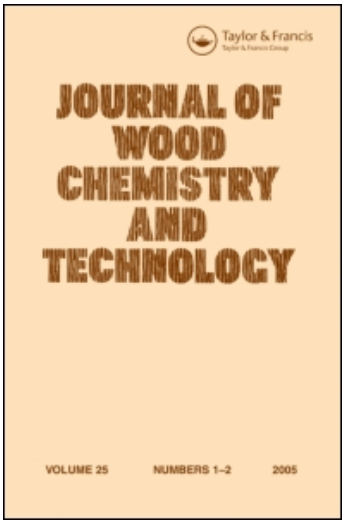

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^{31}P NMR Spectroscopy in Wood Chemistry. I. Model Compounds

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^{31}P NMR SPECTROSCOPY IN WOOD CHEMISTRY PART I - MODEL COMPOUNDS

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ABSTRACT

The quantitative reaction in an NMR tube of 1,3,2-dioxaphospholanyl chloride (I) with compounds bearing active hydrogens was explored as a simple method for derivatizing the labile centres known to occur in lignins. Derivatives of phenols, alcohols and carboxylic acids with (I) gave ^{31}P chemical shifts which appeared in different ranges of the NMR spectra. Any *ortho* substitution onto the aromatic ring of phenols significantly affected the magnitude of the ^{31}P NMR chemical shifts, while *para* and/or *meta* substituents had a much smaller effect. A clear distinction between guaiacyl, syringyl and unsubstituted phenolic hydroxyls can thus be made in mixtures of model compounds. Primary alcohols were clearly distinguished from secondary and tertiary alcohols in their derivatives with (I), while different ^{31}P NMR signals for derivatives of *erythro* and *threo* forms of lignin-like model compounds were identified. While alcohols, phenols and simple carboxylic acids on reaction with (I) gave derivatives that were substitution products, aldehydes reacted via a distinctly different addition mechanism; ketones did not react at all.

INTRODUCTION

Lignins are known [1] to contain relatively small proportions of phenolic, aldehydic, carboxylic, alcoholic, and, in some derivatives, sulphonic acid functionalities, which are all characterized by the labile nature of their protons. Proton NMR spectroscopy alone has not been adequate for determining these groups in lignins. The situation has been improved by the

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concerted use of proton and ^{13}C NMR spectroscopy [2] with attempts to obtain resolvable spectra by substituting the labile protons with suitable groups. The two most common methods are acetylation [3,4] followed by ^{13}C NMR spectroscopy, and, to a lesser extent, silylation [5-7] followed by ^{29}Si NMR spectroscopy.

Acetylation of lignins is relatively simple to achieve under well established and mild conditions [2]. It has been used to estimate the total hydroxyl context in various lignin preparations from proton and ^{13}C NMR spectra [2,3,4]. The ^{13}C NMR spectra may also reveal other structural features of lignins. These spectra permit the distinction to be made between aromatic and aliphatic hydroxyls, and reveal the primary and secondary character of aliphatic hydroxyls [3,4].

The use of silylation to characterize the labile proton-containing groups in lignins, [5-7] is based on the well established procedures of silylation of labile proton-containing compounds employed prior to gas chromatographic analysis [8]. The ^{29}Si NMR spectra of such derivatized lignins contain features that may distinguish between aromatic and aliphatic hydroxyls and carboxylic protons. The method, however, requires large sample concentrations and long instrument times. This is due to the relatively low natural abundance of the silicon 29 nuclei (4.7%), their low magnetic moment, and their high relaxation time.

A major limitation of both methods is that they cannot distinguish between hydroxyls of lignin itself and those of the carbohydrate contaminants, thus requiring sample purification prior to derivatization.

Our particular needs to identify and quantify functional groups in lignins have prompted us to examine other NMR-active nuclei and derivatization procedures which may provide new structural information and overcome the previous limitations. In selecting NMR-active nuclei that may be used to label functional groups in lignins one must consider 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.

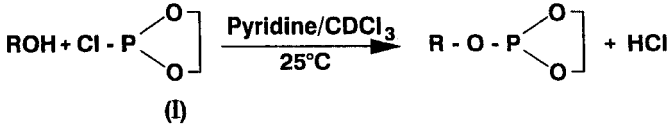
Phosphorus-31 is a nucleus that largely meets these criteria. Its 100% natural abundance makes it ideal for NMR studies. The sensitivity of a ^{31}P NMR experiment is only about 15 times less than that of a proton NMR experiment. The reported range of ^{31}P chemical shifts is more than 1000 ppm and the average line width is about 0.7 Hz [9]. Many classes of

organophosphorus compounds give signals within narrow ranges specific to the state of the phosphorus nuclei. Good relationships have been identified between chemical shift information and structure. In some instances, even stereochemical details are revealed [10,11]. Phosphorus compounds exhibit a rather versatile chemistry because phosphorus may assume all the coordination numbers ranging from 1 to 6. Most of the reactions of phosphorus compounds are governed by the high nucleophilicity of tricoordinated phosphorus towards a wide variety of electrophiles, the strong bonds that are formed (with oxygen, nitrogen, sulphur, halogens and carbon), and the stabilization of the adjacent anions by the phosphorus center. The readily available phosphorus halides [12,13] react rapidly and quantitatively with functional groups bearing labile protons to form an -XP bond where X may be oxygen, sulphur, or nitrogen [10-13].

$$P\text{-}Cl + H\text{-}X \xrightarrow[base]{} P\text{-}X + HCl\text{-}base$$

The range of chemical shifts of the ^{31}P nuclei in such P-X compounds may permit differentiation of the different environments attached to the phosphorus atoms. These techniques have been employed by Verkade's group [14-17], who used 1,3,2-dioxaphospholanyl chloride (I) and other reagents, to derivatize coal extracts, condensates, and pyrolysates in order to examine their functional group distributions.

This report describes our initial attempts to develop qualitative and semiquantitative methods of lignin functional group determination based on ^{31}P NMR spectrometry of phosphite lignin esters. In order to identify the ^{31}P chemical shift ranges of a number of labile centres in lignins, we have examined a variety of model compounds. They were derivatized by means of the quantitative reaction shown in Scheme 1.

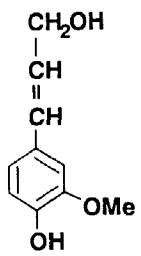


(I)

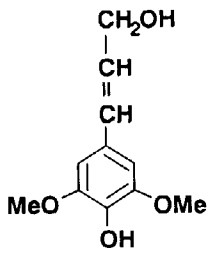
Where R=Residues of Phenols, Alcohols, Aldehydes, Sugars, Carboxylic Acids

Scheme 1. The derivatization reaction used throughout this work, i.e., the reaction of 1,3,2-dioxaphospholanyl chloride (I) with active hydrogen compounds.

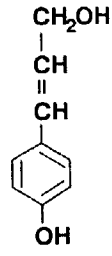
In 1962, Freudenberg *et al.* [18] found that a mixture of coniferyl alcohol (II), synapyl alcohol (III) and p-coumaryl alcohol (IV), in proportions 80:6:14, added to an aqueous solution of a dehydrogenating enzyme, gave an extremely complex macromolecular substance, which is generally accepted to resemble lignin.



Coniferyl Alcohol
(II)



Sinapyl Alcohol
(III)



p-Cumaryl Alcohol
(IV)

In natural lignins, the molar ratio of these phenylpropane building blocks mainly depends on the nature of the plant species. Thus, gymnosperm (softwood) lignins are derived mostly from coniferyl alcohol (II), while angiosperm (hardwood) lignins are produced from coniferyl (II) and sinapyl (III) alcohols. Grass lignins are derived from all three alcohols. More specifically, softwood lignins are composed almost exclusively of guaiacylpropane units, but also contain 4-hydroxyphenylpropane units and syringylpropane units. About 70% of the guaiacylpropane units are etherified at the position *para* to the side chain while the rest bear free phenolic hydroxyl groups [19-21].

The primary objective of this work was to develop simple and reliable techniques which would resolve the three fundamental environments in lignins. The model compounds thus selected contained most of the structural features of subunits (II), (III), and (IV) and some of their copolymerization products.

EXPERIMENTAL

Derivatizing Reagent

All solvents and chemicals used were of reagent or analytical grade. The derivatizing reagent, 1,3,2-dioxaphospholanyl chloride (I) (commercially available, Fluka Chem. Co.), was

synthesized [12] from phosphorus trichloride (99.9%) and anhydrous ethylene glycol (99%). After purification by vacuum distillation, the yield was approximately 74%. Its purity was confirmed by its proton and ^{31}P spectra, and those of its derivative with methanol. The ^{31}P chemical shift of (I) was 167.3 $\pm$ 0.3 ppm as described in the literature [22].

Derivatization Procedure

Model compounds were derivatized in a 1:1 mixture of deuterated chloroform and anhydrous pyridine which acted as both solvent and acid acceptor. The chloroform prevented the precipitation of the pyridine hydrochloride byproduct which could otherwise interfere with the reaction itself and the process of obtaining the spectra. Typically, samples were derivatized in a 10-mm NMR sample-tube or in a 4-mL vial equipped with a small magnetic bar. A solution of the active-hydrogen model compound (0.06-0.08 mmol) in 400 microlitres of pyridine/$CDCl_3$ mixture was first prepared and 150 microlitres of (I) was added with stirring. The reaction was complete within a few minutes. Finally, a small amount of triphenylphosphine was added to serve as an internal standard, when required. Mixtures of model compounds were derivatized in 10 mm sample tubes. This was done when small differences in chemical shifts were to be evaluated. The total amount of the model compounds used was about 0.18-0.2 mmoles, and 200-400 microlitres of (I) was added. The total volume of the solution was adjusted with $CDCl_3$ to approximately 3.0 ml.

NMR Spectroscopy

The ^{31}P and ^{13}C NMR spectra were determined, in 10 or 5 mm sample tubes, on a Varian XL-300 NMR spectrometer operating at 121.5 and 75.4 MHz, respectively. The internal deuterium lock was provided by the deuterium atoms present in the deuterated solvent. The external standard was 85% H_3P0_4, and a sweep width of 10000 Hz was employed. For qualitative studies a pulse delay of 0.5 seconds, with a pulse width corresponding to a 45° flip angle, and a total of 128 transients were sufficient. For quantitative work a pulse delay of 8 seconds and a pulse width of 90° were used. All downfield shifts from H_3P0_4 were considered positive. When model compounds were individually derivatized, the reproducibility of their ^{31}P chemical shifts was found to be about 0.1 ppm. Proton NMR spectra were recorded on a Varian XL-200 spectrometer.

RESULTS AND DISCUSSION

A variety of lignin-related model phenols, alcohols, carboxylic acids and aldehydes, were derivatized with (I) and their ^{31}P NMR chemical shifts were recorded (Tables 1,2,3,4).

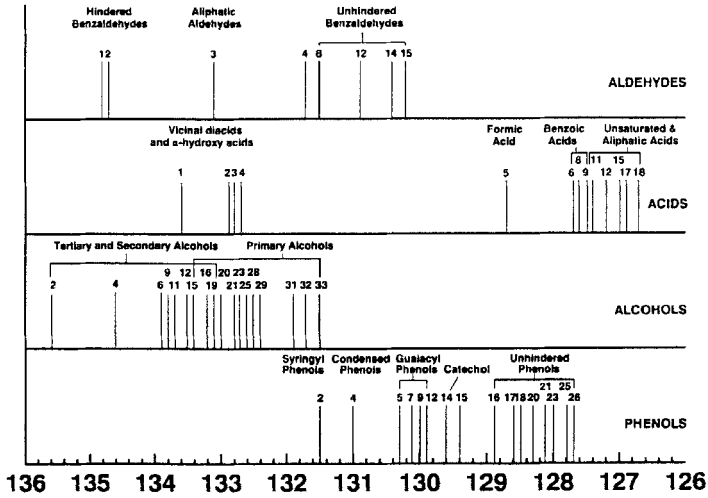
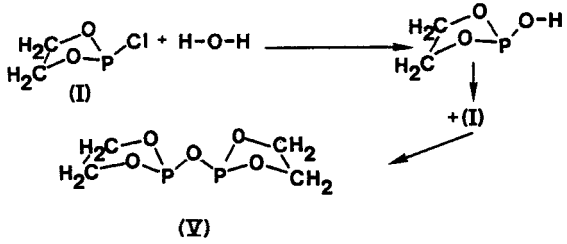


Figure 1. Schematic representation of ^{31}P NMR chemical shifts for the various classes of model compounds examined. The numbers on the simulated signals correspond to the compound numbers in Tables 1 to 4.

Figure 1 summarizes the information given in Tables 1 to 4 and also allows for a clear distinction of the chemical shift ranges observed for the various chemical environments.

Preliminary Experiments

The ^{31}P NMR spectrum of guaiacol (2-methoxyphenol) derivatized with (I) exhibited sharp signals at 130.1 ppm and at 121.1 ppm. Most of the model compounds examined in this work displayed a ^{31}P signal characteristic of the chemical environment around their labile centre, together with a signal at 121.1 ppm. This latter signal was independent of the nature of the compound and the experimental conditions. Its intensity correlated with the moisture content in the sample. When (I) was mixed with wet pyridine in the absence of any other compound, it gave a single signal at 121.1 ppm which varied in intensity with the amount of water added. Thus, it is likely that the signal at 121.1 ppm was due to the product of a reaction between (I) and water, small amounts of which are always present in hydrophylic phenols, acids, and alcohols, as well as in lignins, Scheme 2.



Scheme 2. The reaction of 1,3,2-dioxaphospholanyl chloride (I) with water.

The ^{31}P chemical shift of (V) has been reported to be 121.4 ppm [15]. This signal is rather sensitive to the presence of moisture, because the compound contains two phosphorus atoms so that the intensity of its ^{31}P signal is augmented for every molecule of water present (Scheme 2). The intensity of this signal was also found to be more easily controlled than that of triphenylphosphine which needs to be accurately metered in the sample tube for a good working signal to appear. It was also found that the position of this line was not affected by experimental conditions, and hence was more suitable reference signal than that of triphenylphosphine for assigning the resonances of the compounds. The suitability of this shift is because compound (V) chemically resembles the phosphitylated derivatives and its signal appears close to theirs. This proximity facilitates the procedure of expanding the regions of interest.

Phenols

The ^{31}P NMR chemical shifts of almost all phosphorylated phenols examined are within the narrow range of 127.7 to 131.5 ppm (Table 1). Only 2,6-di-*tert*-butyl phenol, after phosphorylation, gave rise to signals outside of this range, at 135.2 ppm.

Within the region of 127.7-131.5 ppm most of the derivatized phenols formed two groups with similar ^{31}P NMR chemical shifts. The first being the *meta* and/or *para* substituted phenols and the second *ortho* and di-substituted phenols. Unhindered phenols gave ^{31}P chemical shifts centred around 128.0 ppm and the introduction of any substituent (OH,OMe,Me or phenyl) in the *meta* and/or *para* position caused small deviations from this centre. In contrast *o*-substitution resulted in significant downfield shifts ranging from 0.8 to 7.1 ppm depending on the number of o-substituents and their size. Thus, the ^{31}P NMR chemical shifts in 1,3,2-dioxaphospholanyl derivatives of structures resembling lignin units (II), (III) and (IV) are very sensitive to the presence of one or two substituents *ortho* to the phenolic hydroxyl.

Table 1. ^{31}P NMR Chemical Shifts of Phenols Derivatized with (I).

No.	Phenol	Chemical Structure	Chemical Shift ppm
1	2,6-di-*tert*butylphenol	2,6-$((CH_3)_3C)C_6H_3OH$	135.2
2	2,6-dimethoxyphenol	2,6$(OCH_3)_2C_6H_3OH$	131.5
3	Syringaldehyde	4-$(OH)C_6H_2$-3,5-$(OCH_3)_2CHO$	131.5
4	2,2′-dihydroxy-3,3′-dimethoxy 5,5′-dipropylbiphenyl		131.0
5	2-methoxyhydroquinone	2-OCH_3-C_6H_4-1,4-$(OH)_2$, Guaiacyl OH	130.3
6	2-methoxy-4-methylphenol	2-OCH_3-4-Me-C_6H_3OH	130.1
7	Eugenol	4-$H_2C=CH(CH_2)C_6H_3$-2-$(OCH_3)OH$	130.1
8	2-methoxyphenol (Guaiacol)	2-MeO-C_6H_4-OH	130.1
9	Vanillin	4-$(OH)C_6H_3$-3-$(OCH_3)CHO$	130.0
10	Vanillomandelic acid	DL-4-OH-3-$MeOC_6H_5CH(OH)COOH$	130.0
11	4-hydroxy-3-methoxy-*trans* cinnamic acid	4-OH-3-MeO-$C_6H_5CH=CHCH_2COOH$	130.0
12	Acetovanillone	HO-2-OCH_3-C_6H_3-$COCH_3$	129.9
13	Isoeugenol	$CH_3OC_6H_3$ $(CH=CHCH_3)OH$	129.9
14	Catechol	C_6H_4-1,2-$(OH)_2$	129.6
15	2,6-diphenylphenol	2,6-$(C_6H_5)_2$-C_6H_5OH	129.4
16	Salicyl aldehyde	2-$(OH)C_6H_4CHO$	128.9
17	Methyl salicylate	2-$(OH)C_6H_4COOCH_3$	128.6
18	4-hydroxybenzaldehyde	4-$(OH)C_6H_4CHO$	128.5
19	2-isopropylphenol	2-$(CH_3)_2CH$-C_6H_4OH	128.5
20	*cis*-4-hydroxy cinnamic acid	4-OH-$C_6H_5CH=CHCH_2COOH$	128.3
21	Phenol	C_6H_5OH	128.1
22	4,4′-dihydroxy-2,2′-dimethoxybiphenyl		128.1
23	3,5-dimethoxyphenol	3,5-$(OCH_3)_2C_6H_3OH$	128.0
24	2-methoxyhydroquinone	2-OCH_3-C_6H_4-1,4-$(OH)_2$	128.0
25	3,4-dimethoxyphenol	3,4-$(OCH_3)_2C_6H_3OH$	127.8
26	4-ethoxyphenol	4-(CH_3CH_2O)-C_6H_4OH	127.7

Mono and di substitution of *o*-phenols influenced the ^{31}P NMR chemical shifts of their 1,3,2-dioxaphospholanyl derivatives in the following order:

$$H < -CHO < -isopropyl < -diphenyl < -OH < -OMe < -OMe,Ph < -diOMe < -di-tert-Bu$$

The most significant influence is observed when the two *tert*-butyl groups are the nearest neighbours to the phenolic hydroxyl. The same observation has been made by Wroblewski *et al.* [15], who investigated a variety of alkyl substituted phenols by ^{31}P NMR spectroscopy after phosphorylation with (I). According to their work the order of the ^{31}P NMR chemical shifts in alkyl substituted o-phenols is as follows:

$$H=Et < -Me < -isoPr < -COCH_3 < -Ph < -OH < -OMe < -diOMe < -diMe < -OH,Me < -OMe, -Me < -OH, isoPr < di-tert-Bu$$

In general, therefore, any *ortho* substitution to the ring (with the exception of ethyl or methyl groups) is the main contributor to the magnitude of the ^{31}P NMR chemical shifts. Substitution at the *para* and/or *meta* positions results in only small changes. The observed sensitivity towards *ortho* substitution may be due to a combination of steric and electronic factors. Steric effects are highly probable when a *tert*-butyl group is attached at the *ortho* position of a phenol. Interactions between unshared electron pairs of *ortho* substituents bearing oxygen and those of phosphorus of the 1,3,2-dioxaphospholanyl group are also likely. This may be demonstrated by the observation that introducing the relatively bulky isopropyl group onto the *ortho* position of phenol caused only a minor downfield shift of the ^{31}P chemical shift, while the introduction of a methoxy group at the *ortho* position of phenol, despite its smaller size, caused a much more sizable downfield shift.

A clear distinction between unsubstituted phenolic, guaiacyl and syringyl hydroxyls can thus be made. All three environments can readily be identified in their mixtures as well as guaiacyl type structures containing a second *ortho* substituent.

All compounds that contained two hydroxyls in different chemical environments gave ^{31}P NMR spectra with two signals whose positions were not affected by the presence of the other reaction centre. For example, phosphorylated 2-methoxy hydroquinone exhibited one signal, appearing as expected, in the phenol region (128.0 ppm), and another in the guaiacol region (130.3 ppm). Phosphorylated catechol gave only one signal at 129.6 ppm which was shifted 0.5

Table 2. ^{31}P NMR Chemical Shifts of the Phosphorylated Alcohols.

No.	Alcohol	Chemical Structure	Chemical Shift ppm
1	Dimethyl-L-tartate	$CH_3OOCCOHCOHCOOCH_3$	136.2
2	1,3-dititylglycerol	Ph_3-C-O-CH_2-CH-(OH)-CH_2-O-C-Ph_3	135.6
3	2-chloroethanol	CH_2ClCH_2OH	134.4
4	*cis*-1,2-cyclohexanediol	$C_6H_{10}(OH)_2$	134.6
5	*trans*-1,2-cycloheptanediol	$C_7H_{12}(OH)_2$	134.2
6	Phenyl-1,2-diol	$C_6H_5CH(OH)CH_2(OH)$, (2°OH)	133.9
7	(VII)		133.9
8	(S) (+) 1-phenyl 1,2-ethanediol 2-tosylate	$CH_3C_6H_4SO_3CH_2CH(C_6H_5)OH$	133.9
9	*tert*-butyl alcohol	$(CH_3)_3COH$	133.8
10	Methyl 2-hydroxyisobutyrate	$(CH_3)_2C(OH)COOCH_3$	133.8
11	Cyclohexane-1,4-diol	$C_6H_{10}(OH)_2$	133.7
12	Benzhydrol	$(C_6H_5)_2CHOH$	133.5
13	Methyl mandelate	$C_6H_5CH(OH)COOCH_3$	133.5
14	Phenyl-1,2-ethylene-diol	$C_6H_5CH(OH)CH_2(OH)$ (1°OH)	133.5
15	2-benzyloxyethanol	$C_6H_5CH_2OCH_2CH_2OH$	133.4
16	*sec*-phenylethyl alcohol	$C_6H_5CHOHCH_3$	133.2
17	1,4-dioxane-2,3-diol	2,3-$(OH)_2CH_2CH_2OCH_2CH_2O$	133.2
18	(VI)		133.2
19	1-phenyl-1-propanol	Ph-(OH)-CH-CH_2CH_3	133.1
20	4-OH,3-methoxy benzyl alcohol	4-OH-3-MeO-$C_6H_3CH_2OH$	133.0
21	1,4-dihydroxymethylbenzene	$C_6H_4(CH_2OH)_2$	132.8
22	Triphenylmethanol	Ph_3C-OH	132.7
23	Cinnamyl alcohol	$C_6H_5CH=CHCH_2OH$	132.7
24	3-methoxy-benzyl alcohol	3-MeO-$C_6H_3CH_2OH$	132.7
25	Ethylene glycol	CH_2OH-CH_2OH	132.6
26	Polyethylene glycol		132.6
27	Cyclohexylmethanol	$C_6H_{11}CH_2OH$	132.5
28	Propan-1-ol	CH_3-CH_2-CH_2-OH	132.5
29	3,4-dimethoxybenzyl alcohol	3,4-$(MeO)_2C_6H_3CH_2OH$	132.4
30	Phenylethyl alcohol	$C_6H_5CH_2CH_2OH$	131.9
31	Ethanol	CH_3CH_2OH	131.9
32	o-methoxy-phenethyl alcohol	2-$MeOC_6H_4$-CH_2-CH_2-OH	131.7
33	Methanol	CH_3OH	131.5

ppm upfield of that of phosphorylated guaiacol. This can be due to the symmetrical structure of the phosphorylated catechol in which both substituents have identical chemical environments.

Alcohols

The hydroxyls in aliphatic and benzylic alcohols were clearly distinguishable from phenolic hydroxyls in the spectra of the 1,3,2-dioxaphospholanyl derivatives of the model

compounds. Excluding 2,6-di-*tert*-butylphenol (which, in any case is not a structure likely to occur in lignin), the range of the ^{31}P NMR chemical shifts of derivatized alcohols was found to be about 5.0 ppm (131.5 to 136.2 ppm, Table 2). This region is about 0.5 ppm downfield from the phenolic region (Figure 1).

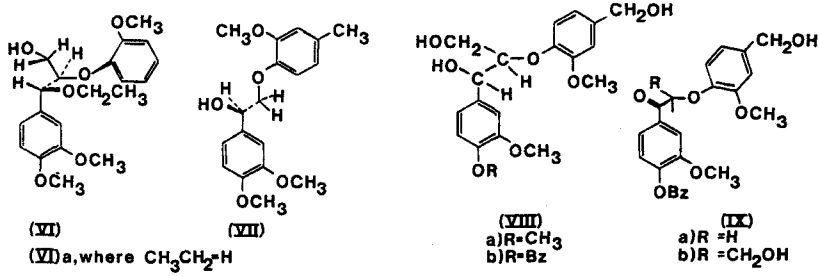
Most of the ^{31}P NMR chemical shifts of phosphorylated primary alcohols span a range of about 2.0 ppm. In general, secondary alcohols gave signals located about 0.5-2.5 ppm downfield from those of primary alcohols. A small overlap between primary and secondary alcohols exists in the region 133.1-133.4 ppm (Figure 1).

The results have shown that substitution of alcohols at C-1 influenced the ^{31}P NMR chemical shifts of their 1,3,2-dioxaphospholanyl derivatives in the following order.

$$H < CH_3 < PhCH_2 < CH_3CH_2,\ Cyclo\text{-}C_6H_{12} < tri\text{-}Ph,$$
$$C_6H_5\text{-}CH{=}CH < C_6H_5\text{-}OCH_2 < di\text{-}Ph < tri\text{-}CH_3 < CH_2ClCH_2 < CHCH_2OCPh_3$$

Steric and electronegativity factors both seem to affect the magnitude of the chemical shift.

There are various types of linkages between the phenylpropane units in lignin, the most frequent being those of arylglycerol-beta-aryl ethers (β-0-4 linkages) [1,2]. It became of interest, therefore, to examine whether the present technique could distinguish between primary and secondary hydroxyls in β-0-4 models.



Phosphorylation of compounds (VI) and (VII), which each bear only one hydroxyl group, gave derivatives with ^{31}P NMR signals located at rather different positions, 133.9 and 133.2 ppm respectively. When, however, compounds (VIII)a and (VIII)b, which each have 3 free hydroxyls, were reacted with (I) their ^{31}P NMR spectra contained five lines at 134.6, 133.7,

132.9, 132.8, 132.3, and at 134.7, 133.9, 133.0, 132.9, 132.5 ppm, respectively. The intensity of the first four lines was approximately equal while the intensity of the last line was double. It is likely that the appearance of five lines, instead of the expected three, is due to the coexistence of *erythro* and *threo* isomers in these β-0-4 compounds. Stereochemical isomeric forms of phosphorus compounds have been observed to give rise to such phenomena [10,11]. *Erythro* and *threo* isomers are also known to give different signals in the proton and ^{13}C NMR spectra [23,24]. The de-ethoxylated analogue of (VI), i.e (VI)a, which was in its *threo* form after reaction with (I), gave rise to two signals at 133.9 and 133.0 ppm assigned to the primary and secondary hydroxyls of the de-ethoxylated molecule. Hence it became possible to assign the signals at 134.6 and at 134.7 ppm in the ^{31}P NMR spectra of derivatized (VIII)a and (VIII)b to their *erythro* forms. It is thus possible that the method under discussion may yield information on *erythro* and *threo forms* within lignins.

Compounds (IX)a and (IX)b may also represent certain structures in lignin. These compounds, after phosphorylation with (I), gave signals at 133.0 ppm (IXa) and 133.4 and 132.8 ppm (IXb).

Derivatization with (I) of symmetrical alcohols containing two hydroxyls attached to adjacent carbons gave only one ^{31}P signal; for example, ethylene glycol, 1,4-dioxane-diol, *cis*-cyclohexane-1,2-diol and *trans*-cycloheptane-1,2-diol (Table 2). Their ^{31}P chemical shifts were shifted downfield by about 0.7 ppm to what one would expect for their monosubstituted analogues, as evidenced by ethylene glycol and ethanol (Table 2).

Such downfield shifts are more pronounced in glycols containing a substituent at C-2, as in phenyl-1,2-diol $\{C_6H_5CH(OH)CH_2(OH)\}$. This compound, when reacted with (I), gave a ^{31}P NMR spectrum composed of two lines of similar intensity at 133.5 and 133.9 ppm. These signals were assigned, on the basis of similar molecules to phenyl 1,2-diol containing either only one primary or only one secondary hydroxyl instead of both. The signal, therefore, at 133.5 ppm was assigned to the primary hydroxyl of phenyl-1,2-diol on the basis of the signal at 131.9 ppm obtained from phenyl ethyl alcohol $\{C_6H_5CH_2CH_2OH\}$, while the signal at 133.9 ppm was assigned to the secondary hydroxyl of phenyl-1,2-diol on the basis of the signal at 133.2 ppm obtained from *sec*-phenylethyl alcohol $\{C_6H_5CHOHCH_3\}$. Furthermore compound 8 (Table 2) in which the primary hydroxyl is blocked gave a single signal at 133.9 ppm.

Table 3. ^{31}P NMR Chemical Shifts of Acids Phosphorylated with (I).

No.	Acid	Chemical Structure	Chemical Shift ppm
1	Phthalic acid	C_6H_4-1,2-$(COOH)_2$	133.6, 127.6
2	Oxalic acid	HOOCCOOH	132.9
3	Glycolic acid	$HOCH_2COOH$	132.8, 139.5
4	Salicylic acid	HOC_6H_4COOH	132.7, 124.8
5	Formic acid	HCOOH	128.7
6	Phenylsuccinic acid	$COOHCH_2CH(C_6H_5)COOH$	127.7
7	Terephthalic acid	1,4-$COOH(C_6H_4)$	127.7
8	3,4-dimethoxy benzoic acid	3,4-$(MeO)_2C_6H_3COOH$	127.6
9	Benzoic acid	C_6H_5COOH	127.5
10	Dibenzoyl-L-tartaric acid	$[C_6H_5COOCH(COOH)-]_2$	127.4
11	Maleic acid	$HOO_2CCH=CHCOOH$	127.4
12	Muconic acid	$HOO_2CCH=CHCH=CHCOOH$	127.2
13	3,5-dimethoxy cinnamic acid	3,5-OMe-$C_6H_3CH=CHCOOH$	127.2
14	Cyclohexyl carboxylic acid	$C_6H_{11}COOH$	127.2
15	Phenylsuccinic acid	$COOHCH_2CH(C_6H_5)COOH$	127.0
16	4-hydroxy, 3-methoxy-*trans*-cinnamic acid	4-OH-3-MeO-$C_6H_3CH=CHCO_2H$	127.0
17	*cis*-4-hydroxy cinnamic acid	4-OH-$C_6H_4CH=CHCOOH$	126.9
18	Acetic acid	CH_3COOH	126.7

Glycols therefore, containing a substituent at C-2 when derivatized with (I), appeared to cause significant (about 1.5 ppm) downfield shifts at the phosphorus atoms attached at their primary centres, secondary centres being somewhat less downfield-shifted.

Low-molecular polyethylene glycol, after derivatization with (I), gave one sharp signal at 132.6 ppm, at the same position as the signal of ethylene glycol itself.

Carboxylic Acids

Table 3 shows the ^{31}P NMR chemical shifts of derivatives of selected carboxylic acids. Both aromatic and aliphatic carboxylic acids lie within a range of about 7 ppm, within which two separate groups of acids may be distinguished. The first group, located upfield, consists of acids which have no other functional groups undergoing phosphorylation close to the carboxylic centre. The second group includes 1,2-diacids or 1,2-hydroxyacids.

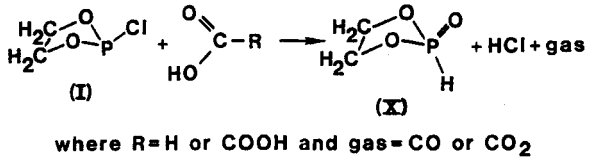
Among the chemical shifts of monofunctional acids shown in Table 3 benzoic acids in general are slightly shifted downfield compared to their saturated and unsaturated olefinic counterparts.

The phosphorylated derivative of phenylsuccinic acid {$COOHCH_2CH(C_6H_5)COOH$} gave two ^{31}P NMR signals: a downfield signal at 127.7 ppm, and an upfield signal at 127.0 ppm. Wroblewski *et al.* [15] reported that phosphorylated 2-hydroxysuccinic acid {$HO_2CCH_2CH(OH)CO_2H$} gave three ^{31}P signals: 128.2 ppm (1-COOH), 127.6 ppm(3-COOH) and 136.8 ppm (2-OH). Two diffferent signals were also observed for the two unsymetrically located derivatized carboxylic hydroxyls in 2,2-dimethylglutaric acid {$HOOCCH_2CH_2C(CH_3)_2COOH$} while phosphorylated 3,3-dimethylglutaric acid {$HOO_2CCH_2C(CH_3)_2CH_2COOH$} gave only one ^{31}P signal at 127.5 ppm [15]. These observations suggest that aliphatic dicarboxylic acids derivatized with (I) give two ^{31}P chemical shifts when substituents are introduced to the carbon *alpha* to the carboxyl groups. The downfield signal is due to this carboxyl group. It is thus possible to assign the signals obtained from both carboxyl groups in phosphorylated phenylsuccinic acid: 127.7 ppm due to 1-COOH, and 127 ppm due to 4-COOH.

The reactivity of (I) was found to be rather different towards saturated and unsaturated acids. Unsaturated acids, such as cinnamic, muconic, and maleic, reacted smoothly with (I) via their carboxyl hydroxyls. No signals were observed in the phosphate region that may indicate other reactions which might cause alteration in the coordination state of the phosphorus atom. Such reactions have been reported in the literature [25]. They took place during storage of the derivatives at room temperature for more than 12 hours.

Saturated aliphatic acids such as formic or oxalic were found to be considerably more reactive towards (I) than unsaturated acids. When an excess of acid was used, gaseous products evolved during derivatization, and several signals in the phosphate region were observed, with the most intense located at approximately 24.5 ppm. These signals are likely to be due to the formation of phosphate esters according to the reaction proposed in Scheme 3.

Compound (X) has been reported to give rise to a ^{31}P NMR chemical shift at 25.3 ppm [15]. The importance of carefully derivatizing aliphatic acids, under stirring and cooling, may be emphasised by the observation that, when oxalic acid was reacted with (I) in the absence of cooling, the intensity of the line at 133 ppm, due to the expected derivative (Table 3), was found



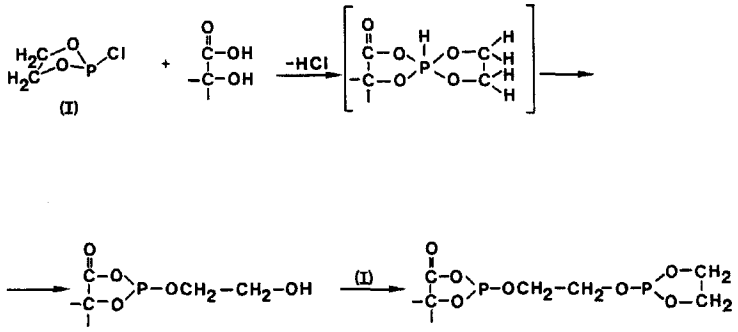
Scheme 3. A reaction that may occur between 1,3,2-dioxaphopholanyl chloride and formic or oxalic acid.

to be substantially reduced. Accordingly, the derivatization of an unknown compound or mixture with (I) should be done carefully under continuous stirring and cooling.

Vicinal diacids and *alpha*-hydroxy acids were found to react with (I) in a peculiar manner. The derivative of oxalic acid, for example gave a single ^{31}P signal at 132.9 ppm which is very close to that of ethylene glycol (132.6 ppm) while no signals were observed around the 127 ppm region characteristic for aliphatic acids (Figure 1). The derivative of phthalic acid, on the other hand gave rise to two ^{31}P signals at 127.6 and 133.6 ppm, the upfield signal being, as expected, in the region of *ortho*-benzoic acids. Terephthalic acid was found to react smoothly with (I), resulting in a single signal at 127.7 ppm (Table 3).

The derivatives of DL-tartaric {HOOCCOHCOHCOOH}, DL-vanillomandelic {4-OH-3-MeOC_6H_5CH(OH)COOH} and DL-mandelic {C_6H_5CH(OH)COOH} acids gave ^{31}P NMR spectra with many lines, of different intensities, ranging between 133 and 142 ppm with no signals observed at around 127 ppm. In contrast, the dimethyl ester of L-tartaric acid (dimethyl-L-tartrate) or the dibenzoyl ether of L-tartaric acid (dibenzoyl-L-tartaric acid) gave single signals at 136.2 and 127.4 ppm for their hydroxyls and carboxyl groups respectively. This observation implies that the reaction proceeds smoothly when the neighbouring reactive group is blocked. Similarly the derivative of salycilic acid gave two ^{31}P signals, at 132.7 and 124.8 ppm, while its methyl ester (methyl salycilate) gave a single signal at 128.6 ppm. The simplest hydroxy acid, i.e. glycolic acid, gave two ^{31}P signals at 132.8 and 139.5 ppm, the upfield signal being in the region of the primary alcohols (Figure 1).

These observations may be due to a transesterification reaction of the phosphite esters that may form when vicinal diacids and/or *alpha*-hydroxy acids react with (I) (Scheme 4). This is a known reaction usually catalysed by acids. Wroblewski *et al.* [16] observed the appearance

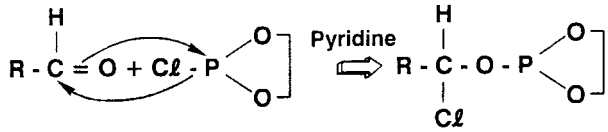


Scheme 4. The possible transesterification reaction that may occur between 1,3,2-dioxaphospholanyl chloride, (I) and vicinal diacids and/or *alpha*-hydroxy acids.

of three signals at 148.0, 135.0, and 132.1 ppm when (I) was reacted with pinacol $\{HOC(CH_3)_2C(CH_3)_2OH\}$. They attributed the signal at 148.0 ppm to be the product of an intramolecular 1,3,2-dioxaphospholanyl ring-opening transesterification. The opening of the ring creates primary hydroxyl groups which, on further reaction with (I), result in ^{31}P signals appearing in the primary alcoholic region.

Aldehydes

The reagent 1,3,2-dioxaphospholanyl chloride (I) was found to react in a peculiar manner with aliphatic and aromatic aldehydes. This class of compounds was the only one among those investigated that yielded intensely coloured solutions after reaction with (I). The derivatization reaction of phenols, acids or alcohols, when performed in pure pyridine, in the absence of chloroform, resulted in the instantaneous formation of a bulky precipitate of pyridine hydrochloride, i.e. the substitution byproduct of the phosphorylation depicted in Scheme 1. When aldehydes, however, were in contact with (I) in pure pyridine, they gave no pyridine hydrochloride. Instead, coloured solutions were obtained, varying in colour according to the aldehyde, and in intensity according to the time elapsed from the onset of the reaction. In the absence of pyridine no reaction took place. In general, the intensity of the colours increased smoothly and reached a maximum after several hours. Coloured products were also formed when the derivatization was done under nitrogen. The ^{13}C NMR spectra of freshly derivatized aldehydes revealed almost complete elimination of the carbon resonance in the carbonyl region,



Where R=Alkyl or Phenyl

Scheme 5. The addition reaction that may occur between 1,3,2-dioxaphospholanyl chloride (I) and aldehydes.

Table 4. ^{31}P NMR Chemical Shifts of Phosphorylated Aldehydes.

No	Aldehyde	Chemical Structure	Chemical Shift ppm
1	2-methoxybenzaldehyde	2-$MeOC_6H_4CHO$	134.8
2	2-hydroxybenzaldehyde	2-OH-C_6H_4CHO	134.7
3	propionaldehyde	CH_3CH_2CHO	133.1
4	*trans*-cinnamaldehyde	$C_6H_3CH{=}CHCHO$	131.7
5	vanillin	4-(OH)3-$MeOC_6H_3CHO$	131.7
6	4-nitrobenzaldehyde	4-NO_2-C_6H_3CHO	131.6
7	phthaladehyde	$C_6H_4(CHO)_2$	131.5
8	benzaldehyde	C_6H_4CHO	131.5
9	5-nitro-vanillin	4-(OH)3-(MeO)5(NO_2)C_6H_3CHO	131.2
10	4-hydroxy-3-methoxybenzaldehyde	4-(OH)3-(MeO)C_6H_3CHO	131.0
11	4-bromobenzaldehyde	4-(Br)C_6H_4CHO	130.9
12	4-hydroxybenzaldehyde	4-(OH)C_6H_4CHO	130.9
13	5-chlorovanillin	4-(OH)3-MeO-5(Cl)C_6H_3CHO	130.6
14	3,4-dimethoxybenzaldehyde	3,4-$(MeO)_2C_6H_3CHO$	130.4
15	4-benzyloxy, 3-OMe-benzaldehyde	$C_6H_5CH_2OC_6H_3$(MeO)CHO	130.2

and indicated that phosphorylation proceeded rather quickly and affected the carbonyl group. Furthermore, ^{31}P NMR spectra of various phosphorylated aldehydes (Table 4) revealed the appearance of single lines in the region 130 to 137 ppm, which may be attributed to the derivatized aldehyde group. These observations can be explained only if an addition reaction between aldehydes and (I) takes place rather than a substitution reaction. Such an addition reaction may occur through an attack of the chlorine atom in 1,3,2-dioxaphospholanyl chloride on the carbon of the carbonyl group of the aldehyde. The role of pyridine may be to polarize the P-Cl bond, thus facilitating the reaction (Scheme 5).

Additional experiments were done in order to further examine the hypothesis in Scheme 5. Proton-decoupled ^{13}C NMR spectra of propan-1-ol and propionaldehyde exhibited singlets at 63.3 and 200.7 ppm, respectively, for the carbons attached to the functional groups of these molecules. Proton-decoupled ^{13}C NMR spectra of propan-1-ol and propionaldehyde derivatized with (I) gave a doublet at 62.7 ppm (doublet due to coupling with ^{31}P) for the -C-O-P fragment in propan-1-ol and a doublet at 88.7 ppm for the -C-O-P fragment in propionaldehyde. Thus the chemical shifts of the functional carbons within these two derivatized compounds appeared to differ by 26 ppm. The significant downfield shift given by the -C-O-P fragment in the derivatized propionaldehyde is evidence of a strong electron-withdrawing group attached to this part of the molecule. Furthermore, the ^{31}P chemical shifts of these two derivatives of (I) were 132.1 and 133.2 ppm, for propan-1-ol and propionaldehyde respectively. The 1.1 ppm downfield shift of the phosphorylated propionaldehyde is an additional indication of an electron-withdrawing group being attached to the -C-O-P fragment of the derivatized propionaldehyde. In accordance with the mechanism of addition of (I) onto aldehydes, as depicted in Scheme 5, this electron-withdrawing group is chlorine. In linear alkanes, substitution of a proton by chlorine causes the ^{13}C signal of this carbon to be downfield shifted by about 31 ppm [26].

In order to further probe the environments of the functional carbons of these two phosphorylated propanolic derivatives, their ^{13}C NMR spectra were recorded with the proton decoupler switched off. The derivative of propan-1-ol exhibited a triplet of doublets at 62.7 ppm for the $-CH_2$-O-P fragment in this molecule. This indicates that the carbon in this grouping is coupled to two protons. However, the proton-coupled ^{13}C NMR spectrum of the phosphorylated derivative of propionaldehyde gave a doublet of doublets in the range 88-89 ppm for its -CHCl-O-P fragment, thus indicating that the carbon is coupled to one proton only.

The proton NMR spectra of derivatized propan-1-ol and propionaldehyde were also examined. Propan-1-ol gave the expected signals due to (I) and the protons of the phospholan ring, and also three multiplets, at 3.07, 0.95 and 0.3 ppm, with intensity ratios 2:2:3 in accord with the number of protons at C-1, C-2 and C-3 within the derivatized propan-1-ol. The phosphorylated propionoaldehyde also exhibited three multiplets, with two of them located upfield at 1.4 and 0.3 ppm (intensity ratio 2:3) while the third was downfield at 6.7 ppm with a relative intensity corresponding to one proton. This evidence offers additional confirmation of the conclusion that in derivatized propionoaldehyde the C-1 atom bears only one proton, and

it is shifted downfield due to the influence of a strong electron-withdrawing group attached to it.

The derivatization of the carbonyl groups in benzaldehyde, *trans*-cinnamaldehyde and propionaldehyde gave rise to ^{13}C NMR signals at 87.7, 88.9 and 88.7 ppm respectively. These are, most probably, characteristic signals for the C-1 (-C-O-P) fragment and may be used to verify the structures of phosphorylated species obtained from carbonyl compounds.

The events depicted in Scheme 5, however, may represent only the first step in a sequence. The adducts of (I) with carbonyl-bearing compounds appeared to be relatively unstable and to undergo further transformation, as evidenced by a decrease in signal intensity in their ^{31}P and ^{13}C NMR spectra after several hours. Such transformations gave rise to ^{31}P NMR signals in the phosphate region, thus indicating an increase in the oxidation and coordination states of the phosphorus. It is likely that the intense colours observed during the derivatization of aromatic and unsaturated aldehydes with (I) were due to the decomposition of the intermediate adducts followed by oligomerization leading to extensive conjugation. This hypothesis is strengthened by the observation that solutions of aliphatic aldehydes and phenylacetaldehyde in contact with (I) were found to be considerably less coloured than those of aromatic and olefinic aldehydes.

Ketones were found to be unreactive with (I) under the experimental conditions employed in this work. However, the reaction between phosphites and ketones is possible. This is, however, of little significance for the present study, because such reactions are characterized by very slow reaction rates and the products usually appear in the phosphate region of the ^{31}P NMR spectra.

CONCLUDING REMARKS

1. The reaction of 1,3,2-dioxaphospholanyl chloride with phenols, alcohols and simple carboxylic acids yields derivatives whose ^{31}P NMR signals appear in regions that are sufficiently well separated to permit distinguishing amongst them.

2. Any *ortho* substitution onto the aromatic ring of phenols significantly affects the magnitude of the ^{31}P NMR chemical shifts, while *para* and/or *meta* substituents have a much smaller effect. A clear distinction between guaiacyl, syringyl and unsubstituted phenolic hydroxyls can thus be made in mixtures of model compounds.

3. Primary alcohols may be distinguished from secondary and tertiary alcohols in their derivatives with 1,3,2-dioxaphospholanyl chloride.

4. Different ^{31}P NMR signals for derivatives of *erythro* and *threo* lignin-like model compounds have been identified.

5. *Alpha*-hydroxy acids and *ortho* benzoic acids react with 1,3,2-dioxaphospholanyl chloride, most likely via a transesterification reaction followed by the opening of the dioxaphosholane ring, which amongst others, give rise to ^{31}P NMR signals in the region of primary alcohols.

6. Aldehydes react with 1,3,2-dioxaphospholanyl chloride via an addition mechanism onto their carbonyl groups which is distinctly different from the substitution mechanism observed among alcohols, phenols and simple carboxylic acids.
7. Ketones were found uncreactive towards 1,3,2-dioxaphospholanyl chloride.

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