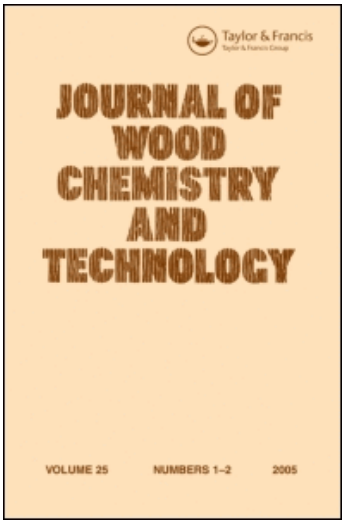

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^{31}P NMR Spectroscopy in Wood Chemistry Part V. Qualitative Analysis of Lignin Functional Groups

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^{31}P NMR SPECTROSCOPY IN WOOD CHEMISTRY PART V. QUALITATIVE ANALYSIS OF LIGNIN FUNCTIONAL GROUPS

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ABSTRACT

Dioxane lignin isolated from a hardwood (birch, *betula verrucosa*) and milled wood lignin isolated from a softwood (black spruce, *picea mariana*) were subjected to a variety of selective reactions to modify some of their functional groups. All the lignins were then treated with 1,3,2-dioxaphospholanyl chloride (I) and the ^{31}P NMR spectra of the derivatives were recorded. Most of the ^{31}P NMR signals were assigned from the chemical shifts previously obtained from model compounds. The signals arising from derivatizing the labile protons in carbohydrates, *erythro* and *threo* forms in β-O-4 structures, primary hydroxyls in a variety of lignin structures, syringyl and guaiacyl phenolic hydroxyls and those of carboxylic acids were assigned. The *alpha* benzylic hydroxyls in β-O-4 structures within birch lignin were less reactive toward alkylation than those in spruce lignin, consistent with the findings of previous workers; a difference attributable to the aryl part of the β-O-4 ether. The ^{31}P NMR spectroscopy of lignins derivatized with 1,3,2 dioxaphospholanyl chloride is thus shown to have considerable potential for the structural elucidation of lignins. This simple method is recommended for the rapid analysis of soluble lignins.

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INTRODUCTION

In recent years nuclear magnetic resonance spectroscopy has attained widespread application for the characterization of lignins. NMR spectra of lignins may provide detailed compositional characteristics that cannot be obtained by other analytical methods.

Most of the early work in this field was focused on proton NMR spectroscopy (1). The 100% natural abundance of the ^{1}H nucleus, resulting in high sensitivity during an NMR experiment, is among the advantages of proton NMR spectroscopy. Very little material is needed for analysis: usually 30 - 50 mg for lignins, and even less for model compounds (2). In addition instrument times are relatively short, varying from several minutes to a maximum of one hour, and since the relaxation times are small, the spectra are quantitative.

Nevertheless, there are some essential limitations to proton NMR spectroscopy. The rather limited range of chemical shifts of protons (12 ppm) results in poor spectral resolution and extensive signal overlapping. This is a particular drawback in the analysis of lignins, which contain a large variety of protons in similar, but not identical, chemical environments. In addition, signal overlapping is further increased by signal multiplicity due to J scalar couplings between adjacent proton nuclei.

The general limitation of proton NMR spectroscopy is that it can reveal information only on proton-containing groups. The chemical nature of carbon-containing groups is beyond its reach. Furthermore, proton NMR spectroscopy is not suitable for the investigation of labile proton functionalities (e.g. OH, COOH, SH), since their resonance lines are usually broad and rather sensitive to experimental conditions which affect hydrogen bond formation, proton exchange and other processes.

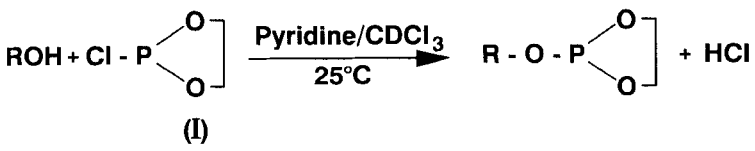
In contrast to proton NMR, ^{13}C NMR spectroscopy provides information on all the carbons present within a molecule. The ^{13}C NMR spectra are not complicated by spin-spin coupling phenomena such as ^{1}H-^{13}C coupling which are

eliminated by selective proton decoupling during the ^{13}C NMR experiment, thus providing a single signal for each ^{13}C nucleus. ^{13}C NMR spectroscopy is significantly less sensitive than its proton counterpart (1/5800) because of the low natural abundance (1.1%) of the ^{13}C nucleus and its low magnetic moment. Consequently, the amount of sample and instrument time required are increased by almost an order of magnitude. Quantitative analysis is subject to more severe limitations due to the Nuclear Overhauser Effect (NOE) and the various relaxation profiles of the different carbons present in the molecule under investigation (2). Experiments on materials such as lignins require at least 10 to 11 seconds delay time between successive pulses when a 90° pulse width is used (3,4). Furthermore, at least 10,000 transients are necessary for a sample concentration as high as 200 mg/mL in deuterated dimethyl sulphoxide. The required instrument time therefore, may be as high as 30 hours.

Two other techniques that have been used for the structural elucidation of lignin, are acetylation and silylation, aimed at derivatizing the labile protons in lignins, followed by ^{13}C and ^{29}Si NMR spectroscopy, respectively. Their advantages and limitations have been discussed in Part I of this series of papers (5).

In order to develop an improved technique, we have been examining the potential of ^{31}P NMR spectroscopy for characterizing lignin model compounds (5) and carbohydrates (6) whose labile protons were derivatized with 1,3,2-dioxaphospholanyl chloride (I). (Scheme I).

This reaction proceeded rapidly and quantitatively. Side products were obtained only from vicinal hydroxy acids and diacids. Such species, however, are unlikely to be found in lignins. The ^{31}P NMR spectra of the derivatized model compounds described in parts I and II of this series were found to be rather simple. Each functional group usually gave rise to sharp, single ^{31}P signals, and the chemical shifts were found to be rather sensitive to the chemical environment of the phosphitylated centre. Phenols, alcohols and simple carboxylic acids gave derivatives whose ^{31}P signals appeared in regions sufficiently well separated to permit distinguishing among them.



(I)

Where R=Residues of Phenols, Alcohols, Aldehydes, Sugars, Carboxylic Acids

Scheme 1: The reaction of 1,3,2-dioxaphospholanyl chloride (I) with active hydrogen compounds.

Furthermore, the method showed promise for determining fine structural details in lignin model compounds. For example, it was possible to distinguish primary, secondary, and tertiary alcohols, as well as *erythro* and *threo* forms, in arylglycerol-β-aryl ethers (5). Molecules that contained adjacent hydroxyl groups gave rise to signals positioned well downfield from their monofunctional analogues. These observations permitted the characterization of a number of mono- and disaccharides, as well as a variety of derivatives of cellulose (6).

We also found that the spin lattice relaxation times (T_1) of the ^{31}P nuclei and the concentration dependence of the chemical shifts of a variety of model compounds and birch dioxane lignin affected spectral quality. Accordingly the resolution of the spectra of lignins can be significantly improved by taking advantage of small solvent effects. The signals from primary and secondary alcohol groups in lignin may readily be resolved by appropriately adjusting the solvent concentration during spectral acquisition (7).

Having laid the foundations of this new technique by investigating the spectra of model compounds, we have now turned our attention to lignins. This paper describes how a variety of selective reactions were performed on two lignins (a hardwood and a softwood lignin), so that some of their functional groups were selectively modified. These modified lignins were derivatized with (I), and the ^{31}P

NMR spectra were recorded. Assignments from the spectra of the model compounds (5,6,7) permitted the assignment of most of the ^{31}P NMR signals of the lignins.

EXPERIMENTAL

Preparation and Analyses of Lignins

Birch *(Betula verrucosa)* dioxane lignin (BDL) was prepared according to the method of Pepper *et. al* (8). Black spruce (*Picea mariana)* milled wood lignin was prepared from an acetone-preextracted thermomechanical pulp. Ball milling was done according to Brownell (9) while the isolation and purification of the lignin was achieved according to Bjorkman (10). The elemental analyses were carried-out at Schwarzkopf microanalytical laboratories, Woodside N.Y. by using a Perkin Elmer 2400 carbon, hydrogen, nitrogen analyzer. The methoxyl content was determined by a method essentially identical to Tappi T209 SU-69. The carbohydrate and ash contents were determined by the chemical analysis section of the Pulp and Paper Research Institute of Canada , using standard methods.

Purification of Birch Dioxane Lignin (BDL)

Birch dioxane lignin was purified by liquid-liquid extraction using a slight modification of the procedure of Lundquist *et. al.* (11). The lignin (4.2 g.) was initially extracted with diethyl ether (2 x 500 mL) and then dissolved in 130 mL of a mixture containing pyridine, acetic acid and water (9:1:4). This solution was then extracted with 330 mL of chloroform. The aqueous layer contained about 40 mg of product (part of it in the form of a precipitate). The lignin in the organic layer was precipitated in diethyl ether (1L). It was washed with diethyl ether (3 x 200 mL), and dried under reduced pressure.

Acetylation of Lignin

Dried lignin samples (about 200 mg) were dissolved in a mixture of 2 mL of acetic anhydride and 2 mL of pyridine. After 24 hours at room temperature,

the reaction mixture was poured into about 20 g of crushed ice. The pH of the resulting mixture was adjusted to 3 by adding a solution of 6M HCl. The precipitated lignin was washed with water, filtered, and dried under reduced pressure.

Deacetylation of Acetylated Birch Dioxane Lignin

The acetylated BDL (200 mg) was dissolved in 4 mL of dioxane and deacetylation was started by adding 3 mL of pyrrolidine (12, 13). Samples (1.4 mL) were withdrawn after 10, 20, 60 and 120 min. They were each poured into 150 mL of diethyl ether, and the precipitated lignin was filtered, washed thoroughly with ether, and dried under reduced pressure prior to derivatization with 1,3,2-dioxaphospholanyl chloride (I).

Methylation of Lignin

Birch dioxane lignin and spruce milled-wood lignin were methylated with methanol in dioxane at 40°C, using 0.15 M p-toluenesulfonic acid as a catalyst, in accordance to Adler *et. al.* (14). The reaction was allowed to continue for four days, was then quenched by adding solid sodium bicarbonate. After solvent evaporation at reduced pressure, the methylated lignins were precipitated in a 1% solution of sodium bicarbonate, filtered, and dried in vacuo.

Reduction of Lignin

Lignin samples (200 mg) were reduced with 200 mg of sodium borohydride in 0.1 N NaOH solution for one week with efficient stirring. The reduced lignin was then precipitated from the alkaline solution by acidification with acetic acid, washed thoroughly with water, and dried, under reduced pressure, at 50-55°C.

Derivatizing Reagent (I) and Derivatization Procedure

All solvents and chemicals used were of reagent or analytical grade. The derivatizing reagent 1,3,2-dioxaphospholanyl chloride (I) was synthesized from

phosphorus trichloride (99.9%) and anhydrous ethylene glycol (99.0%), as described elsewhere (15), and purified by vacuum distillation.

Lignins (18-25 mg) were dissolved in 200-400 μL of pyridine-d_5, (acting as acid acceptor) and then diluted with 200-1000 μL of deuterated chloroform. The relative ratio of pyridine to chloroform in the mixture significantly affects the resolution of the spectra, specially in the downfield region (7). For this reason the actual acquisition conditions for each spectrum are recorder in the caption of each relevant figure. To the chloroform solution a known amount (0.5-1.0 mg/mL) of freshly resublimed dimethyl-L-tartrate was added to serve as an internal standard for quantification purposes, when required. To this mixture 50 μL of (I) was added with stirring.

After derivatization of the lignin, the reaction mixture was immediately transferred into a 5-mm NMR sample tube for subsequent analysis.

NMR spectroscopy

The ^{31}P NMR spectra were obtained by the inverse gated decoupling sequence on a Varian XL-300 NMR spectrometer operating at 121.5 MHz in the Fourier transform mode. An internal deuterium lock was provided by the deuterated chloroform solvent. The external standard was 85% H_3PO_4. All downfield shifts from H_3PO_4 were considered positive. A sweep width of 10,000 Hz was observed, and spectra were accumulated with a time delay of 25 s between pulses. A pulse width causing a 90° flip angle was used. Line broadening of 2 Hz was used in processing the spectra. All chemical shifts reported in this paper were referenced by the product of (I) reacting with water; this product has been observed to give a sharp ^{31}P signal at 121.1 ppm, relative to the external standard (5, 6, 7).

^{13}C NMR spectra were recorded on a Varian XL-300 NMR spectrometer operating at 75.4 MHz. Lignin samples were placed in 10-mm NMR sample tubes as 18% solutions (w/v) in dimethyl sulphoxide-d_6 and the spectra run at 50°C. NOE was eliminated by applying the inverse gated proton decoupling sequence,

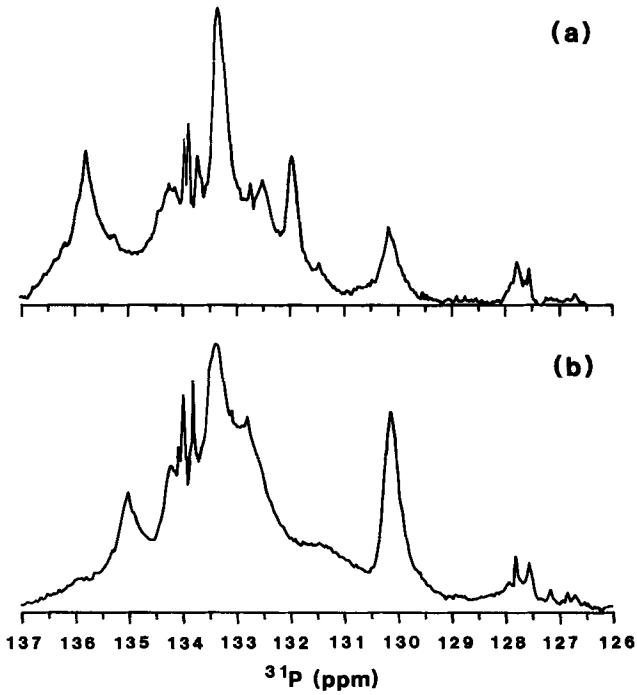


Figure 1: ^{31}P NMR spectra of (a) Birch dioxane lignin and (b) Black spruce milled wood lignin derivatized with (I). Acquisition conditions: lignin 18-25 mg, pyridine 200 μL, $CDCl_3$ 500 μL, (I) 50 μL.

while the time delay was 11 s between 90° pulses. A total of 6,000 - 10,000 transients were accumulated.

Proton NMR spectra were recorded on a Varian XL-200 NMR spectrometer operating in the Fourier transform mode.

RESULTS AND DISCUSSION

Qualitative ^{31}P NMR Spectra of Lignins Derivatized with (I) and Assignment of Main Signals.

For the purpose of the present investigation two lignin samples were examined; a hardwood dioxane lignin *(birch)* and a softwood milled wood lignin

(black spruce). Both lignins were characterized by elemental analysis, carbohydrate and ash content, and by proton and ^{13}C NMR spectroscopy. Such spectra for both lignins agreed with the published spectra of similar samples (2,3,16,17).

Typical ^{31}P NMR spectra of derivatized birch dioxane lignin and black spruce milled wood lignin are shown in Figure 1. The broad and sharp signals of varying intensities may contain information on secondary and primary alcohols, mono- and di-*ortho*-methoxyl phenols, *ortho*-aryl phenols, unsubstituted phenols, and carboxylic acids.

The signals at around 127.5, 130, and 132 ppm can quite definitely be assigned to derivatives of carboxylic, guaiacyl, and syringyl hydroxyls, respectively (5). The assignment, however, of the secondary and primary alcohol derivatives present in a variety of lignin structures and within carbohydrate contaminants is considerably less clear.

In order to assign signals, these lignins were chemically modified, and birch dioxane lignin was purified according to known procedures. Phosphitylated derivatives of the modified and purified samples yielded ^{31}P NMR spectra which allowed signal assignment.

In the following discussion the spectra are analyzed commencing with downfield signals and proceeding to upfield signals; i.e, in order of decreasing chemical shift values.

Assignment of ^{31}P Signals Due to Residual Carbohydrates in Lignin.

Gas chromatographic analysis of the BDL sample revealed that it contained about 2% of carbohydrate composed almost entirely of xylose (Table 1). This result is to be expected, because the amount of residual carbohydrates in milled wood lignin from birch wood has been reported to be considerably higher than in the corresponding preparation from spruce (18). The predominant carbohydrate in birch lignin is xylan (19,20). Since about 90% of this xylan can be removed by a mild alkaline treatment, it has been deduced (19,20) that the lignin and xylan are connected by ester linkages.

Table 1. Elemental Composition, Carbohydrate, Methoxyl and Ash Content of the Examined Lignins

Lignin	Carbon (%)	Hydrogen (%)	Methoxyl (%)	Carbohydrate (%)	Ash (%)
Birch Dioxane Lignin	57.9	6.00	21.6	2.15	0.09
Black Spruce Milled Wood Lignin	60.8	5.66	15.89	0.0	0.00

Figure 2 shows spectra of BDL in which the pyridine content was optimized as discussed in Part IV of this series (7). The purified lignin does not show the presence of carbohydrate contaminants, seen in the spectrum of the unpurified lignin as sharp, low intensity signals in the downfield end at 134.9, 134.8, 134.3, 133.8 and 133.6 ppm.

The last three values are in good agreement with those obtained from xylose derivatives which, after reaction with (I), gave signals at 135.7, 134.4 133.8 and 133.5 ppm (6). In xylan, however, the number of signals depends on the number of xylose units in the polysaccharide, since the xylose units within the chain bear only two hydroxyls; whose ^{31}P NMR chemical shifts depend on their relative distance from the end groups. Derivatives of di- and tri- saccharides with (I) were found (6) to exhibit more complex ^{31}P NMR spectra than monosaccharides. A xylan with a degree of polymerization of greater than, say 10, composed only of xylose linked by 1,4 glycosidic bonds, would result in no more than two broad signals due to phosphitylated hydroxyls at C-2 and C-3. Polysaccharides bound to lignin, however, are thought to contain about three monomer units so that relatively sharp ^{31}P signals are to be expected.

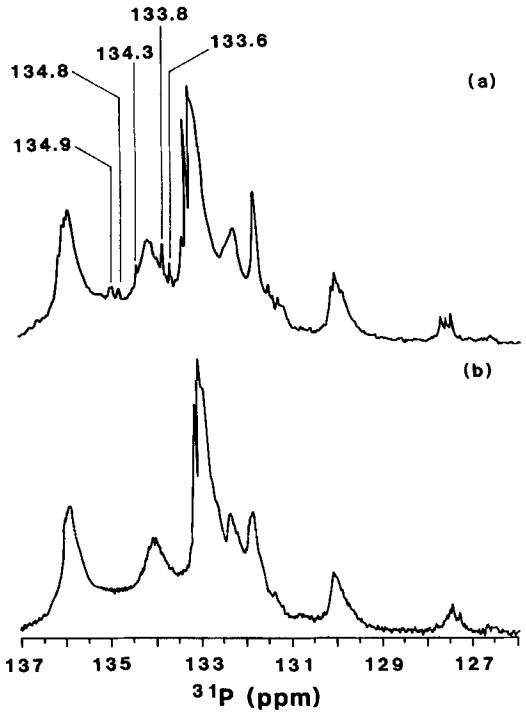


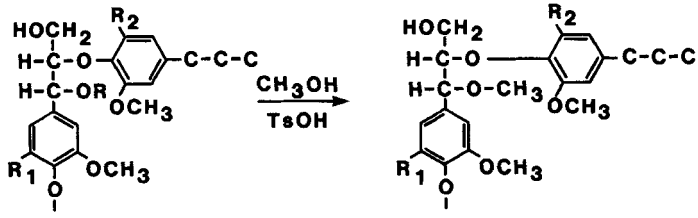
Figure 2: ^{31}P NMR spectra of derivatized birch dioxane lignins; (a) Unpurified sample (b) Purified from carbohydrates by liquid-liquid extraction (11). Acquisition conditions: lignin 18-25 mg, pyridine 400 μL, $CDCl_3$ 200 μL, (I) 50 μL.

The spectrum of derivatized black spruce milled wood lignin (Figure 3a) showed no signals due to carbohydrate hydroxyls in agreement with the analysis for carbohydrate content in Table 1.

Assignment of ^{31}P Signals Due to β-O-4 Structures Present in Lignin.

Lignins have been alkylated under mild conditions (14) with methanol in the presence of p-toluenesulphonic acid (Scheme 2).

This reaction methylated β-0-4 structures in model compounds at the *alpha* hydroxyl position (14). When the *alpha* hydroxyls were etherified (i.e, R=ethyl),



R = H, alkyl or aryl
R_1 = H or OCH_3
R_2 = H or OCH_3
TsOH = toluene sulphonic acid

Scheme 2: The alkylation reaction aimed at methylating the *alpha* hydroxyl groups in β-O-4 structures in lignins (14).

the reaction was found to proceed toward complete transetherification. Furthermore, aldehydes were partially converted into acetals. Nevertheless reactions with model compounds have generally supported the introduction of the methoxyl group in the *alpha* position of β-O-4 models (14).

Alkylation experiments on spruce milled wood lignin (softwood) introduced about one methyl group on every second phenylpropane unit, as revealed by proton NMR spectra. Groups susceptible to alkylation under these conditions were found to be the benzyl alcohols and their ethers. About half of the aldehyde groups were also assumed to be converted into acetals. In similar alkylation experiments on birch lignin (hardwood), a fraction of the benzyl hydroxyls was found unalkylated, even though the alkylation treatment was maintained for 5 days (14).

In part I of this series it was observed that derivatives of (I) with the *alpha* hydroxyl groups in lignin model compounds resembling β-O-4 structures gave distinct signals characteristic of their *erythro* and *threo* forms (5). The solvent effects altering the chemical shifts and the resolution of these signals were examined in model compounds and lignin (7). The accumulated evidence pointed

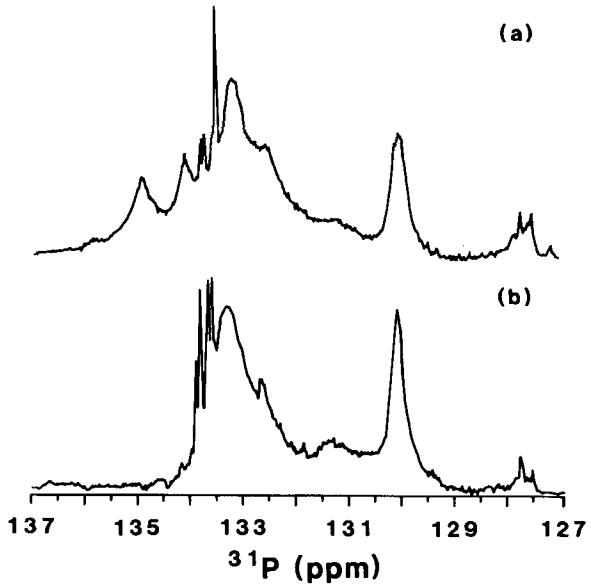


Figure 3: The ^{31}P NMR spectra of (a) black spruce milled wood lignin, (b) methylated black spruce milled wood lignin, derivatized with (I). Acquisition conditions: lignin 18-25 mg, pyridine 400 μL, $CDCl_3$ 500 μL, (I) 50 μL.

toward assigning the signals appearing at around 136, 135 and 134 ppm as being due to the *erythro* and *threo* forms in derivatized *alpha* hydroxyl groups present in β-O-4 structures in lignin.

Supporting evidence for this assignment was sought by using the lignin alkylation studies of Adler, Brunow, and Lundquist (14). Figure 3 shows how methylation of black spruce milled wood lignin resulted in the complete elimination of the two broad peaks at 135.0 and 134.2 ppm attributed to the *erythro* and *threo* forms, respectively, of the derivatized *alpha* hydroxyl groups in β-O-4 structures. This result agrees well with the alkylation scheme proposed by Adler, Brunow, and Lundquist (14).

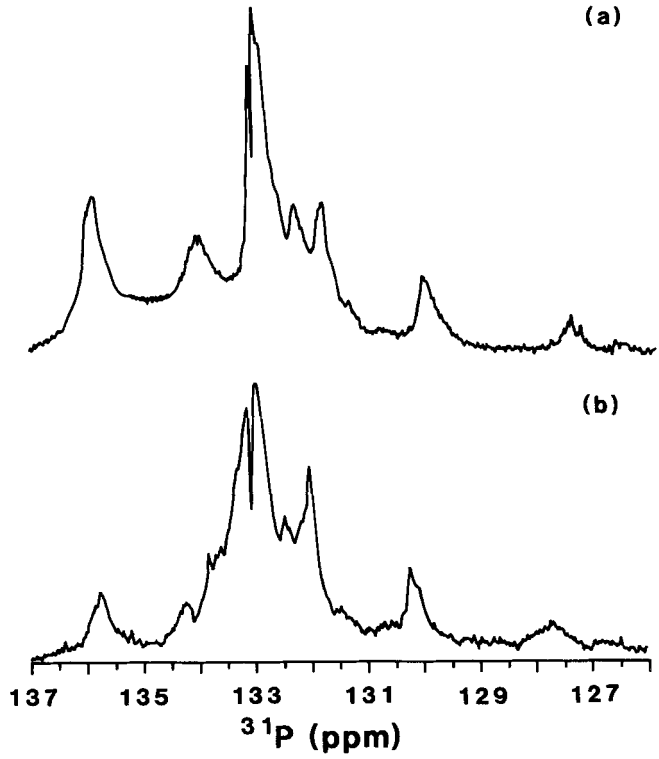


Figure 4: The ^{31}P NMR spectra of (a) birch dioxane lignin, (b) methylated birch dioxane lignin, derivatized with (I). Acquisition conditions: lignin 18-25 mg, pyridine 400 μL, $CDCl_3$ 200 μL, (I) 50 μL.

The integration of the signal at around 130 ppm, due to the derivatized guaiacyl phenolic hydroxyls, remained unaltered after methylation, thus providing supporting evidence for the selectivity of this reaction. Clearly, the *alpha* benzylic hydroxyls in aryl-β-O-4-guaiacyl structures (predominant in softwood lignins) are completely methylated when the reaction proceeds at 40°C for a period of four days.

The methylation of purified birch dioxane lignin (Figure 4) was not as efficient as that of black spruce milled wood lignin. A relatively intense residual

signal was apparent at around 135.8 ppm in the spectrum of the methylated sample (Figure 4b). Integration of the signal at around 135.8 ppm before and after methylation revealed that about 70% of the *alpha* benzylic hydroxyls were methylated. This finding implies that the *alpha* benzylic hydroxyls in β-O-4 structures within birch lignin are less reactive toward alkylation than those in spruce lignin. This difference can be attributed to the aryl part of the β-O-4 ether. In spruce lignins the aryl part of the β-O-4 ether is composed entirely of guaiacyl structures while in birch lignins these parts are both of the guaiacyl and the syringyl type (21-25). The decreased reactivity toward methylation observed in the hardwood lignin is also consistent with the work of Adler, Brunow, and Lundquist (14).

It is also to be noted that the presence of the second methoxyl group in the aryl part of the β-O-4 ether caused a strong downfield shift of the phosphitylated *erythro alpha* hydroxyls in birch dioxane lignin (hardwood) compared to those in black spruce milled wood lignin (softwood) (see Figures 3a and 4a and Table 2).

Assignment of ^{31}P Signals Due to Phenolic Hydroxyls in Lignin.

Lignin samples may contain different types of phenolic hydroxyls depending on wood species and their treatment during isolation. Birch dioxane lignin, being isolated from a hardwood under acidolysis conditions is expected to contain syringyl and guaiacyl phenolic groups together with some diphenyl ether and condensed biphenyl structures. The latter may arise from condensation reactions occurring during dioxane acidolysis (26).

Figure 1 showed the ^{31}P NMR spectra of birch dioxane lignin and of black spruce milled wood lignin derivatized with (I). One distinct difference between these spectra is the presence of a signal at around 131.9 ppm in the spectrum of birch dioxane lignin and its absence from that of the black spruce milled wood lignin.

The ^{31}P signals of model compounds of guaiacyl phenolic hydroxyls derivatized with (I) were invariably found at around 130 ppm, while their syringyl

Table 2 ^{31}P NMR Chemical Shift Ranges and Solvent Concentration-Dependency of Various Functionalities in Lignins after Derivatization with (I).

High pyridine concentration mole fraction: 0.4-0.5 ^{31}P Chemical shift (ppm)	Low pyridine concentration mole fraction: 0.2-0.3 ^{31}P Chemical shift (ppm)	Lignin Functionality
136.5-135.8	136.7-136.2	Hydroxyl groups in xylan
136.8-135.2	136.2-135.4	*Erythro alpha*-hydroxyls in arylglycerol-β-syringyl units
135.2-135.4	135.4-134.7	*Erythro alpha*-hydroxyls in arylglycerol-β-guaiacyl units, xylan or LCC .
134.5-133.7	around 134.7	*Threo alpha*-hydroxyls in arylglycerol-β-syringyl and guaiacyl units, LCC
133.7-133.2	134.3-133.8	*Gamma* hydroxyls in *alpha*-carbonyl containing units, cinnamyl alcohol, LCC
133.2-132.7	133.7-132.8	*Gamma* hydroxyls in arylglycerol-β-aryl units
132.7-132.1	132.8-132.3	Primary aliphatic hydroxyls (probably phenylcoumaran structures)
132.1-131.6	132.3-131.6	Phenolic hydroxyls in syringyl structures.
131.6-131.0	131.6-131.0	Phenolic hydroxyls in biphenyl units, cinnamic aldehydes.
130.4-129.7	130.5-129.9	Phenolic hydroxyls in guaiacyl structures.
129.7-129.3	129.9-129.5	Phenolic hydroxyls in guaiacyl and catechol structures
27.1-126.5	27.1-126.5	Carboxylic hydroxyls in aliphatic acids, cynnamic acid.

counterparts always gave ^{31}P signals positioned downfield at about 131.5 ppm (5). From this evidence the signal at around 131.9 ppm in the spectrum of the birch dioxane lignin may be assigned to the syringyl phenolic hydroxyls, and the signal at around 130 ppm to its guaiacyl hydroxyls. The absence of a clear signal at around 131.9 ppm in the spectrum of the softwood (black spruce) milled wood lignin strongly supports this assignment, since the proportion of syringyl units in softwood lignins is known to be rather small.

Despite this strong evidence for the assignment of the predominant phenolic structures in lignins, it is likely that some of the signals in these spectra may overlap or be obscured by the high intensity broad signals arising from the derivatized alcoholic hydroxyls that also appear in the 132-133 ppm region.

The aminolysis of acetylated lignin model compounds and of lignins with pyrrolidine quantitatively deacetylates the phenolic hydroxyls (12). Certain alcoholic acetates may also deacetylate in the presence of pyrrolidine, but at a much slower rate than that of deacetylation of the phenyl acetates (12).

For this reason a further attempt was made to establish the actual position of the ^{31}P signals due to the phenolic hydroxyls by deacetylating an acetylated lignin sample (12-13) with pyrrolidine (aminolysis) followed by derivatization with (I). This reaction has been applied to the determination of phenolic hydroxyl groups in lignins and wood tissue with considerable success (13).

In the present work acetylated birch dioxane lignin was deacetylated according to the procedure described by Gellerstedt and Lindfors (13). Aliquots of the reaction mixture were withdrawn at various time intervals and the deacetylation reaction was quenched. The deacetylated lignins were then derivatized with (I) and their ^{31}P NMR spectra were obtained. This procedure made it possible to observe the changes in the distribution of the functional groups in birch dioxane lignin as the deacetylation reaction proceeded with time (Fig. 5).

The ^{31}P NMR spectra of the deacetylated lignins, derivatized with (I), shown in Figure 5, indicate that both phenolic and alcoholic hydroxyls are liberated within 10 minutes of the onset of the aminolysis reaction. Furthermore, the deacetylation of alcoholic hydroxyls seems to be a complex sequence of

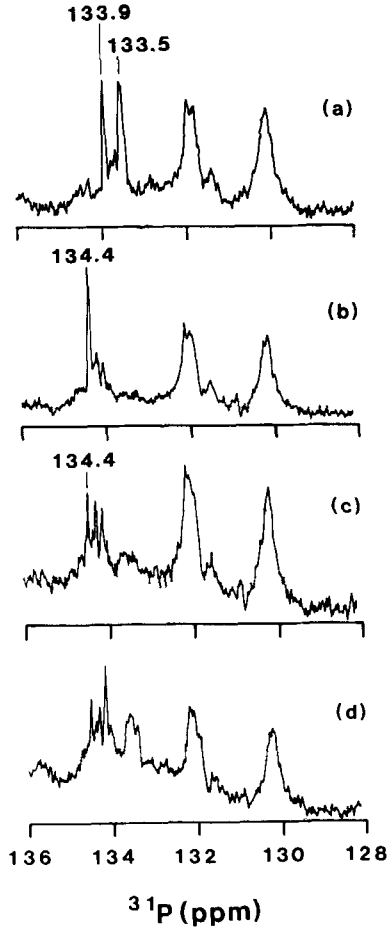


Figure 5: ^{31}P NMR Spectra of acetylated birch dioxane lignin which has been deacetylated with pyrrolidine for various periods of time and then derivatized with (I), (a) deacetylated for 10 minutes, (b) 20 minutes, (c) 60 minutes, (d) 120 minutes. Acquisition conditions: lignin 18-25 mg, pyridine 200 μL, $CDCl_3$ 500 μL, (I) 50 μL.

reactions, possibly including acetyl group migration during the early stages of aminolysis.

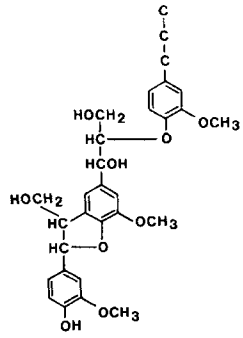
These conclusions are suggested by comparing the signals in the alcoholic hydroxyl region (between 135-133 ppm) to those in the phenolic hydroxyl region (at around 130.0 and 132.0 ppm) in all the spectra of Figure 5 . Relatively minor changes in signal shape and intensity occurred within the phenolic hydroxyl region, but the changes within the alcoholic region were quite dramatic.

Within 10 minutes of the onset of aminolysis (Figure 5a) two strong signals appeared at 133.9 and 133.5 ppm. An additional 10 minutes of reaction (Figure 5b) substantially reduced the intensity of these signals while a strong signal appeared at 134.4 ppm. The relative intensity of the alcohol region (135-133 ppm) was, however, increased from 43% at 10 minutes of reaction, to 55% at 20 minutes of reaction. This may be due to possible migration of acetyl groups or to other side-reactions. After one hour of deacetylation (Figure 5c) the relative intensity of the alcohol region further increased to 60% and new, sharp signals appeared in the 134.7-134.0 ppm region, while the signal at 134.4 ppm was again partially reduced in intensity.

Growth of signal intensity was also observed in the 134.0-133.3 ppm region and was further augmented after 2 hours of aminolysis (Figure 5d), while the relative intensity of the alcoholic hydroxyls further increased to 70%. This region can be tentatively assigned to primary hydroxyls with oxygen-containing substituents, i.e. hydroxymethyl groups in aryl-glycerol-β-aryl ether units.

At the same time there was almost no increase in signal intensity in the region between 133.4 - 132.4 ppm. This is the region where primary aliphatic alcoholic hydroxyls are expected to appear, hydroxymethyl groups in phenylcoumaran units (II).

The ratio of signal intensities of the syringyl hydroxyls to guaiacyl was about 0.9 after 10 minutes of aminolysis, and became 1.1 after 20 minutes. This may imply that the guaiacyl acetates are deacetylated somewhat faster than the syringyl acetates.

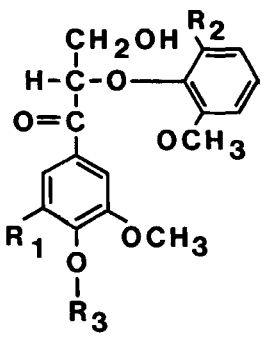


(II)

^{31}P NMR chemical shifts in biphenyl units (condensed lignin structures) should be close to that of the 2,2′-dihydroxy-3,3′-dimethoxy-5,5′-dipropylbiphenyl model compound which gave a sharp signal at about 131.0 ppm (5,7). In lignins, however, the relative stereochemical orientation of the two benzene rings might be different from that of the model compound because they are part of a rigid polymer structure. Therefore the ^{31}P NMR signals of the phosphitylated hydroxyls within such units may not appear close to those of the model, and may vary according to the substituents in the propyl chain and the relative orientation of the benzene rings. For these reasons, the small broad peak (at 131.4 ppm) located between syringyl and guaiacyl hydroxyls (Figures 1,3,4,5) may be partly attributed to the phenolic hydroxyls in biphenyl units. This region, however, may also contain signals from aldehydes present in lignins (5).

Assignment of ^{31}P Signals Due to Hydroxyls Neighbouring Carbonyl Groups.

The treatment of lignins with sodium borohydride is a well-established procedure that reduces aldehyde and ketone groups. During the present investigation the ^{31}P NMR spectra of reduced lignins derivatized with (I) were determined in order to identify the labile protons whose neighbouring groups are carbonyls.



III R_1,R_2=H or OCH_3, R_3=H or Aryl
IV R_1=R_2=H, R_3=CH_2-Phenyl

Since the *alpha* carbonyl structures (III) in lignins are rather resistant to borohydride reduction, birch dioxane lignin, purified from carbohydrates, was reduced for one week in the presence of a relatively high concentration of sodium borohydride. The ^{31}P NMR spectrum of the derivatized product is shown in Figure 6b together with that of the starting sample (Figure 6a). The main difference between these two spectra is that the intensities of the sharp signals at around 134 ppm are considerably less in the reduced sample, while the area of the broad signal at around 133.4 is somewhat enhanced.

It is likely, therefore, that the sharp signals appearing between 134.4-133.8 ppm are due to hydroxyls with neighbouring carbonyl groups, or to *alpha*-carbonyl structures with terminal hydroxymethyl groups. On reduction, the carbonyl groups become alcohols. As such the environment of the neighbouring hydroxyls is transformed in such a way that the ^{31}P NMR signals of their derivatives appear within the broad envelope that ranges between 133.7-133.1 ppm.

In the expanded spectrum of birch dioxane lignin (Figure 6a), three clear signals are present at 134.33, 134.28 and 134.11 ppm. The two 134.33 and 134.11 ppm were drastically decreased in intensity after reduction (Figure 6b). In order to further investigate the nature of these signals, the model compound (IV), containing an *alpha*-carbonyl group, was added to the sample of Figure 6a. A

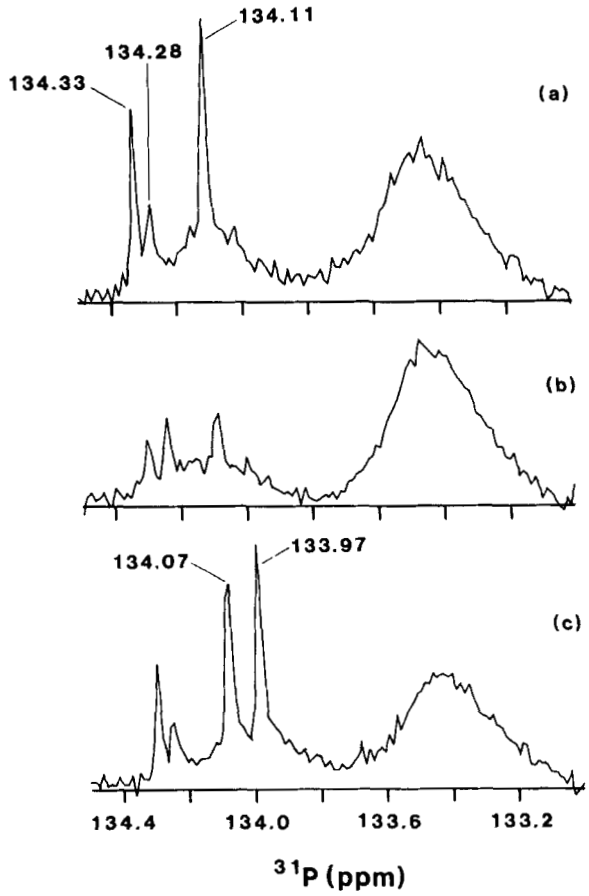


Figure 6: The expanded region (134.4-133.0 ppm) of ^{31}P NMR spectra of (a) birch dioxane lignin (purified from carbohydrates), (b) birch dioxane lignin reduced with sodium borohydride, (c) birch dioxane lignin with the addition of 1-2 mg of compound (IV) and cinnamyl alcohol. Acquisition conditions: lignin 10 mg, pyridine 400 μL, $CDCl_3$ 1000 μL, (I) 50 μL.

signal at 134.07 ppm appeared (Figure 6c). Thus, it is likely that the signal at 134.11 ppm in lignin may be due to derivatized hydroxyls neighbouring carbonyl groups. The slight difference in chemical shifts is most likely due to the somewhat different structure of the carbonyl-containing fragment in the model compound and the lignin.

In a further effort to assign these sharp signals, the sample of derivatized birch dioxane lignin, with compound IV added to it, was further spiked with cinnamyl alcohol and 3,4,5-trimethylbenzyl alcohol. These compounds gave signals at 133.97 ppm and 133.79 ppm, respectively. These signals overlapped with the signal of the *alpha* carbonyl-containing model compound (III). Cinnamyl alcohols and cinnamic aldehydes are structures present in low abundance in lignins (16,25,27). The ^{31}P NMR signals due to *alpha* carbonyl-containing structures appear to overlap with the signals due to cinnamyl alcohol structures in lignin.

Furthermore, adding *trans*-cinnamic aldehyde in the same sample gave rise to a ^{31}P NMR signal at 131.46 ppm. The small, relatively broad shoulder at 131.35 ppm that appears in the ^{31}P NMR spectrum of the purified birch dioxane lignin of Figures 1a and 2b was almost completely eliminated after the sample was reduced with sodium borohydride. It is thus likely that the origin of this signal may, at least partially, be due to cinnamic aldehydes.

The Concentration Dependency of ^{31}P NMR Chemical Shifts of the Various Functionalities Present in Lignins.

In part IV of this series the position of the ^{31}P NMR chemical shifts of a number of the functional groups found in lignins were found to be somewhat affected by the amount of solvent present (7). In order to relate these results to the assignment of the ^{31}P NMR signals from lignins derivatized with (I), the solvent effect was also addressed during the present investigation. Table 2 summarizes the ^{31}P NMR chemical shift ranges of a variety of functional groups in lignins at two levels of pyridine concentration. This table may serve as a guide

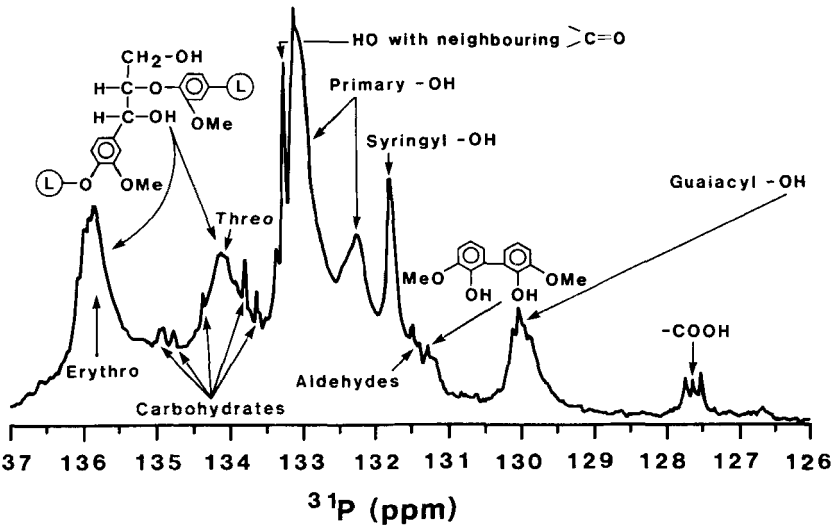


Figure 7: The ^{31}P NMR spectrum of birch dioxane lignin derivatized with (I), and the assignment of the signals. Acquisition conditions: lignin 18-25 mg, pyridine 400 μL, $CDCl_3$ 200 μL, (I) 50 μL.

for assigning the ^{31}P NMR chemical shifts received when lignins are derivatized with 1,3,2-dioxaphospholanyl chloride (I).

From the results of this and previous work (5,6,7), the 31P NMR spectrum of birch dioxane lignin derivatized with (I) is assigned in Figure 7, which provides a general guide to interpreting most of the main 31P NMR signals of lignins derivatized with (I).

CONCLUDING REMARKS

The results in this paper show that the derivatization with 1,3,2-dioxaphospholanyl chloride, followed by ^{31}P NMR spectroscopy, has considerable potential for the structural analysis of lignins. The method offers information on a wide variety of functional groups that bear labile protons and are present in

lignins. The merit of this method is its simplicity, and is thus recommended for the rapid analysis of soluble lignins.

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