Characterization of Lignin Functionality by NMR

Fluorine(19)–NMR of Lignin

Carbonyl functional groups in lignin were determined by using a modification of the ^{19}F–NMR procedure developed by Sevillano *et al.* [Sevillano, R., Barrelle, M., Mortha, G., and Lachenal, D., "^{19}F-NMR Spectroscopy for the Quantitative Analysis of Carbonyl Groups in Lignin", Proceedings, Fifth European Workshop on Lignocellulosics and Pulp, 1998, p. 469.]. Approximately 60 mg of lignin was dissolved in 500 μl DMF, then 1 mL of 50% DMF/water (v/v) containing 110 mg 4–trifluoromethylphenylhydrazine (Aldrich Chemical Company, recrystallized from pentane) was added. The mixture was kept at room temperature, in the dark, for 12 hours. The derivatized lignin was precipitated by the addition of ~20 mL of water (pH = 2.0 adjusted with 36% HCl). The aqueous layer was discarded and the lignin was freeze–dried. The resulting lignin was Soxhlet extracted with dichloromethane for 2 hours, then dried under vacuum over P_2O_5.

Approximately 25 mg of derivatized lignin was dissolved in 450 μL DMSO–d_6 containing 3–trifluoromethoxybenzoic acid (0.5 mg/mL, δ -57.19 ppm, Lancaster Synthesis Incorporated) as an internal standard. Quantitative NMR parameters used were: 90° pulse without proton decoupling, 10–second pulse delay, and approximately 400 acquisition transients. Chemical shifts were adjusted to CCl_3F (δ 0.00 ppm) used as an external standard. Integration was accomplished by lineshape analysis using NUTS–NMR Transform Utility Software (Acorn NMR Incorporated).

Phosphorus(31)–NMR *Ortho*– and *Para*–Quinone Content

A procedure was developed to measure the combined *ortho*– and *para*–quinone contents of isolated lignins. Dry residual lignin (30 mg) was derivatized with 250 μL trimethylphosphite and 250 μL anhydrous DMF under an argon atmosphere at room temperature for 2 days. Lignin samples were previously dried under vacuum at 40°C for 24 hours. NMR grade trimethylphosphite (Aldrich Chemical Company) was either used from a freshly opened bottle or purified by distillation from solution containing sodium metal.

Derivatized lignin samples were prepared for analysis by removing excess trimethylphosphite under vacuum at 40°C for 3 hours. The treated lignins were dissolved in 450 μL of solvent consisting of 60% DMSO–d_6/pyridine (v/v) containing tri–*meta*–tolylphosphate (0.7 mg/mL) and chromium–acetylacetonate (0.9 mg/mL). Derivatized lignin–quinone structures were hydrolyzed to the open–chain phosphate ester by the addition of 5 μL water (0.3 mmol per 30 mg lignin). After 12 hours, the ^{31}P–NMR spectrum of the resulting solution was acquired with a Bruker 400 MHz NMR spectrometer.

Phosphorus–NMR spectra were acquired under quantitative conditions at 305°K. A 90° pulse was utilized with a 5–second pulse delay along with inverse–gated broad–band proton decoupling. A line–broadening factor of 5 Hz was used and the time domain (TD) size was 64K. For each spectrum ~1500 scans were collected. The internal standard tri–

m–tolylphosphate (δ -16.3 ppm) was used both for quantification and as a shift reference. The ^{31}P–NMR chemical shift of tri–*meta*–tolylphosphate in DMSO–d_6 was determined with the aid of 85% H_3PO_4 as an external shift reference. Previously, the chemical shift of tri–*meta*–tolylphosphate was reported as δ -17.3 ppm ($CDCl_3$ solvent) [146]. Quantification of lignin–quinone content was achieved by integrating the areas of the internal standard, δ -15.3 to -17.1 ppm, and the phosphate–ester (quinone adduct) resonance at δ -0.3 to -6.0 ppm (Figure 1).

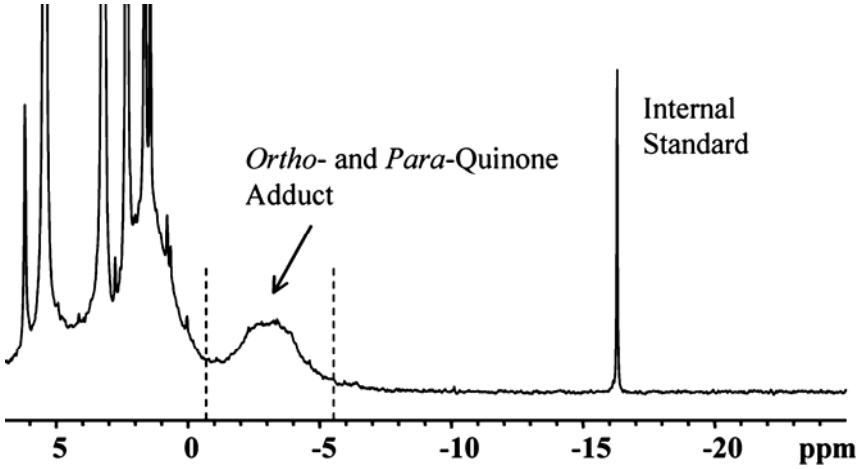

Figure 1. Phosphorous–NMR spectrum of trimethylphosphite treated D_0 residual lignin.

^{31}P–^{1}H heterocorrelation experiment was performed using the COLOC (Correlation *via* Long–Range Couplings) pulse sequence. The following acquisition parameters were used: selected ^{31}P–^{1}H coupling constant ($^{3}J_{POCH}$) of 11.0 Hz, ^{1}H sweep width of 16.92 ppm, center of ^{1}H channel at 6.18 ppm, ^{31}P sweep width of 29.91 ppm, center of ^{31}P channel at -9.89 ppm, Waltz–16 ^{1}H decoupling, 1.0 second pulse delay, 160 scans acquired, 16 dummy scans, and 64 experiments.

^{1}H–, ^{13}C–, and ^{31}P–NMR Spectroscopy for Lignin Analysis.

The analytical techniques of ^{1}H–NMR, ^{13}C–NMR, and ^{31}P–NMR are essential tools for the determination of residual lignin structural features impacting upon delignification and brightening reactions. Below is a brief review of the application of NMR spectroscopy for lignin analysis.

Proton–NMR is able to quantify a number of important residual lignin structural features including: carboxylic acid (δ 12.6–13.5 ppm), aldehyde (δ 9.4–10.0 ppm), phenolic hydroxyl (δ 8.0–9.4 ppm), β–5 phenolic hydroxyl (δ 8.99 ppm), syringyl C5 phenolic hydroxyl (δ 8.0–8.5 ppm), aromatic protons (δ 6.3–7.7 ppm), and aliphatic protons [Lundquist, K., "NMR Studies of Lignins. 3. ^{1}H-NMR Spectroscopic Data for Lignin Model Compounds", *Acta Chemica Scandinavica*, B33, 418, (1979); [238]Lundquist, K., "On the Occurence of β-1 Structures in Lignins", *Journal of Wood Chemistry and Technology*, 7 (2), 179, (1987). Li, S. and Lundquist, K., "A New Method for the Analysis of Phenolic Groups in Lignins by ^{1}H-NMR", *Nordic Pulp and Paper Research*

Journal, 3, 191, (1994); Lundquist, K., "NMR Stduies of Lignins. 5. Investigation of Non-derivatized Spruce and Birch Lignin by ^{1}H-NMR Spectroscopy", *Acta Chemica Scandinavica*, B35, 497, (1981); Lundquist, K. and Olsson, T., "NMR Studies of Lignins. I. Signals Due to Protons in Formyl Groups", *Acta Chemica Scandinavica*, B31, 788, (1977)].

Proton-NMR has also been used for the quantification of structures in lignin related humic acid and fulvic acid samples. The major advantages of ^{1}H–NMR are no modification of the residual lignin is required and the high intrinsic sensitivity allows for the use of a small sample size and a short acquisition time. Figure illustrates a typical ^{1}H–NMR spectrum of a nonacetylated kraft softwood residual lignin.

DMSO–d_6 is an excellent lignin solvent and the chemical shift of hydroxyl protons in this solvent is characteristic and proton exchange is slow. Li and Lundquist have stated that ^{1}H–NMR spectrometric analysis of lignin–phenolic groups in DMSO–d_6 solvent is possible if the following conditions are maintained: the amount of water present is 'almost bone dry', no acid is present except for the small number of lignin–carboxylic acid groups, and no base is present.

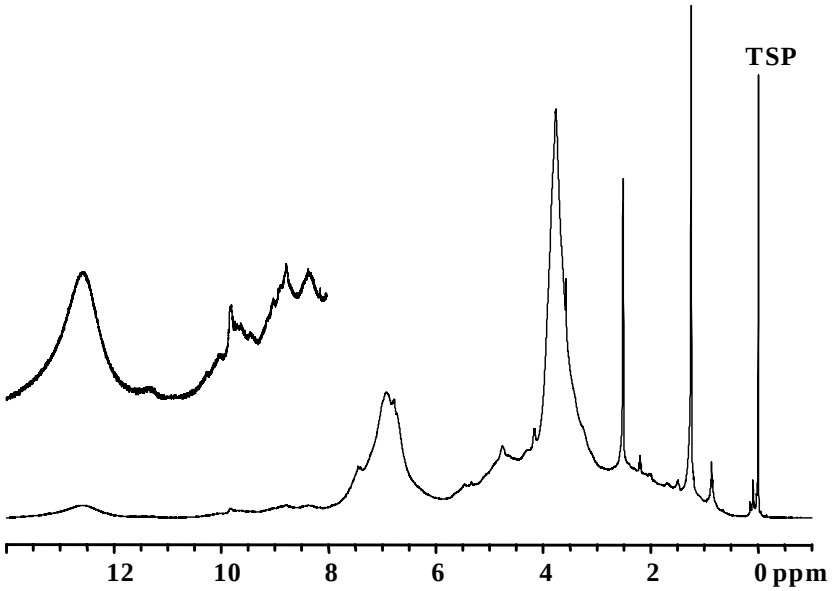

Figure 2. Quantitative ^{1}H–NMR spectrum of a residual isolated from an oxygen delignified softwood kraft pulp (brownstock, kappa = 47, prepared in this study).

The analysis of underivatized lignins also allows for the quantification of carboxylic acid groups which have a well separated signal at approximately δ 12. Integrating this signal has been found to yield valuable data on the quantity of carboxylic acid groups in lignins, as will be demonstrated in this dissertation. In some circumstances phosphitylating (and acetylating) lignin with high carboxylic acid contents seems to be difficult making the analysis of lignins with high content of these functional groups difficult.

^{1}H-NMR of Acetylated Lignin samples provides improved spectral resolution of key lignin functionality. Table 1 lists the structural assignments for the chemical shifts in this spectrum, which are based on extensive model compound studies. The small, broad peak at $\delta \sim 6.0$ can be principally assigned to arylglycerol units with a β-aryl ether substituent, however, β-1 structures and arylglycerol units also have been shown to contribute to this peak. Additional types of β-aryl ether structures (e.g. derivatized arylglycerol β-aryl ethers with an ether group at the α position) do not contribute to the peak at $\delta \sim 6$. It is possible, however, to make rough but accurate estimates on the percentage of β-aryl ether linkages in the lignin by integrating this peak. The signals at δ 2.3 and 2.0 are assigned to the phenolic and aliphatic acetate groups, respectively. However, it has been shown that phenolic acetate signals from biphenyl structures have a chemical shift at 2.08-2.11 ppm. Quantifying phenolic hydroxyl groups using this technique must be performed with the realization that some free phenolic groups are contributing to the aliphatic acetate signal which will result in a slight underestimate of the total phenolic content and an overestimate of the aliphatic hydroxyl content (Lundquist, K. ^{1}H-NMR Spectral Studies of Lignins. Quantitative Estimates of Some Types of Structural Elements. Nordic Pulp and Paper Research Journal 3:140-146 (1991)).

Table 1. Assignments of signals in the ^{1}H NMR spectrum of acetylated spruce lignin shown in Figure 24 (Lundquist, K. Proton (^{1}H) NMR Spectroscopy. In: Lin, S.Y. and Dence, C.W. (eds.) Methods in Lignin Chemistry, Springer Verlag Berlin Heidelberg 242-249 (1992)).

δ ppm	Assignment
1.26	Hydrocarbon contaminant
2.01	Aliphatic acetate
2.28	Aromatic acetate
2.62	Benzylic protons in β-β structures
3.81	Protons in methoxyl groups
4.27	Hγ in several structures
4.39	Hγ in, primarily, β-O-4 structures and β-5 structures
4.65	Hβ in β-O-4 structures
~4.80	Inflection possibly due to Hα in pinoresinol units and Hβ in noncyclic benzyl aryl ethers
5.49	Hα in β-5 structures
6.06	Hα in β-O-4 structures (Hα in β-1 structures)
6.93	Aromatic protons (certain vinyl protons)
7.29	Chloroform (solvent)
7.41	Aromatic protons in benzaldehyde units and vinyl protons on the carbon atoms adjacent to aromatic rings in cinnamaldehyde units
7.53	Aromatic protons in benzaldehyde units
9.64	Formyl protons in cinnamaldehyde units
9.84	Formyl protons in benzaldehyde units

Carbon(13)–NMR is a powerful technique capable of revealing a large amount of lignin structural information (see Fig. 3) including the presence of aryl ether, condensed and uncondensed aromatic and aliphatic carbons [Pan, D., Tai, D., and Chen, C.-L., "Comparitive Studies on Chemical Composition of Wood Components in Recent and Ancient Woods of Bischofia polycarpa", *Holzforschung*, 44 (1), 7, (1990); Gellerstedt, G. and Robert, D., "Quantitative ^{13}C NMR Analysis of Kraft Lignins", *Acta Chemica Scandinavica*, B41, 541, (1987); Hawkes, G., Smith, C., Utley, J., Vargas, R., and Viertler, H., "A Comparison of Solution and Solid State ^{13}C-NMR Spectra of Lignins and Lignin Model Compounds", *Holzforschung*, 47, 302, (1993); Robert, D., *Carbon-13 Nuclear Magnetic Resonance Spectrometry*, in *Methods in Lignin Chemistry*, Lin, S. and Dence, C., Editors, Springer-Verlag, New York, (1992); Kringstad, K. and Morck, R., "^{13}C-NMR Spectra of Kraft Lignins", *Holzforschung*, 37, 237, (1983); Nimz, H. and Ludeman, H., "Kohlenstoff-13-NMR-Spektren von Ligninen, 6.) Lignin- und DHP-Acetate", *Holzforschung*, 30 (2), 33, (1976); Drumond, M., Aoyama, M., Chen, C.-L., and Robert, D., "Substituent Effects on C-13 Chemical Shifts of Aromatic Carbons in Biphenyl Type Lignin Model Compounds", *Journal of Wood Chemistry and Technology*, 9 (4), 421, (1989).]. Table lists an extensive compilation of structural assignments that have been derived from model compound studies. The major disadvantage of ^{13}C–NMR spectroscopy is the inherent low sensitivity which requires that a large sample size and a long acquisition time be used. Nonacetylated lignin samples are dissolved in either DMSO–d_6 or acetone–d_6/D_2O (9:1 v/v) at a concentration of 400–600 mg lignin / 1.8 mL solvent. Functional group chemical shift differences between the two solvent systems are generally less than 1 ppm.

Quantitative ^{13}C–NMR analysis requires a number of conditions to be fulfilled. First, the lignin sample must be free of contaminants such as carbohydrates or extractives. Also, the lignin/solvent solution must be made as concentrated as possible to maximize signal–to–noise and minimize baseline and phasing distortions. Generally, ^{13}C–NMR spectra of concentrated lignin/DMSO–d_6 are acquired at 50ºC in order to reduce viscosity. A 11–second pulse delay has been used which is five times the longest lignin–carbon T_1 relaxation time [Robert, D., "Quantitative Structural Analysis of Lignins by ^{13}C NMR Analysis", Proceedings, Canadian Wood Chemistry Symposium, Niagara Falls, Canada, 1982, p. 63]. Finally, the inverse–gated decoupling sequence is used which involves turning off the proton decoupler during the recovery between pulses so that the NOE effect is avoided.

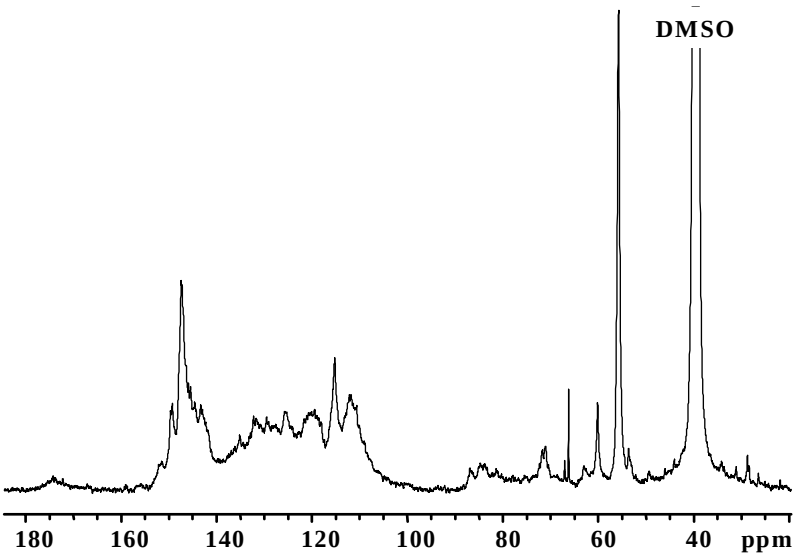

Figure 3. Quantitative ^{13}C–NMR spectrum of softwood residual lignin (brownstock, kappa = 47).

Table 2. Chemical shift assignments for ^{13}C–NMR spectrum of lignin.

δ ^{13}C–NMR (ppm)	Structure [a]
193.4	C=O in ϕ–CH=CH–CHO, C=O in ϕ–C(=O)CH(–O ϕ)–C–
191.6	C=O in ϕ–CHO
169.4	Ester C=O in R–C(=O)OCH_3
166.2	C=O in ϕ–COOH, Ester C=O in ϕ–C(=O)OR
156.4	C–4 in H-units
152.9	C–3/C–3’ in etherified 5–5 units, C–α in ϕ–CH=CH–CHO units
152.1	C–3/C–5 in etherified S units and B ring of 4–O–5 units
151.3	C–4 in etherified G units with α–C=O
149.4	C–3 in etherified G units
149.1	C–3 in etherified G type β–O–4 units
146.8	C–4 in etherified G units
146.6	C–3 in non-etherified G units (β–O–4 type)
145.8	C–4 in non-etherified G units
145.0	C–4/C–4’ of etherified 5–5 units
143.3	C–4 in ring B of β–5 units, C–4/C–4’ of non–etherified 5–5 units
134.6	C–1 in etherified G units
132.4	C–5/C–5’ in etherified 5–5 units
131.1	C–1 in non–etherified 5–5 units
129.3	C–β in ϕ–CH=CH–CHO
128.0	C–α and C–β in ϕ–CH=CH–CH_2OH
125.9	C–5/C–5’ in non–etherified 5–5 units
122.6	C–1 and C–6 in ϕ–C(=O)C–C units
119.9	C–6 in G units
118.4	C–6 in G units
115.1	C–5 in G units

114.7	C–5 in G units
111.1	C–2 in G units
110.4	C–2 in G units
86.6	C–α in G type β–5 units
84.6	C–β in G type β–O–4 units (*threo*)
83.8	C–β in G type β–O–4 units (*erythro*)
71.8	C–α in G type β–O–4 units (*erythro*)
71.2	C–α in G type β–O–4 units (*threo*), C–γ in G type β–β
63.2	C–γ in G type β–O–4 units with α–C=O
62.8	C–γ in G type β–5, β–1 units
60.2	C–γ in G type β–O–4 units
55.6	C in ϕ–OCH_3
53.9	C–β in β–β units
53.4	C–β in β–5 units

[a] data from [247], G = guaiacyl, S = syringyl.

Phosphorous–NMR has been exploited to determine hydroxyl functional groups in various substrates [Hulst, R., Kellogg, R., and Feringa, B., "New Methodologies for Enantiomeric Excess (ee) Determination Based on Phosphorous NMR", *Recueil des Travaux Chimiques des Pays-Bas*, 114 (4-5), 115, (1995)] including coal [Mohan, T. and Verkade, J., "Determination of Total Phenolic Concentrations in Coal Liquefaction Resids by ^{31}P NMR Spectroscopy", *Energy and Fuels*, 7, 222, (1993); Wroblewski, A., Lensink, C., Markuszewski, R., and Verkade, J., "^{31}P NMR Spectroscopic Analysis of Coal Pyrolysis Condensates and Extracts for Heteroatom Functionalities Possessing Labile Hydrogen", *Energy and Fuels*, 2, 765, (1988)], and isolated lignin [Argyropoulos, D., "Quantitative Phosphorus-31 NMR Analysis of Six Soluble Lignins", *Journal of Wood Chemistry and Technology*, 14 (1), 65, (1994); Argyropoulos, D., Bolker, H., Heitner, C., and Archipov, Y., "^{31}P-NMR Spectroscopy in Wood Chemistry Part V. Qualitative Analysis of Lignin Functional Groups", *Journal of Wood Chemistry and Technology*, 13 (2), 187, (1993); Argyropoulos, D., "Quantitative Phosphorus-31 NMR Analysis of Lignins, A New Tool for the Lignin Chemist", *Journal of Wood Chemistry and Technology*, 14 (1), 45, (1994)]. Trivalent and pentavalent phosphorous reagent have been used. The largest diastereomeric shift differences and substituent influences are observed with trivalent phosphorous reagents.

Hydroxyl functional groups in isolated lignins have been identified by a ^{31}P–NMR technique that involves derivatization with the phosphorylating agent 2–chloro–4,4,5,5–tetramethyl–1,3,2–dioxaphospholane (TMDP). The reaction of TMDP with hydroxyl functional groups is illustrated in Figure 4. TMDP reacts with hydroxyl functional groups to give phosphite products which are resolvable by ^{31}P–NMR into separate regions arising from aliphatic hydroxyl, phenolic, and carboxylic acids groups. Figure illustrates typical spectra of a TMPD treated softwood residual lignin

sample. Table 5 gives a compilation of integration region that have been used for the TMPD/^{31}P–NMR analysis of softwood isolated lignins.

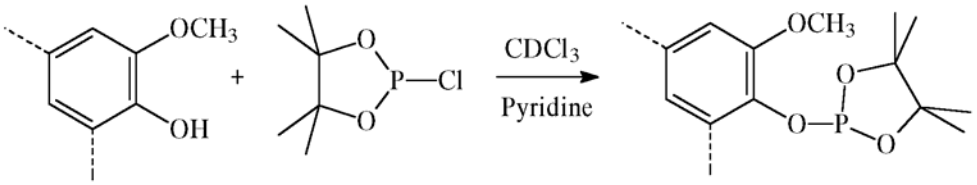

Figure 4. Derivatization of phenolic structures with 2–chloro–4,4,5,5–tetramethyl–1,3,2–dioxaphospholane (TMDP).

The major advantages of the TMPD/^{31}P–NMR is that the technique is well developed and a database of model compound spectral information is available [256]. An additional derivatizing agent, 2–chloro–1,3,2–dioxaphospholane, has been reported to allow for the discrimination between primary and secondary hydroxyl groups and also to differentiate between *erythro*– and *threo*–conformations. Quantitative information gained from the technique has been verified against other techniques (benzyl acetate/GC, ^{1}H–NMR, ^{13}C–NMR and ^{31}P–NMR) during a recent international round robin lignin study.

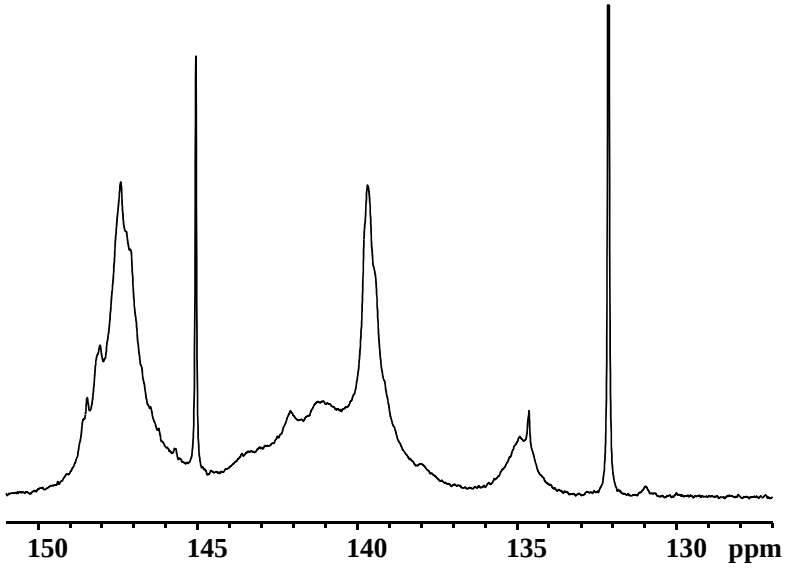

Figure 5. Quantitative ^{31}P–NMR spectrum of softwood residual lignin (brownstock, kappa = 47, prepared in this study, derivatized with TMDP).

Table 5. Integration regions for TMDP treated SW lignins.

Functional Group	**Integration Region (ppm)**
Cyclohexanol (internal standard)	145.6 – 144.4
Aliphatic OH	149.0 – 145.6
Condensed phenolic OH	144.4 – 140.4
Guaiacyl phenolic OH	140.4 – 137.6
Carboxyl OH	136.0 – 133.8