including dibenzodioxocins. Within residual kraft lignin, 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 GC was very small in the residual kraft lignin, but large in the milled wood lignin. 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. The same approach was used latter by the same research group to define the abundance of dibenzodioxocin moieties in lignin [45b].

Recently, the Argyropoulos group has applied the combination of DFRC with quantitative ^{31}P NMR to determination the arylglycerol-β-aryl ether linkages in enzymatic mild acidolysis lignins (EMAL) [74a–c] and further compare the DFRC/^{31}P NMR protocol with thioacidolysis [45c]. More specifically, enzymatic mild acidolysis lignins [74a–c], isolated from different species of softwood and *Eucalyptus globules*, were submitted to comparative analysis that included thioacidolysis, DFRC, and DFRC followed by quantitative ^{31}P NMR (DFRC/^{31}P NMR) [45a]. Gas chromatography (GC) was used to determine the monomer yields from both thioacidolysis and DFRC; ^{31}P NMR was used to quantified the various phenolic hydroxyl groups released by DFRC [45a]. The monomer yields from thioacidolysis and DFRC were substantially different; thioacidolysis resulted in higher yields. In contrast, an excellent agreement was obtained in the total number of β-aryl ether structures determined by thioacidolysis and DFRC/^{31}P NMR. These results indicate that the lower monomer yields derived from DFRC are due to a limitation of using GC alone for detecting DFRC monomers, rather than an inefficiency in the DFRC protocol's chemistry.

To further validate this data, we applied both thioacidolysis and DFRC/^{31}P NMR to better understand the lignin isolation process from wood. The results show that mild rotary ball milling minimizes, but does not prevent the degradation of β–O–4 structures, during the early stages of wood pulverization. The extent of such degradation was found to be higher for *E. globulus* than for a variety of softwoods examined. However, the combination of enzymatic hydrolysis and mild acid hydrolysis [74a–c] with low-intensity ball milling protocols resulted in high lignin isolation yields, with

less degradation, than traditional lignin isolation protocols. Furthermore, the structures of the EMALs isolated at yields ranging from 20 to 62% were very similar, indicating structural homogeneity in the lignin biopolymer within the secondary wall [45c].

Another acidolytic method of residual lignin isolation, proposed in the mid-1990s [46], has been widely accepted for the isolation of residual lignins from kraft and other pulps. However, it is not known to what extent the isolated lignins are affected by the process of acidolysis. To address this issue, Jiang and Argyropoulos [47] isolated residual lignin from various softwood kraft pulps using a dioxane acidolysis process in batch and flow-through reactors. The lignin was characterized using a variety of wet-chemical and ^{31}P NMR spectroscopic techniques. The dioxane acidolysis lignins, isolated from kraft pulps using the flow-through reactor, were found to have similar functional group distributions and elemental compositions as those of the acidolysis lignins obtained from the same pulps using the batchwise process. The data offers evidence that the structure of residual lignin in kraft pulps is not altered significantly during a dioxane acidolysis isolation process.

The formation of diphenylmethane (DPM) moieties in lignin during conventional kraft, soda and modified extended modified continuous cooking (EMCC) pulping conditions has also been probed using ^{31}P NMR [48a]. This effort confirmed the assignment of a ^{31}P NMR signal due exclusively to the presence of phosphitylated DPM phenolic hydroxyl groups. Softwood milled-wood lignin was subjected to kraft pulping conditions in the presence and absence of varying amounts of formaldehyde; the ^{31}P NMR spectra of the recovered lignins revealed selective signal growth in the region between 142.8 and 144.3 ppm. These signals were assigned to DPM ArOH groups, in accordance with previous model compound work and calculations based on the Hammett principles [31,40]. The DPM moieties in lignin accumulate in significantly higher proportions in an isothermal (120°C) soda pulping experiment than in a comparable kraft pulping experiment. DPM structures were also found to prevail among the condensed phenolic units of a conventionally cooked pulp compared to one produced using a modified cooking protocol (EMCC), providing additional evidence that modern modified pulping technologies alter the structure of residual kraft lignin beneficially [48a]. This effort was extended further with detailed computational analyses [48b]. Similar conclusions were also reached by Froass et al., who used quantitative ^{31}P NMR, together with ^{1}H and ^{13}C NMR spectroscopies, to investigate the structure of residual lignins from conventional and modified cooking protocols [49].

The stereoselective cleavage of the β–O–4-structures under conventional kraft pulping conditions has also been studied using quantitative ^{31}P NMR [50]. Quantitative spectra of residual and dissolved softwood kraft lignins, isolated at various degrees of delignification, confirmed earlier observations that the *erythro* isomers cleave faster than their *threo* counterparts [50]. In subsequent work, Ahvazi and Argyropoulos [51] measured the remaining β–O–4 ether structures present in softwood milled-wood lignin at various points along a kraft cook. This effort was focused at deriving the various absolute fundamental thermodynamic parameters that govern the stereoselective degradation of β-ethers under homogeneous kraft pulping conditions. The absolute rates of scission of the two diastereomers followed two kinetic regimes: an initial fast phase, followed by a slower phase. In agreement with previous accounts, the rate constants for these scission reactions were found to follow a pseudo-first-order rate law during both phases. Rate constant data invariably indicated that the *erythro*-isomers of the β–O–4 units of softwood milled-wood lignins cleave faster than their *threo* counterparts during both phases.

A number of research efforts have been focused at using mixtures of organic solvents with water as the pulping medium. The Alcell® process represents one such process, with the inherent limitation that it cannot be used for the pulping of softwoods. In recent work by Liu et al.[52], quantitative ^{31}P NMR was used for the comparative analysis of dissolved and residual lignins after conventional kraft and Alcell cooking of softwood and hardwood species. At all degrees of delignification, the ArOH group content for both wood species was: (a) higher for the solubilized kraft lignins than for the solubilized Alcell lignins and (b) lower in the residual kraft lignins than in the residual Alcell lignin. The authors rationalized these findings on the basis of the greater solvating abilities of alkaline aqueous media (toward inducing solubilization of the phenolic moieties) as opposed to those of ethanol. The condensed phenolic units in residual lignins formed in greater abundance in softwood rather than hardwood pulps for both processes. Condensed phenolic structures were formed most rapidly in the residual lignins of the softwood Alcell® pulps and in particular during the early and later phases of delignification. The condensation reactions, induced under the acidic conditions and elevated temperatures, may be responsible for the deceleration of the delignification observed for softwoods during the Alcell® process.

The fundamental changes of softwood and hardwood kraft lignins during high-pressure oxygen and low-pressure oxygen-peroxide reinforced delignification (Eop) stages have also been elucidated using quantitative ^{31}P NMR [53]. Conventional kraft pulps from a hardwood and a softwood, which were oxygen delignified using similar conditions, were subjected to residual and solubilized lignin isolation procedures. The formation of carboxylic acids and the degradation of condensed phenolic structures were found to be the main pathways that account for lignin dissolution during the oxygen or the Eop delignification stages. Significant degradation of the uncondensed and condensed phenolic units occurred during the oxygen and the Eop stages. Presumably, the formation of carboxylic acids compensated for the elimination of phenolic hydroxyl groups by keeping the lignin fragments hydrophilic enough for dissolution in alkaline media. In general, the chemical and physicochemical considerations that affect the efficiency of oxygen and Eop stages were found to be similar. However, due to the milder nature of the Eop stage, the resultant residual lignins were found to be less affected than their high-pressure oxygen counterparts [53].

An isolated softwood residual kraft lignin was systematically oxidized at different times and temperatures in an attempt to understand the complex interactions of this lignin with oxygen at elevated temperatures, pressures, and pH [54]. Quantitative ^{31}P NMR spectra of oxidized lignins, obtained after phosphitylation with reagent II, showed the formation and/or elimination of the various functional groups as a function of time and temperature. For all temperatures studied, two regimes described the rate of carboxylic acid group formation: an initial rapid phase that dominates the process for the first 20 minutes (at all temperatures), followed by a slower phase. This is not an unusual finding, since similar observations were also reported by Renard et al. [55,56], who studied the kinetics of oxidation of cuoxam lignin. The rate of

carboxylic acid group formation was found to dramatically increase as the reaction temperature increased. At reaction temperatures, typical of conventional commercial oxygen delignification installations (80–100°C), only minor oxidation occurred within the residual kraft lignin. In general, the efficiency of oxidation of residual kraft lignin was found to significantly increase above 100°C, even though these experiments were conducted under idealized two-phase homogeneous conditions. The technological ramifications of these data imply that a three-phase oxygen delignification system, operating at temperatures below 100°C, causes only minor oxidative changes in the structure of the lignin on the fiber.

Quantitative ^{31}P NMR spectra of the oxidized lignin samples described above [54] also showed characteristic signals of catechols in the region 138.6–139.1 ppm [29,31,50]. Quantification of the catechol signals, as a function of time and temperature, showed that the catechol concentration was relatively constant over the whole temperature range examined, implying that catechols were oxidation intermediates. Their participation in an oxygen delignification was previously suggested by Gellerstedt and Lindfors [57] and Renard et al. [55].

Sun and Argyropoulos [58], using quantitative ^{31}P NMR, mapped the reactivity and efficiency of several bleaching agents: chlorine dioxide, ozone, dimethyldioxirane and alkaline hydrogen peroxide. Residual lignin isolated from a conventional softwood kraft pulp was reacted with varying charges of these reagents, followed by quantitative ^{31}P NMR analyses. Chlorine dioxide and ozone were found to be the most efficient reagents in causing the formation of carboxylic acids; alkaline hydrogen peroxide was less efficient. Guaiacyl phenolic units were found to be the major sites of attack of all the oxidative treatments. The results confirmed the reactivity of ozone toward both free and etherified phenolic structures; the reactivity of chlorine dioxide and hydrogen peroxide was mainly directed toward free phenolic structures. The elimination of condensed phenolic structures was also examined for all oxidative treatments. At a given reagent charge, the relative efficiencies of elimination of condensed phenolic moieties were ozone > chlorine dioxide > alkaline hydrogen peroxide. Ozone and chlorine dioxide exhibited relatively high reactivity toward condensed phenolic units. For these cases, the rate of elimination/unit charge of reagent was similar to that observed for the elimination of guaiacyl phenolic units.

Senior et al. used quantitative ^{31}P NMR spectroscopy to compare the effectiveness of chlorine dioxide (D) and hydrogen peroxide (P) in the DEDP and DEPD bleaching sequences (E = extraction stage) as applied to softwood and hardwood kraft pulps [59]. The amount of condensed and uncondensed phenolic units in the residual lignins after different bleaching stages was determined and an attempt was made to correlate the units with the Kappa number and the brightness of the pulp. The analysis demonstrated that the second D (D_2) stage reduced the content of the free phenolic units in lignin from softwood pulp by 60%, while the P stage had no such effect. For the hardwood pulp, the D_2 stage reduced the content of free phenolic units in lignin by 90–95%. Based on these findings, Senior et al. concluded that the chlorine dioxide stage should precede hydrogen peroxide. This complies with the findings of van Lierop et al. [60] that a peroxide bleaching stage can decrease the efficiency of a subsequent chlorine dioxide stage by degrading phenolic hydroxyl units, which are considered to be sites of attack by chlorine dioxide.

Quantitative ^{31}P NMR spectroscopy has also been used to study the mechanism of light-induced yellowing. Photochemically induced degradation, condensation, and rearrangement reactions were observed during irradiation [61]. Milled wood lignin produced from alkaline hydrogen peroxide bleached softwood TMP fibers was adsorbed on pure cellulose and irradiated for various times under oxygen and nitrogen. The absolute amounts of β–O–4 ethers, phenolic hydroxyl groups, carboxylic acids and various condensed phenolic units were nondestructively quantified, using ^{31}P NMR spectroscopy. The ability of quantitative ^{31}P NMR to determine the concentration of both diastereomeric forms of the β–O–4 linkages in lignins provided a unique opportunity to further investigate the kinetics of this scission and of any possible salient stereochemical effects. Irradiation of milled wood lignin caused severe cleavage of the β–O–4 structures. These structures were eliminated faster in the presence of excess oxygen compared to nitrogen. This research provided additional evidence on the extreme reactivity of phenoxy radicals formed by homolytic bond scission within the β–O–4 structures during irradiation. These radicals may further condense or disproportionate to yield chromophoric centers.

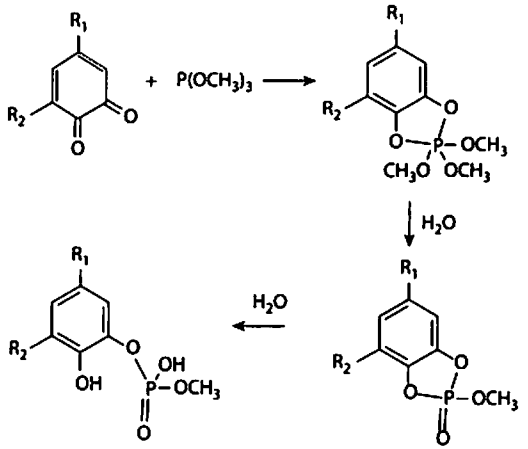
The kinetic profiles of formation and/or elimination of various condensed phenolic structures formed *via* radical coupling reactions in irradiated milled wood lignin were also followed using quantitative ^{31}P NMR [61]. A net increase of the C5-related condensed phenolic units occurs during irradiation of milled wood lignin. The build up of the condensed phenolic units was more rapid in the presence of excess oxygen than in the presence of nitrogen. This work also revealed a well-resolved signal at 144.2 ppm that appeared in the ^{31}P NMR spectra of irradiated milled wood lignins. The identity of this signal was found to be due to the formation of Cα–C5 and/or Cβ–C5 phenolic moieties in lignin during irradiation. The relative amounts of these units were found to gradually increase and did not diminish in the presence of oxygen or nitrogen.

^{31}P NMR SPECTROSCOPY FOR THE DETECTION OF QUINONES

Carbonyl groups of simple aliphatic aldehydes, *ortho* and *para* quinones, α,β-unsaturated carbonyls and cyclic aromatic anhydrides are known to condense with trimethyl phosphite, producing phosphite esters [62]. Lebo and Lonsky used trimethyl phosphite to determine the light-induced formation of *ortho*-quinonoid functional groups in refiner mechanical pulp [63].

Solid state ^{31}P NMR spectra of mechanical and ultra-high-yield pulps treated with trimethyl phosphite showed useful information on the type and amount of carbonyl groups. A thorough investigation of this reaction by Argyropoulos's group [64,65] revealed a number of salient features, which has led to the development of a technique for the quantitative determination of *ortho*-quinones present in solid lignocellulosic materials [65] and a semiquantitative protocol for their detection in soluble lignin [66].

A strong signal at 10 ppm in the ^{31}P solid state cross polarization/magic angle spinning (CP/MAS) NMR spectrum of stone-ground wood pulp (SGW) treated with trimethyl phosphite, has been attributed to a cyclic phosphite ester formed *via* hydrolysis of an oxyphosphorane adduct of trimethyl phosphite and *ortho*-quinones present in lignin, as shown in Scheme 6.2 [64–66].



SCHEME 6.2 Reactions of *ortho*-quinones with trimethyl phosphite and the possible transformation products (Argyropoulos, D. S. and Heitner, C., *Holzforschung*, 48 (Suppl.), 112–116, 1994; Argyropoulos, D. S. and Zhang, L., *J. Agric. Food Chem.*, 46(11), 4628–4634, 1998.)

The oxyphosphorane adducts of *ortho*-quinones with trimethyl phosphite, when synthesized in the absence of water, are known to give rise to a signal at –45.8 ppm [64]. Such an adduct, however, was found to be unstable in a typical pulp sample because traces of water and hydroxyl groups seem to hydrolyze oxyphosphoranes to cyclic phosphite esters, resulting in a signal at 10 ppm [65,66]. The assignment the ^{31}P chemical shift at 10 ppm to cyclic phosphite esters formed from adducts of trimethyl phosphite and *ortho*-quinones present in lignin is supported by the significant increase in signal intensity when SGW was oxidized with potassium nitrosodisulphonate (Fremy's salt).

The broad signal extending over the 20–40 ppm region in the ^{31}P solid state NMR spectra of pulps treated with trimethyl phosphite has particular interest. It is known that trimethyl phosphite reacts with 3-benzylidene-2,4-pentanedione to rapidly form a stable adduct at room temperature and give rise to a ^{31}P NMR signal 27.9 ppm [64]. The 20–40 ppm broad signal in the pulps was assigned to conjugated structures (e.g., α,β-unsaturated aldehydes and ketones) present in lignin within high-yield pulps [66]. Further independent evidence supporting this assignment was obtained from the solid state diffuse reflectance UV/visible difference spectra of thermomechanical pulp (TMP) and TMP treated with trimethyl phosphite [66]. Carboxylic acids are known to be present in high-yield pulps and the amount increases after alkaline hydrogen peroxide treatment [69]. Some signals in the ^{31}P NMR spectra of bleached pulps may be due to trimethyl phosphite reacting with these carboxylic acid groups. Carboxylic acids and trialkyl phosphites adduct formation has been reported by a number of other workers [68–70].

Carboxylic acids in mechanical pulp also can undergo oxyphosphorylation with trimethyl phosphite; the signals from the adducts may interfere with those attributed to *ortho*-quinones. The adduct formation between carboxylic acids and trimethyl

phosphite in oxyphosphorylated mechanical pulp was investigated with solid state ^{31}P NMR spectroscopy [65]; it was shown that complete ionization of the carboxylic acid groups in pulp prior to oxyphosphorylation prevents adduct formation and, thus, NMR signal overlap with *ortho*-quinone derivatives.

The oxyphosphorylation technique was used to probe the light-induced brightness reversion of black spruce SGW samples irradiated with a weak light source as a function of time [71]. The intensity of the central ^{31}P NMR signal at around 10 ppm due to *ortho* quinones was found to increase substantially after one and two days of irradiation. However, the rapid formation of *ortho*-quinones during early irradiation was followed by a reduction of these species at later phases. The data suggests that during early photochemistry, *ortho*-quinones are produced in high-yield pulps and subsequently react, creating other more complex chromophores that do not possess the quinone character.

To extend the technique for the detection of quinones in soluble lignins, Argyropoulos and Zhang [66] carried out detailed measurements and observations with model *ortho*- and *para*-quinones. These compounds, in dry organic solvents, were shown to form adducts with trimethylphosphite in quantitative yield and have ^{31}P NMR signals around –46 ppm and –2 ppm for *ortho*- and *para*-quinones, respectively. The adducts of *ortho*-quinones, in the presence of moisture and lignin, were shown to hydrolyze to the open ring product, dimethylphenylphosphate (–2 ppm), at an overall yield of about 70%. Similarly, the yield of the hydrolysis of *para*-quinone adducts with trimethylphosphite was about 70%. Consequently, a number of important issues (internal standard, and spin-lattice relaxation considerations for model compounds and lignins) were investigated, which allowed for the development of an experimental semiquantitative protocol recommended for spectral acquisition [66].

Lignin samples were then subjected to the developed protocol to determine the reliability of the technique in semiquantitatively detecting the changes in quinone content in accord with known chemistry. The accumulated data are tabulated in Table 6.3. The quinone content in MWL was 0.4 per 100 C9 units, which is lower than results obtained in other previous studies [67,72]. Furthermore, oxygen delignification was found to increase the quinone content within kraft lignin, possibly via demethylation reactions as recently demonstrated by Asgari and Argyropoulos [54].

TABLE 6.3
Total Quinone Content of Different Lignin Samples Detected in This Work

Lignin Sample	Quinone Content (mmol/g)
Black spruce MWL	0.020
Softwood solubilized kraft lignin	0.029
Indulin	0.023
Dimethyldioxirane-treated hardwood residual kraft lignin	0.24
Oxygen-treated kraft lignin	0.055

Source: Ramirez, F., *Pure and Appl. Chem.*, 9, 337–369, 1964.

Finally, a treatment of a hardwood (aspen) solubilized kraft lignin with dimethyldioxirane resulted in a dramatic increase in its quinone content as expected from its actual reactivity with lignin model compounds [60,73].

^{31}P NMR SPECTROSCOPY FOR THE DETECTION OF FREE RADICALS

In recent years our group has also emabrked at developing quantitative ^{31}P NMR spin trapping techniques that can be used as effective tools for the detection and quantification of many free radical species. Free radicals react with a nitroxide phosphorus compound, 5-diisopropoxy-phosphoryl-5-methyl-1-pyrroline-N-oxide (DIPPMPO) to form stable radical adducts, which are suitably detected and accurately quantified using ^{31}P NMR [77]. Initially, the ^{31}P NMR signals for the radical adducts of oxygen-centered ($^{\bullet}OH$, $O_2^{\bullet-}$) and carbon-centered ($^{\bullet}CH_3$, $^{\bullet}CH_2OH$, $CH_2{}^{\bullet}CH_2OH$) radicals were assigned [77]]. Subsequently, the quantitative reliability of the developed technique was demonstrated under a variety of experimental conditions. The ^{31}P NMR chemical shifts for the hydroxyl and superoxide reaction adducts with DIPPMPO were found to be 25.3 and 16.9, 17.1 ppm (in phosphate buffer), respectively. The ^{31}P NMR chemical shifts for $^{\bullet}CH_3$, $^{\bullet}CH_2OH$, $^{\bullet}CH(OH)CH_3$ and $^{\bullet}C(O)CH_3$ spin adducts were 23.1, 22.6, 27.3 and 30.2 ppm, respectively [77].

The same system was also applied for the detection of phenoxy radicals, as an alternative to traditional EPR techniques. More specifically, the phenoxy radicals were produced via the oxidation of different phenols by $K_3Fe(CN)_6$. The ^{31}P NMR signals for the radical adducts of phenoxy radicals ($PhO^{\bullet}$) were assigned and found to be located at 25.2 ppm [78]. Subsequently, this spin trapping system was applied to the oxidation of various phenols in the presence of peroxidases and 1-hydroxybenzotriazole (HBT) as a mediator: the 2,4,6-trichlorophenol and 2,4,6-tri-tert-butylphenol were oxidized and only phenoxy radical adducts were detected, whereas during the oxidation of 2,4-dimethylphenol and isoeugenol, other adducts were detected and related to radical delocalization [78].

This powerful system was also applied for the trapping of ketyl radicals, which are very difficult intermediates to be detected and quantified with traditional techniques (i.e. EPR). Ketyl radicals were initially produced using photochemical reactions of acetophenone, whose excited triplet state is able to abstract hydrogen from an H donor [79]. As such, the ^{31}P NMR signals for the radical adducts of the DIPPMPO spin trap with the ketyl radicals were assigned. Furthermore in efforts to confirm the structure of these adducts, their mass spectra and fragmentation patterns were carefully examined under GC-MS conditions [80]. Subsequently, the DIPPMPO spin trapping system was applied to the oxidation of 1-(3,4-dimethoxyphenyl)ethanol in the presence of horseradish peroxidase, hydrogen peroxide and 1-hydroxybenzotriazole as the electron carrier (mediator) [79]. Our work confirmed that the mechanism consists of a hydrogen abstraction reaction from the α position, involving the ketyl radical: during the oxidation, the hydroxyl, hydroperoxyl and ketyl radical intermediates were all detected.

These efforts demonstrate the efficacy of our methodology that provides for the first time a facile means for the detection of otherwise elusive radical species, with important implications in biology, chemistry and biochemistry.

CONCLUDING REMARKS

The selective and quantitative tagging of lignin with various heteronuclear spectroscopic reporter groups, followed by quantitative NMR data acquisition, has the potential to provide some truly unique insights toward understanding the complex and variable reactions of lignin under conditions of natural or commercial transformations. Many of the selective lignin chemical transformations reviewed here permit specific enquiries to be made on well-defined lignin functional groups. The described heteronulcear NMR approach has distinct advantages over its carbon and proton counterparts, since it allows for rapid quantitative acquisitions for well defined lignin moieties. This information, when coupled with careful ^{13}C NMR acquisitions, may offer a comprehensive detailed understanding of the complex lignin macromolecule. The remaining challenges of probing structural information of lignin present on the fiber, without the need of its prior isolation, may also be eventually addressed by modern advances of quantitative ^{31}P NMR of wood and pulps dissolved in ionic liquids.

REFERENCES

1. Brezny, R., and Schraml, J., "Silicon-29 NMR spectral studies of kraft lignin and related model compounds," *Holzforschung* **41**(5), 293–298 (1987).
2. Nieminen, M.O.J., Pukkinen, E., and Rahkamaa, E., "Determination of hydroxyl groups in kraft pine lignin by silicon-29 NMR spectroscopy," *Holzforschung* **43**(5), 303–307 (1989).
3. Herzog, H., Burba, P., and Buddrus, J., "Quantification of hydroxylic groups in a river humic substance by Si-29-NMR," *Fresenius J. Anal. Chem.*, **V354**, N3, 375–377 (1996).
4. Chen, C.-L., and Robert, D., in *Methods in Enzymology*, 161, Part B, pp. 137–174, W.A. Woods and S.T. Kellogg (eds.), Academic Press, New York (1988).
5. Potthast, A., Schiene, R., and Fischer, K., "Structural investigations of n-modified lignins by N-15-NMR spectroscopy and possible pathways for formation of nitrogen-containing compounds related to lignin," *Holzforschung* **50**(6), 554–562 (1996).
6. Knicker, H., Ludemann, H. D., and Haider, K., "Incorporation studies of NH4 + during incubation of organic residues by N-15-CPMAS NMR-spectroscopy," *Eur. J. Soil Sci.* 4(3), 431–441 (1997).
7. Kögel-Knabner, I., "C-13 and N-15 NMR spectroscopy as a tool in soil organic matter studies," *Geoderma* **80**(3–4), 243–270 (1997).
8. Neirinck, V., Robert, D., and Nardin, R., "Use of indirect Hg-199 NMR detection for aromatic compounds of biological interest," *Mag. Reson. in Chem.* **31**(9), 815–822 (1993).
9. Kolehmainen, E., Paasivirto, J., Kauppinen, R., Otollinen, T., and Kasa, S., "^{119}Sn-NMR study on bis(tri-butiltin)oxide derivatives of phenolic compounds," *Intern. J. Environ. Anal. Chem.* **43**, 19–24 (1990).
10. Barrelle, M., "A new method for the quantitative fluorine-19 NMR spectroscopic analysis of hydroxyl groups in lignins," *Holzforschung* **47**(3), 261–267 (1993).
11. Barrelle, M., Fernandes, J. C., Froment, P., and Lachenal, D., "An approach to the determination of functional groups in oxidized lignins by fluorine-19 NMR," *J. Wood Chem. Technol.* **12**(4), 413–424 (1992).
12. Lachenal, D., Fernandes, J. C., and Froment, P., "Behavior of residual lignin in kraft pulp during bleaching," *J. Pulp Paper Sci.* **21**(5), J173–J177 (1995).

13. Sevillano, R. M., Mortha, G., Froment, P., Lachenal, D., and Barrelle, M., "^{19}F NMR spectroscopy for the quantitative analysis of carbonyl groups in lignin," 4th European Workshop Lignocellulosicsa and Pulp, Stresa, Italy, 292 (1996).
14. Ahvazi, B., and Argyropoulos, D. S., "^{19}F Nuclear magnetic resonance spectroscopy for the elucidation of carbonyl groups in lignins: Part I: Model compounds," *J. Agric. Food Chem.* **44**(8), 2167–2175 (1996).
15. Ahvazi, B., Crestini, C., and Argyropoulos, D. S., "^{19}F Nuclear magnetic resonance spectroscopy (NMR) for the quantitative detection and classification of carbonyl groups in lignins," *J. Agric. Food Chem.* **47**(1), 190–201 (1999).
16. Verkade, J. G., and Quin, L. D., "Phosphorus-31 NMR spectroscopy in stereochemical analysis of organic compounds and metal complexes," in *Methods of Stereochemical Analysis*, M. Grayson and E. J. Griffith (eds.), **7**, VCH Publ. (1987).
17. Anderson, R. C., and Shapiro, M. Y., "2-Chloro-4(R), 5(R)-dimethyl-2-oxo-1,3,2,-dioxaphospholane, a new chiral derivatizing agent," *J. Org. Chem.* **49**, 1304 (1984).
18. Johnson, C. R., Elliot, R. C., and Penning, T. D., "Determination of enantiomeric purities of alcohols and amines by a ^{31}P NMR technique," *J. Am. Chem. Soc.* **106**, 5019 (1984).
19. Schiff, D. E., Verkade, J. G., Metzler, R. M., Squires, T. G., and Vernier, C. G., "Determination of alcohols, phenols, and carboxylic acids using phosphorus-31 NMR spectroscopy," *Applied Spectroscopy* **40**(3), 348 (1986).
20. Wroblewski, A. E., Markuszewski, R., and Verkade J. G., "A novel application of ^{31}P NMR spectroscopy to the analysis of organic groups containing -OH, -NH, and -SH functionalities in coal extracts and condensates," *Am. Chem. Soc. Div. of Fuel Chem.* **32**(3), 202 (1987).
21. Wroblewski, A. E., Lensink, C., Markuszewski, R., and Verkade, J. G., "^{31}P NMR spectroscopic analysis of coal pyrolysis condensates and extracts for heteroatom functionalities possessing labile hydrogen," *Ener. Fuels* **2**, 765 (1988).
22. Lensink, C., and Verkade, J. G., "Identification of labile hydrogen functionalities in coal derived liquids by ^{31}P NMR spectroscopy," *Am. Chem. Soc. Div. of Fuel Chem.* **33**(4), 906 (1988).
23. Lucas, H. J., Mitchell, F. W., and Scully, C. N. "Cyclic phosphites of some aliphatic glycols," *J. Am. Chem. Soc.* **72**, 5491 (1950).
24. Argyropoulos, D. S., "^{31}P NMR in wood chemistry: A review of recent progress," *Research on Chemical Intermediates* **21**(3–5), 373–395 (1995).
25. Archipov, Y., Argyropoulos, D. S., Bolker, H. I., and Heitner, C., "^{31}P NMR spectroscopy in wood chemistry. Part I. Lignin model compounds," *J. Wood Chem. Technol.* **11**(2), 137–157 (1991).
26. Adler, E., Brunow, G., and Lundquist, K. "Investigation of the acid-catalysed alkylation of lignin by means of NMR spectroscopic methods," *Holzforschung* **41**, 199 (1987).
27. Argyropoulos, D. S., Bolker, H. I., Heitner, C., and Archipov, Y. "^{31}P NMR spectroscopy in wood chemistry. Part V. Qualitative analysis of lignin functional groups." *J. Wood Chem. Technol.* **13**(2),187–212 (1993).
28. Argyropoulos, D. S., Archipov, Y., Bolker, H. I., and Heitner, C., "^{31}P NMR spectroscopy in wood chemistry. Part IV. Lignin models: Spin lattice relaxation times and solvent effects in ^{31}P NMR," *Holzforschung* **47**, 50–56 (1993).
29. Granata, A., and Argyropoulos, D. S, "2-Chloro-4,4,5,5-Tetramethyl-1,3,2-Dioxaphospholane a reagent for the accurate determination of the uncondensed and condensed phenolic moieties in lignins," *J. Agric. Food Chem.* **43**(6), 1538–1544 (1995).
30. Sun, Y., and Argyropoulos, D. S., "Fundamentals of high pressure oxygen and low pressure oxygen-peroxide (Eop) delignification of softwood and hardwood kraft pulps: A comparison," *J. Pulp Paper Sci.* **21**(6), J185–190 (1995).

31. Jiang, Z. H., Argyropoulos, D. S., and Granata, A., "Correlation analysis of ^{31}P NMR chemical shifts with substituent effects of PHENOLS," *Magnetic Resonance in Chemistry* **33**, 375–382 (1995).
32. Mazúr, M., and Argyropoulos, D. S., "^{31}P NMR spectroscopy in wood chemistry. Part VII. Studies toward elucidating the phosphorus relaxation mechanism of phosphitylated lignins," *Cellulose Chem. Technol.* **29**(5), 589–601 (1995).
33. Argyropoulos, D. S., "Quantitative phosphorus-31 NMR analysis of lignins: A new tool for the lignin chemist," *Journal of Wood Chemistry and Technology* **14**(1), 45–63 (1994).
34. Faix, O., Argyropoulos, D. S., Robert, D., and Neirinck, V., "Determination of hydroxyl groups in lignins: Evaluation of ^{1}H-, ^{13}C-, ^{31}P-NMR, FTIR and wet chemical methods," *Holzforschung* **48**(5), 387–394 (1994).
35. Argyropoulos, D. S., "Quantitative phosphorus-31 NMR analysis of six soluble lignins," *J. Wood Chem. Technol.* **14**(1), 65–82 (1994).
36. Heuts, L., PhD Thesis, Royal Institute of Technology (1998).
37. Argyropoulos, D. S., Hortling, B., Poppius-Levlin, K., Sun, Y., and Mazur, M., "MILOX pulping, lignin characterization by ^{31}P NMR spectroscopy and oxidative degradation," *Nordic Pulp and Paper Research J.* **10**(1), 68–73 (1995).
38. Saake, B., Argyropoulos, D. S, and Faix, O., "Structural investigations of synthetic lignins (DHP's) as a function of molar mass and mode of preparation," *Phytochemistry* **43**(2), 499–507 (1996).
39. Jurasek, L., "Molecular modelling of fibre walls," *J. Pulp Paper Sci.* **24**(7), 209–212 (1998).
40. Jiang, Z., and Argyropoulos, D. S., Coupling ^{31}P NMR with the Mannich reaction toward the quantitative analysis of lignins" *Can J. Chem.* **76**(5), 612–622 (1998).
41. Smit, R., Suckling, I. D., and Ede, R. M., "A new method for the quantification of condensed and uncondensed softwood lignin structures." Proceedings of 9th International Symposium on Wood and Pulping Chemistry. Montreal, Canada, June 1997, Vol. 1, L4-1–L4-6.
42. Lu, F., and Ralph, J., "Derivatization followed by reductive cleavage (DFRC method), new method for lignin analysis: protocol for analysis of DFRC monomers," *J. Agric. Food Chem.* **45**, 2590–2592 (1997).
43. Lu, F., and Ralph, J., "DFRC method for lignin analysis. 1. new method for β-aryl ether cleavage: lignin model studies," *J. Agric. Food Chem.* **45**, 4655–4660 (1997).
44. Lu, F., and Ralph, J., "The DFRC method for lignin analysis. 2. Monomers from isolated lignins," *J. Agric. Food Chem.* **46**, 547–552 (1998).
45. (a) Tohmura, S., and Argyropoulos, D. S., "Determination of arylglycerol-β-aryl ethers and other linkages in native and technical lignins," *J. Agric. Food Chem.* **49**(2), 536–542 (2001); (b) Argyropoulos, D. S., Jurasek, L., Krištofová, L., Xia, Z., Sun, Y., and Paluš, P., "Abundance and reactivity of dibenzodioxocins in softwood lignin," *J. Agric. and Food Chem.* **50**, 658–666 (2002); (c) Guerra, A., Norambuena, M., Freer, J., and Argyropoulos, D. S., " Determination of arylglycerol-β-aryl ether linkages in enzymatic mild acidolysis lignins (EMAL): Comparison of DFRC/^{31}P NMR with thioacidolysis," *Journal of Natural Products* **71**(5), 836–841 (2008).
46. Gellerstedt, G., Pranda, J., and Lindfors, E.-L., "Structural and molecular properties of residual birch kraft lignins," *J. Wood Chem. Technol.* **14**(4), 467–482 (1994).
47. Jiang, Z., and Argyropoulos, D. S., "Isolation and characterization of residual lignin in kraft pulp," *J. Pulp Paper Sci.* **25**(1), 25–29 (1999).
48. (a) Ahvazi, B., Pageau, G. and Argyropoulos, D. S., "On the formation of diphenylmethane structures in lignin under kraft, EMCC, and soda pulping conditions," *Can. J. Chem.* **76**, 506–512 (1998); (b) Jurasek, L., Kristofova, L., Sun, Y., and Argyropoulos, D. S., "Alkaline oxidative degradation of diphenylmethane structures: Activation energy and computational analysis of the reaction mechanism," *Can. J.Chem.* **79**, 1394–1401 (2001).

49. Froass, P. M., Ragauskas, A. J., and Jiang, J.-E., "Chemical structure of residual lignin from kraft pulp," *J. Wood Chem. Technol.* **16**(4), 347–365 (1996).
50. Jiang, Z., and Argyropoulos, D. S., "The stereoselective degradation of arylglycerol-β-aryl ethers during kraft pulping," *J. Pulp Paper Sci.* **20**(7), 183–188 (1994).
51. Ahvazi, B., and Argyropoulos, D. S., "Thermodynamic parameters governing the stereoselective degradation of arylglycerol-β-aryl ether bonds in milled wood lignin under kraft pulping conditions," *Nordic Pulp and Paper Res. J.* **12**(4), 282–288 (1997).
52. Liu, Y., Carriero, S., Pye K., and Argyropoulos, D. S., "A comparison of the structural changes occurring in lignin during alcell and kraft pulping of hardwoods and softwoods," in *Lignin: Historical, Biological and Materials Perspectives,* W. G. Glasser, R. A. Northey, T.P. Schultz (eds.), ACS Symposium Series No. 742, Chapter 22, 447–464 (1999).
53. Sun, Y., and Argyropoulos, D.S., "Fundamentals of high-pressure oxygen and low-pressure oxygen-peroxide (Eop) delignification of softwood and hardwood kraft pulps: A comparison," *J. Pulp Paper Sci.* **21**(6), J185–J190 (1995).
54. Asgari, F., and Argyropoulos, D. S., "Fundamentals of oxygen delignification. Part II. Kinetics of functional group formation/elimination in residual kraft lignin," *Can. J. Chem.* **76**, 1606–1615 (1998).
55. Renard, J. J., Mackie, D. M., and Bolker, H. I., *Paperi Ja Puu* **11**(1) (1975).
56. Renard, J. J., Mackie, D. M., Bolker, H. I., and Clayton, D. W., "Delignification of wood using pressurized oxygen. II. Mechanism of transfer of the reagents into the wood structure," *Cellulose Chem. Technol.* **9**(4), 341–352 (1975).
57. Gellerstedt, G., and Lindfors, E.-L., "Hydrophilic groups in lignin after oxygen bleaching," *Tappi J.* **70**(6), 119–122 (1987).
58. Sun, Y., and Argyropoulos, D. S., "A comparison of the reactivity and efficiency of ozone, chlorine dioxide, dimethyldioxirane and hydrogen peroxide with residual kraft lignin," *Holzforschung* **50**(2), 175–182 (1996).
59. Senior, D.J., Hamilton, J., Ragauskas, A.J., Sealey, J., and Froass, P., "Interaction of hydrogen peroxide and chlorine dioxide stages in ECF bleaching," *Tappi J.* **81**(6), 170–177 (1988).
60. Van Lierop, B., Jiang, Z. H., Chen, J., Argyropoulos, D. S., and Berry, R. M., "On the efficiency of hydrogen peroxide use in ECF bleaching," *J. Pulp Paper Sci.* **26**(7), 255–259 (2000).
61. Argyropoulos, D. S., and Sun, Y., "Photochemically induced solid-state degradation, condensation, and rearrangement reactions in lignin model compounds and milled wood lignin," *Photochem. and Photobiol.* **64**(3), 510–517 (1996).
62. Ramirez, F., "Condensations of carbonyl compounds with phosphite esters," *Pure and Appl. Chem.* **9**, 337–369 (1964).
63. Lebo, S. E., and Lonsky, W.F.W., "The occurrence and light induced formation of *ortho*-quinonoid lignin structures in white spruce refiner mechanical pulp," *J. Pulp Paper Sci.* **16**(5), J139–J143 (1990).
64. Argyropoulos, D. S., Heitner, C., and Morin, F. G., "^{31}P NMR spectroscopy in wood chemistry. Part III. Solid state ^{31}P NMR of trimethyl phosphite derivatives of chromophores in mechanical pulps," *Holzforschung* **46**(3), 211–218 (1992).
65. Argyropoulos, D. S., and Heitner, C., "^{31}P NMR spectroscopy in wood chemistry. Part VI. Solid state ^{31}P NMR of trimethyl phosphite derivatives of chromophores and carboxylic acids present in mechanical pulps: A method for the determination of *ortho*-quinones," *Holzforschung* **48** (Suppl.), 112–116 (1994).
66. Argyropoulos, D. S., and Zhang, L., "On the semiquantitative determination of quinonoid structures in soluble lignins by ^{31}P NMR," *J. Agric. Food Chem.* **46**(11), 4628–4634 (1998).

67. Gierer, J., and Imsgard, F., "The reactions of lignins with oxygen and hydrogen peroxide in alkaline media," *Svensk. Papperstidn.* **80**(16), 510–518 (1977).
68. Pudovik, A. N., Gazizov, T. K., and Kharlamov, V. A., "The reaction of trialkyl phosphites with mineral and carboxylic acids," *Dolkady Academii Nauk SSSR* **227**(2), 376–379 (1976).
69. Krutikov, V. I., Aleinikov, S. F., Kalaverdova, E. A., and Lavrent'ev, A. N., "Some characteristics of the formation of adducts of carboxylic acids with phosphorus acid esters," *Zh Obshch. Khim.* **58**(11), 2490–2493 (1988).
70. Huyser, E. S., and Dietter, J. A., "Synthesis and acetolysis of mixed trialkyl phosphites," *J. Org. Chem.* **33**, 4205–4210 (1968).
71. Argyropoulos, D. S., Heitner, C., and Schmidt, J. A., "Observation of quinonoid groups during the light-induced yellowing of softwood mechanical pulp," *Res. Chem. Intermed.* **21**(3–5), 263–274 (1995).
72. Imsgard, F., Falkehag, S. I., and Kringstad, K. P., "On possible chromophoric structures in spruce wood," *Tappi* **54**(10), 1680 (1971).
73. Argyropoulos, D. S., Sun, Y, Berry, R. M., and Bouchard, J., "Reactions of dimethyldioxirane with lignin model compounds," *J. Pulp Paper Sci.* **22**(3), J84–J90 (1996).
74. (a) Guerra, A., Filpponen, I., Lucia, L., and Argyropoulos, D. S., "A comparative evaluation of three lignin isolation protocols, with different wood species" *J. Agric. Food Chem.* **54**(26), 9696–9705 (2006); (b) Guerra, A., Filpponen, I., Lucia, L., Saquing, C., Baumberger, S., and Argyropoulos, D. S., "Toward a better understanding of the lignin isolation process from wood," *J. Agric. Food Chem.* **54**(16), 5939–5947 (2006); (c) Wu, S., and Argyropoulos, D. S., "An improved method for isolating lignin in high yield and purity," *J. Pulp Paper Sci.* **29**(7), 235–240 (2003).
75. King, A.W.T., Zoia, L., Filpponen, I., Olszewska, A., Xie, H., Kilpeläinen, I., Argyropoulos, D.S. *In-situ* Determination of Lignin Phenolics and Wood Solubility in Imidazolium Chlorides using ^{31}P NMR"; *J. Agric. Food Chem.* 2009, 57, 8236–8243.; King, A., Järvi, P., Kilpeläinen, I., Heikkinen, S., Argyropoulos., D. S., "Hydrophobic Interactions Determining Functionalized Lignocellulose Solubility in Dialkylimidazolium Chlorides, as Probed by ^{31}P NMR"; DOI: 10.1021/bm8010159, Biomacromolecules, *10*, 458–463 (2009).
76. Kilpelainen, I., Xie, H., King, A., Granström, M., Heikkinen, S., and Argyropoulos, D. S., "Dissolution of wood in ionic liquids," *J. Agric. Food Chem.* **55**(22), 9142–9148 (2007).
77. Argyropoulos, D. S., Li, H., Gaspar, A. R., Smith, K., Lucia, L., Rojas, O. J., "Quantitative ^{31}P NMR Detection of Oxygen-centered and Carbon-centered Radical Species." *Bioorganic & Medicinal Chemistry*, Volume 14, Issue 12, 15 June, pp. 4017–4028, (2006).
78. Zoia, L. Argyropoulos., D. S., "Phenoxy Radical Detection Using Quantitative ^{31}P NMR Spin Trapping", *J. Phys. Org. Chem.*, 22 1070–1077, (2009); www.interscience.wiley.com) DOI 10.1002/poc.1561, 2009.
79. Zoia, L. Argyropoulos., D. S., "Ketyl Radical Detection Using Quantitative ^{31}P NMR Spin Trapping"; *J. Phys. Org. Chem.* 2009, DOI:10.1002/poc. 1561.
80. Zoia, L., Argyropoulos D. S., "Characterization of free radical spin adducts of the DIPPMPO using Mass Spectrometry and ^{31}P NMR", European Journal of Mass Spectrometry, To appear January 2010, DOI:10.1155/ejms. 1062.

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