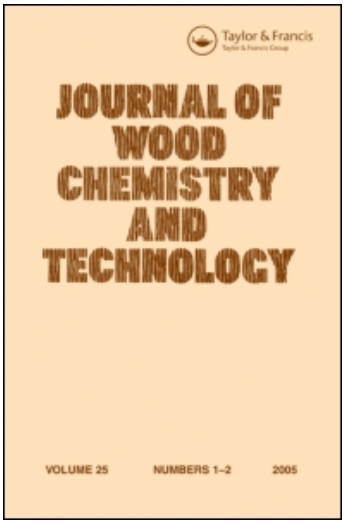

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Quantitative Phosphorus-31 NMR Analysis of Six Soluble Lignins

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QUANTITATIVE PHOSPHORUS-31 NMR ANALYSIS OF SIX SOLUBLE LIGNINS

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ABSTRACT

By using a set of lignin samples, which have been subjected to thorough analyses by the international wood chemistry community, the recently developed quantitative method of ^{31}P NMR spectroscopy was comprehensively examined. The values of total phenolic hydroxyl groups and those of total hydroxyl groups were found to favourably compare with those obtained by other laboratories, applying independent methods of analysis. Furthermore, the application of quantitative ^{31}P NMR spectroscopy offered additional detailed structural information for the examined lignins which was in accord with literature accounts for similar wood species and lignin preparations. More specifically, the steam explosion lignins from aspen and yellow poplar woods and that produced by ball milling/enzyme hydrolysis of cottonwood were found to contain relatively high amounts of β-O-4 structures. In contrast, the kraft, organosolv, and the acid hydrolysis processes were found to induce significant chain scission on the resulting lignins. Ball milled cottonwood lignin contained the highest frequency of β-O-4 bonds and the lowest amount of free phenolic hydroxyls. The *erythro* form of β-O-4 structures were invariably predominant in the lignins from aspen, yellow poplar and cottonwood, in accord with the conclusions of previous reports on hardwood lignins. Thus, the application of quantitative ^{31}P NMR spectroscopy offered the detailed chemical composition of the examined lignins.

INTRODUCTION

Since lignins are known to be heterogeneous materials and their composition is dependent on the method of isolation, the wood species and many other variables, there are no real standards for this highly abundant natural polymer. The international wood chemistry community has thus organized a round robin analytical effort under the auspice of the International Energy Agency (1,2). Six lignin samples were prepared and purified in large quantities and they were supplied to a large number of laboratories around the world. Each laboratory was to perform a set of analyses and report the results to the activity coordinators. These lignin samples have thus become *"de facto"* standards for the international wood chemistry community (1,2).

This effort provided a unique opportunity for the exploration of quantitative aspects of ^{31}P NMR spectroscopy. In a previous paper, a detailed account of most aspects involved in the development of quantitative ^{31}P NMR spectroscopy of lignins has been given (3). The present work addresses itself to the application of this technique on the six round robin lignins. The quantitative ^{31}P NMR data obtained is discussed in the light of the method of lignin preparation and the wood supply. Concurrently the values of total phenolic hydroxyl groups and those of total hydroxyl groups are compared with those obtained in other laboratories, applying independent methods of analysis (1). This comparison presents clear evidence toward the effectiveness of quantitative ^{31}P NMR spectroscopy for the analysis of lignins.

EXPERIMENTAL

Elemental Analyses , Methoxyl, Lignin and Sugar Contents

Elemental and methoxyl analyses, were carried out by the Schwarzkopf microanalytical laboratories, Woodside NY. Prior to analysis the samples were dried for one hour at 105^0 C with an average 3% loss being recorded for all

lignins. A Perkin Elmer 2400 carbon, hydrogen, nitrogen analyzer was employed. The methoxyl content was determined by a method essentially identical to Tappi T 209 SU-69. The Klason and UV soluble lignin was determined by the chemical analysis section at Paprican. The CPPA method G8 was slightly modified in order to accommodate lignin and carbohydrate analyses on the same sample. The lignin sample was dispersed in 72% sulphuric acid and diluted with deionized water to about 3% concentration. The mixture was then autoclaved at 121^{0}C for one hour. The acid insoluble material was filtered dried and weighed, resulting in the reported Klason lignin contents. The acid soluble lignin was determined spectrophotometrically at 205 nm. The path length was 10 mm and the concentration of sulphuric acid 3%. An absorptivity of 110 L/g.cm was used in the calculations as being an average value obtained from a variety of determinations of wood and pulps. The carbohydrate profiles present in these lignins were determined according to the TAPPI standard gas chromatographic method no. T 249 CM84.

Lignin Preparation, Derivatizing Reagent, Derivatization Procedure, NMR Spectroscopy

Details of lignin preparation have been supplied by Chum et al. (1,2), while those of applying quantitative ^{31}P NMR spectroscopy have been described by Argyropoulos (3).

RESULTS AND DISCUSSION

The mean values obtained from four quantitative ^{31}P NMR analyses of the round robin lignins are shown in Table I. The figures obtained for total phenolic and total hydroxyl groups are compared with those obtained by other methods during the international analytical effort. Detailed calculation procedures are described in the appendix. During the international analytical round robin effort the total phenolic hydroxyl contents were determined by four independent

TABLE I -Functional group distributions derived by quantitative ^{31}P NMR analysis for the examined lignins. [a] The figures in parentheses are the averages obtained during the international round robin analytical effort

Lignin Sample	Lignin Functional Group (mol/C_9)								
	-COOH	Guaiacyl -OH	Syringyl -OH	Total Phenolic -OH	Primary -OH	*Alpha*-OH in β-O-4 Structures			Total Hydroxyl Content
						Erythro	*Threo*	Total β-O-4 -OH	
Steam Explosion (Aspen)	0.04	0.14	0.27	0.42[b] (0.45)	0.44	0.24	0.13	0.37	1.27 (1.26)
Steam Explosion (Yellow Poplar)	0.00	0.15	0.24	0.48[b] (0.59)	0.36	0.12	0.10	0.22	1.06 (1.20)
Ball Milled Enzyme (Cottonwood)	0.00	0.04	0.05	0.15[c] (0.18)	0.52	0.39	0.14	0.53	1.20 (1.47)
Alcell™ Organosolv (Mixed hardwoods)	0.06	0.25	0.45	0.70 (0.73)	0.32	0.06	0.10	0.16	1.24 (1.20)
Indulin™ AT Kraft (Mixed Softwoods)	0.06	0.21	0.00	0.57[d] (0.67)	0.41	0.06	0.08	0.14	1.18 (1.23)

[a] Average of four quantitative experiments
[b] Includes condensed biphenolic structures, 0.09 mol/C_9
[c] Includes condensed biphenolic structures, 0.04 mol/C_9 and p-hydroxyphenyl structures, 0.02 mol/C_9
[d] Includes condensed biphenolic structures, 0.36 mol/C_9

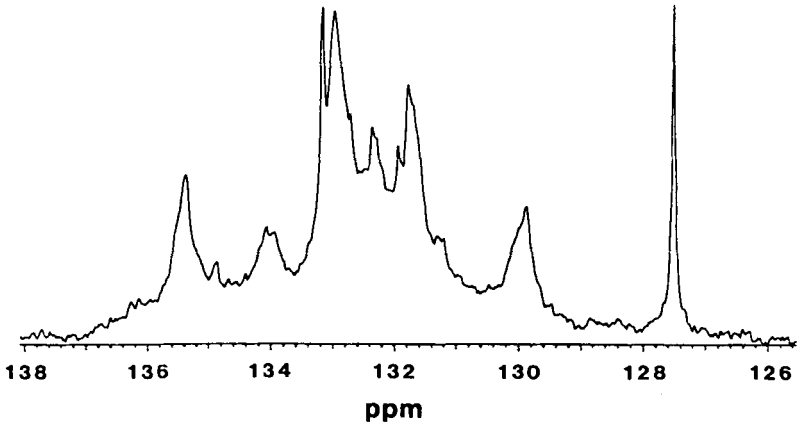

Figure 1: The quantitative ^{31}P NMR spectrum of steam explosion lignin produced from aspen, phosphitylated with (I).

techniques, namely: FTIR/Pyrolysis, proton and ^{13}C NMR spectroscopies, the conductometric titration technique of Chum *et al.* (4) and the aminolysis method of Mansson (5).

Despite some variability in this data, the values obtained by these methods were averaged and compared with the data obtained by quantitative ^{31}P NMR (Table I). The total hydroxyl contents reported during the international round robin (1), were obtained by two techniques, namely: proton NMR and the wet chemical method of Bethge and Lindstrom (6). The figures reported (1) by these two techniques were averaged and compared to those of quantitative ^{31}P NMR.

Steam Explosion Lignin From Aspen

The quantitative ^{31}P NMR spectrum of the round robin steam explosion lignin from aspen wood is shown in Figure 1. The syringyl to guaiacyl free phenolic hydroxyl ratio, as derived from the signals centred at 131.8 and 130.0 ppm, was found to be approximately equal to 2.0 (Table 1). There are twice as many free syringyl phenolic units in this lignin than their guaiacyl counterparts. Robert *et al.* (7) have examined the structural changes occurring in aspen lignin during the steam explosion treatment. They have pointed out that during steam explosion cleavage of the β-O-4 bonds results in the preferential liberation of free syringyl end groups. This is indeed the case observed from the ^{31}P NMR analysis of this sample. These findings are also supported by the work of Obst and Landucci (8) who have examined milled wood lignin from the same wood species using quantitative ^{13}C NMR spectroscopy.

They have reported the ratio of total (etherified plus non-etherified) syringyl/guaiacyl units to be 0.89. It is likely therefore, that the process of steam explosion of hardwoods liberates higher amounts of syringyl free phenolic hydroxyls than guaiacyl.

The total free phenolic hydroxyl content in the steam explosion lignin from aspen was found to be $0.42/C_9$, in good agreement with the average value

obtained during the round robin effort (0.45 molC_9). Similarly, the total hydroxyl groups determined by quantitative ^{31}P NMR (1.27 mol/C_9) were found to be in quantitative agreement with those obtained during the international round robin effort (1.26 mol/C_9).

Since the aryl glycerol β-O-4 structures are known to be the most prominent linkages connecting the C_9 units of lignin (9), their proportion within a lignin preparation may be a qualitative indication of the degree of polymerization. A high abundance of these structures is apparent in the lignin produced via the steam explosion of aspen wood. This becomes evident from the signal intensities centred at 135.4, and 134.0 ppm (Figure 1). These signals are known to be responsible for the secondary *alpha*-hydroxyls in β-0-4 structures in lignins (10). The signals around 134.9 and 136.2 ppm were not integrated since, most likely, they are due to residual phosphitylated carbohydrate hydroxyls (10). This is also supported by the fact that this sample contained a high amount (8.6%) of carbohydrates. (Tables A.1 and A2). Therefore, a total of 0.37 mol/C_9 of β-0-4 structures were determined by quantitative ^{31}P NMR spectroscopy to be present in the round robin steam explosion lignin from aspen (Table I). Furthermore, the amount of *alpha erythro* hydroxyls, present in the β-0-4 structures, was found to be 0.24 mol/C_9. These were found to be considerably more abundant than their *threo* counterparts (0.13 mol/C_9). This is in accord with the work of Nimz *et al.* (11), Hauteville *et al.* (12), and of Brunow *et al.* (13), where the predominance of *erythro* forms in hardwood lignins has been demonstrated by ^{13}C (11) and proton NMR spectroscopies (12,13).

Steam Explosion Lignin From Yellow Poplar

In a manner analogous to the discussion relevant to the steam explosion lignin from aspen, the quantitative ^{31}P NMR analysis of the steam explosion lignin from yellow poplar also showed a relatively high proportion of β-0-4 structures, (signals centred at 135.4 and 134 ppm, Figure 2). However, the total amount of

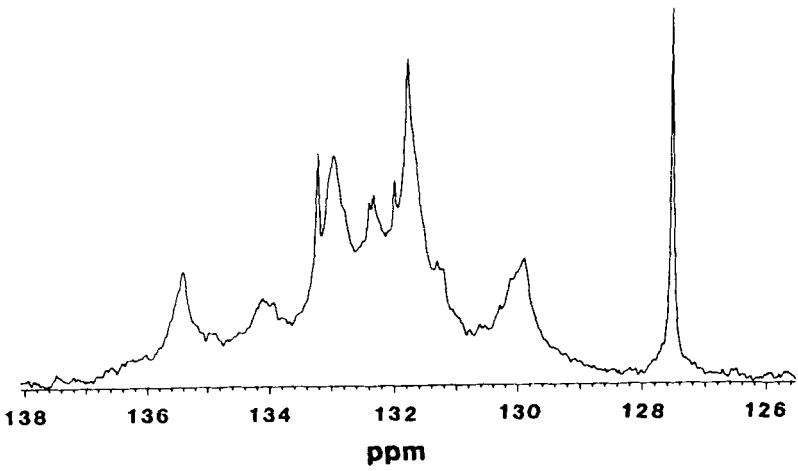

Figure 2: The quantitative ^{31}P NMR spectrum of steam explosion lignin from yellow poplar, phosphitylated with (I).

β-0-4 structures present in this steam exploded lignin was considerably less than that obtained for the aspen steam exploded lignin (Table I). This may be due to the fact that the severity factor (Log R_0) (14), applied during the isolation of the steam explosion of yellow poplar, was equal to 4.3, while the one applied during the isolation of the steam explosion lignin from aspen, was equal to 3.8 (2). This may have caused the scission of a greater proportion of β-0-4 bonds in this lignin (7,15), which eliminated indiscriminately both the *erythro* and *threo* forms of the β-0-4 aryl ether structures. In actual fact, the ratio of *erythro/threo* hydroxyls detected for this lignin was found to be equal to 1.2 (0.12/0.10).

Both steam explosion lignins showed the presence of small amounts of free biphenyl hydroxyl structures, as evidenced form the signals at 131.3 ppm, representing 0.04-0.09 mol/C_9 (Table I). Such structures may also form during the steam explosion treatment. Steam explosion conditions have been shown to cause the formation of carbon-carbon bonds within lignins (7,15). More specifically, Robert *et al.* (7, 15) have concluded that condensation reactions are

prevalent during the steam explosion of aspen wood, involving reactive aromatic carbons *para* to methoxyl groups.

The high methoxyl content (Tables A.1 and A.3) and the ^{31}P NMR signal received at 131.8 ppm (Figure 2) unequivocally classifies the steam explosion lignin produced from yellow poplar wood as being a hardwood lignin. The total free phenolic hydroxyl content, detected by ^{31}P NMR for this lignin (0.48 mol/C_9), was in reasonable agreement with that obtained by ^{13}C NMR (0.52 mol/C_9), during the international round robin analytical effort (1). It was lower, however, by about 20%, compared with the overall average value obtained during the same effort (1).

The total free phenolic hydroxyl content of the steam explosion lignin from yellow poplar, was higher to that obtained for the steam explosion lignin from aspen (Table I). This may also be a consequence of the more severe steam explosion conditions used in the preparation of the yellow poplar lignin. Higher severity factors will most likely cause the formation of higher amounts of syringyl and guaiacyl phenolic hydroxyls.

Ball Milled Enzyme Lignin From Cottonwood

Since milled wood lignins are known to be rather representative of native lignin (9) it is not surprising to find in the ^{31}P NMR spectrum of this preparation, two strong signals at 135.4 and 134 ppm, due to phosphitylated *alpha* hydroxyls of the β-O-4 structures (Figure 3). Four quantitative experiments carried out on this sample estimated the frequency of β-O-4 structures to be 0.53 mol/C_9 (Table I). This is the highest frequency of β-O-4 units determined amongst all examined lignins. Adler (9) reports a frequency of about 0.6 mol/C_9 for (arylglycerol-β-aryl ether) structures to be present in Björkman lignin from birch (*Betula verrucosa*), which is in relatively good agreement with the figure obtained by quantitative ^{31}P NMR, considering that similar lignin preparations but different wood species are compared.

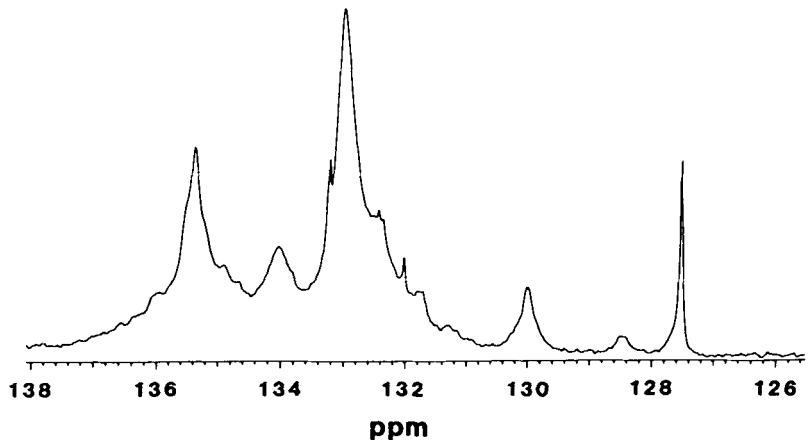

Figure 3: The quantitative ^{31}P NMR spectrum of ball milled enzyme lignin from cottonwood, phosphitylated with (I).

The *alpha* hydroxyls belonging to the *erythro* form of the β-0-4 structures were considerably more abundant than their *threo* counterparts (0.39 and 0.14 mol/C_9 respectively) in accord with the hardwood nature of this sample and previous reports (11-13). The signals around 136.0, and between 134.9 -134.6 ppm were not considered during integration because they most likely are due to residual carbohydrates present in the sample (3.4 %, Tables A.1 and A.2) (10).

Four types of free phenolic hydroxyls, in relatively low amounts, were detected in the ball milled enzyme lignin from cottonwood. An approximately equal abundance of guaiacyl (0.04 mol/C_9) and syringyl (0.05 mol/C_9) free phenolic hydroxyls were determined together with low amounts of biphenolic (0.04 mol/C_9) and p-hydroxyphenyl structures (0.02 mol/C_9) (Table I). Venverloo (16) has confirmed that relatively high amounts of p-hydroxyphenylpropane structures are present in aspen wood, while biphenyl structures bearing both free and etherified hydroxyls have been reported to amount to about 0.045 mol/C_9 in Björkman lignin from birch (*Betula verrucosa*) (9).

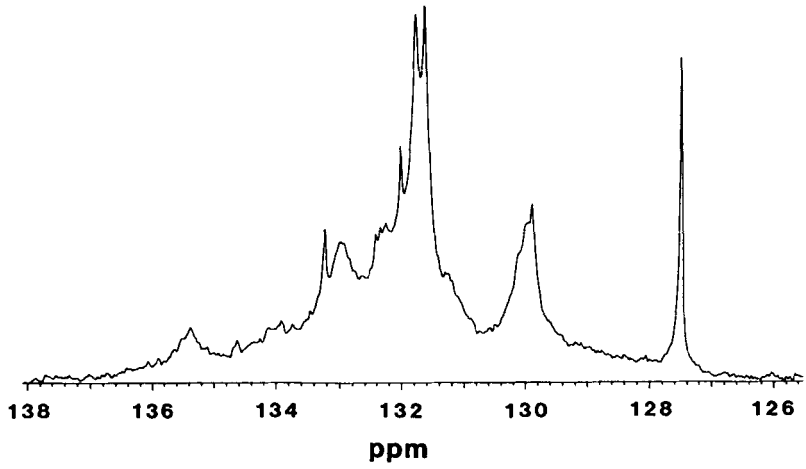

Figure 4: The quantitative ^{31}P NMR spectrum of Alcell™ organosolv lignin from mixed hardwoods, phosphitylated with (I).

The total phenolic hydroxyl content of this lignin, determined by quantitative ^{31}P NMR, was found to be 0.15 mol/C_9, in reasonable agreement with the average obtained during the international round robin analytical effort (0.18 mol/C_9), (Table I). This is a much reduced phenolic hydroxyl content compared to the previously discussed steam explosion lignins. This can be rationalized on the basis that steam explosion is known to cause the cleavage of the aryl glycerol β-O-4 structures (7,15, 17-19). Their cleavage will result in the concomitant increase of the free phenolic hydroxyl content of the lignin, in agreement with the results of this work and those obtained during the international round robin analytical effort (1).

Allcell ™ Organosolv Lignin From Mixed Hardwoods

The effect of chemical degradation during the isolation of Alcell™ organosolv lignin becomes apparent in the quantitative ^{31}P NMR spectrum of Figure 4. The weak signals at around 135.4 and 134 ppm, imply that the

arylglycerol β-0-4 linkages in this sample have been subjected to considerable cleavage. The total amount of secondary *alpha* hydroxyls in β-0-4 structures was determined to be rather low (0.16 mol/C_9, Table I). Consequently, the chemical degradation has caused the formation of relatively high proportions of free syringyl and guaiacyl phenolic hydroxyl groups, as evidenced by the high signal intensities at 131.8 ppm and 130.0 ppm respectively. More specifically 0.45 mol/C_9 syringyl and 0.25 mol/C_9 guaiacyl phenolic groups, giving rise to a total phenolic hydroxyl content of 0.70 mol/C_9, in excellent agreement with the average value of 0.73 mol/C_9 obtained during the international round robin analytical effort. Such a high phenolic hydroxyl content is of the magnitude expected for a lignin preparation isolated from chemically treated wood.

Indulin™ AT Kraft Lignin From Mixed Softwoods

Quantitative ^{31}P NMR analyses of solubilized kraft lignins isolated from the spent liquors at various degrees of delignification of black spruce wood (*picea mariana*), carried out in our laboratory, have shown the gradual elimination of arylglycerol β-0-4 secondary hydroxyls as the degree of delignification was increased. In addition the formation of increasing amounts of biphenyl and diarylmethane phenolic hydroxyls was apparent, due to condensation reactions operating during the pulping process, giving rise to a signal at around 131.2 ppm (10). In both of these respects the Indulin™ AT lignin represents no exemption. In a manner similar to the organosolv process, kraft pulping, cleaves the arylglycerol β-0-4 bonds in lignin (20,21). Consequently, considerably reduced signal intensities in the ^{31}P NMR spectrum of Figure 5 at 134.8 and 134.0 ppm were obtained. The total amount of β-0-4 structures determined by quantitative ^{31}P NMR was 0.14 mol/C_9 (Table I), a figure nearly identical to that obtained for the Alcell™ organosolv lignin (0.16 mol/C_9). Furthermore, condensed biphenyl and diaryl methane structures are apparent from the signal centred at 131.2 ppm. Their amount was determined to be 0.36 mol/C_9. This is also supported by the work of Robert and Bardet (20), Brunow and Miksche (21), who amongst other

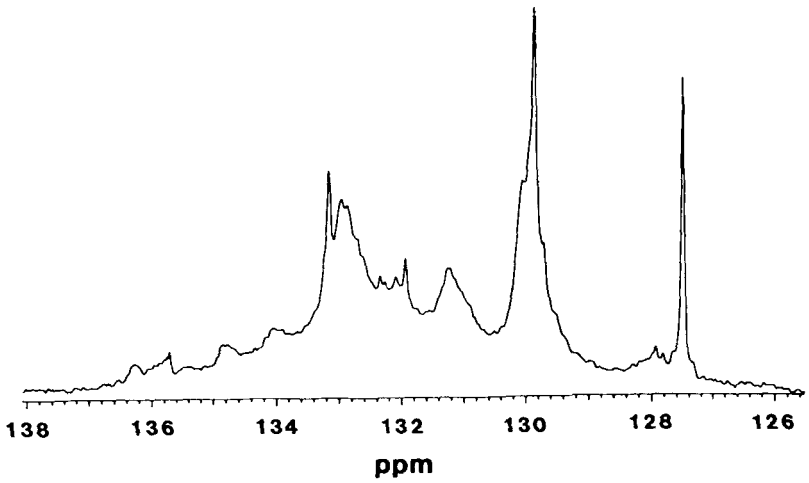

Figure 5: The quantitative ^{31}P NMR spectrum of Indulin™ AT kraft lignin from mixed softwoods derivatized with (I).

workers in the field, have demonstrated the formation of condensed structures during kraft pulping.

The amount of guaiacyl units in this kraft lignin was determined to be 0.21 mol/C_9, in accord with the softwood nature of the wood supply. The total amount of phenolic hydroxyl (including that arising from the condensed structures) was thus found to be 0.57 mol/C_9, in reasonable agreement with the average value obtained during the international round robin analytical effort (0.67 mol/C_9).

Sucrolin ™ Acid Hydrolysis Lignin

The qualitative ^{31}P NMR spectrum of acid hydrolysis lignin from bagasse, Sucrolin™, is shown in Figure 6. Attempts to obtain quantitative spectra for this lignin failed due to its high carboxylic acid content. This precluded the use of benzoic acid as an internal standard. However, a number of qualitative observations can be made for this lignin. Figure 6 shows that this is a highly degraded lignin containing relatively small amounts of β-0-4 structures. The signal

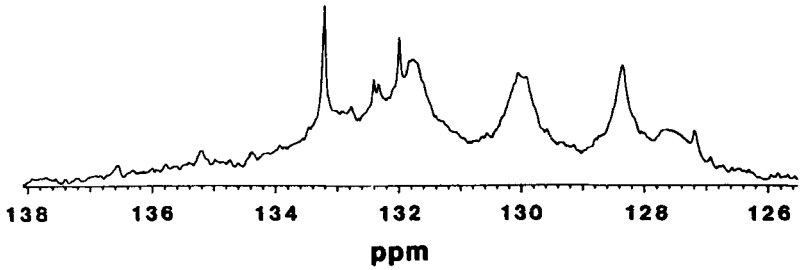

Figure 6: The qualitative ^{31}P NMR spectrum of acid hydrolysis lignin from bagasse, Sucrolin™ phosphitylated with (I).

due to primary hydroxyls (centred around 133.2 ppm) is also relatively weak. Acid hydrolysis lignin from bagasse seems to contain a mixture of free syringyl, guaiacyl and p-hydroxyphenyl units, as evidenced by the signals centred at 131.8, 130, and 128.4 ppm respectively. This is the only lignin, amongst the ones examined, that showed a strong signal at 128.3 ppm due to p-hydroxyphenyl structures (10,22). This is in accord with the fact that grass lignins are known to contain substantial amounts of p-hydroxylphenylpropane units (23-26).

APPENDIX

Calculation Methods and Tables of Results

Since the objective of this work was to employ ^{31}P NMR spectroscopy to determine the functional group profiles, in mol/C_9 units, the molecular formulae of the supplied lignins were calculated (Table A.3). This was done by first taking into account the elemental and methoxyl analyses shown in Table A.1. The contributions of the sugars to the molecular formulae were then calculated from the data of Tables A.1 and A.2 by assuming the molecular weight of xylose as an average for all sugars present. This is because xylan is known to be the predominant carbohydrate associated with hardwood lignins (27,28). The protein

TABLE A.1 - Elemental composition, sugars and protein contents for the examined lignins[1]

Lignin Sample	Carbon (%)	Hydrogen (%)	Oxygen (%)	Methoxyl (%)	Total[2] Sugars (%)	Nitrogen (%)	Protein[3] (%)
Steam Explosion (Aspen)	61.4	6.08	32.5	17.5	8.62	0.15	0.96
Steam Explosion (Yellow Poplar)	63.5	5.83	30.7	20.8	0.00	0.32	2.0
Ball Milled Enzyme (Cottonwood)	58.9	5.95	35.2	20.2	3.82	0.15	0.96
Alcell™ Orgnanosolv (Mixed hardwoods)	67.1	6.03	26.9	17.6	0.00	0.17	1.09
Indulin™ AT Kraft (Mixed softwoods)	66.1	5.83	26.1	16.2	0.18	0.09	0.56
Sucrolin™ Acid hydrolysis (bagasse)	66.5	5.73	27.8	9.2	0.22	0.63	3.93

[1] All values reported are averages of two determinations
[2] For actual carbohydrate profiles see Table A.2
[3] Nitrogen content x 6.24

TABLE A.2- Lignin and carbohydrate analyses for the examined lignin samples

Lignin Sample	Lignin Analyses			Carbohydrate Analyses					
	Klason Lignin (%)	UV Soluble Lignin (%)	Total Lignin (%)	Arabinose (%)	Xylose (%)	Mannose (%)	Galactose (%)	Glucose (%)	Total Sugars (%)
Steam Explosion (Aspen)	88.2	0.66	88.86	N.D.	0.56	0.26	N.D.	7.8	8.62
Steam Explosion (Yellow Poplar)	97.5	0.68	98.20	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Ball Milled Enzyme (Cottonwood)	92.5	0.72	93.22	N.D.	3.08	0.14	N.D.	0.27	3.52
Alcell™ Organosolv (Mixed hardwoods)	97.0	1.05	98.05	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.
Indulin™ AT Kraft (Mixed Softwoods)	97.5	0.78	98.28	0.08	N.D.	N.D.	0.1	N.D.	0.18
Sucrolin™ Acid Hydrolysis (bagasse)	97.9	0.63	98.53	N.D.	N.D.	N.D.	N.D.	0.22	0.22

N.D. = Not Detected

contents shown in Table A.1 were calculated from the nitrogen data of the lignins by using the factor 6.25 to convert this data to protein (20). The contribution of protein to the molecular weights shown in Table A.3 was not taken into account.

The functional groups shown in Table I were calculated from the ^{31}P NMR spectra of the phosphitylated lignins (Figures 1-6). The approximate chemical shift ranges integrated representing each functional group are shown in Table A.4.

TABLE A.3 Molecular formulae of lignins calculated from the data of Tables A.1 and A.2

Lignin Sample	Carbon	Hydrogen	Oxygen	Methoxyl	Molecular Weight (g/mol)
Steam Explosion (Aspen)	9	7.98	2.50	1.20	193
Steam Explosion Yellow Poplar)	9	7.38	2.43	1.31	195
Ball Milled Enzyme (Cottonwood)	9	8.09	3.10	1.42	210
Alcell™ Ognanosolv (Mixed hardwoods)	9	7.69	2.00	1.02	179
Indulin™ AT Kraft (Mixed softwoods)	9	7.63	2.22	0.95	181
Sucrolin™ Acid Hydrolysis (Bagasse)	9	8.20	2.46	0.51	172

Table A.4: Approximate chemical shift ranges integrated in order to quantify the various functional groups.

Functional group	Approximate chemical shift range integrated (ppm)
Internal standard, benzoic acid	128.0-127.2 (or 127.8-127.2 Indulin™)
erythro α-OH in β-O-4	135.9-135.0 (or 136.6-134.9 Alcell™) (or 135.2-134.4 Indulin™)
threo α-OH in β-O-4	134.6-133.6 (or 134.8-133.5 Alcell™) (or 133.7-134.4 Indulin™)
primary-OH	133.6-132.0 (or 133.7-131.7 Indulin™)
Syringyl -OH	132.0-131.4 (or 132.0-131.6 Indulin™)
Guaiacyl-OH	130.5-129.1
p-Hydroxyphenyl-OH	128.8-128
Biphenolic & diarylmethane phenolic structures	131.4-130.5 (or 131.6-130.6 Indulin™)
Carboxylic acids	129.0-128

The calculation of the data of Table I was carried out as follows. The signal area that corresponded to one hydroxyl group present within the introduced amount (0.00134g) of internal standard (benzoic acid) was first calculated by considering that the molecular weight of benzoic acid is 122.12 g/mol. This molecule contains one hydroxyl thus giving rise to one sharp signal. Therefore, $0.00134/(122.12)=1.097x10^{-5}$ is the number of moles of hydroxyl groups present within the internal standard.

Based on the integrated area for the signal of the internal standard, a standard factor was calculated for each spectrum. This factor expresses the number of moles of hydroxyls that give rise to a unit area of signal for each spectrum. ie. if for a spectrum, the integration of the internal standard area was 7% the standard factor was: $1.097x10^{-5}/7=1.567x10^{-6}$ mole -OH/unit area in spectrum.

The standard factor was then multiplied be the integral areas, determined for each functional group, and then divided by the amount of dry lignin sample (in g) weighed prior to derivatization with (I). This gave the mol/g for each functional group present in each lignin ie. If for a spectrum the integration for the carboxylic acids signal was 4.9% and 17.3 mg of lignin sample had been weighed, then: $(4.9x1.567x10^{-6})/0.0173=4.438x10^{-4}$ mol/g COOH were present in this sample. These values were then transformed to mol/C_9 by multiplying them with the molecular weight calculated for each lignin (Table A.3).

A computer program for these calculations can be supplied by the author.

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