^{31}P NMR Analysis of Lignin Hydroxyl Groups

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 . 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. **Error! Reference source not found.** illustrates a typical spectra of a TMPD treated softwood residual lignin sample. **Error! Reference source not found.** gives a comprehensive compilation of integration region that have been used for the TMPD/^{31}P–NMR analysis of softwood isolated lignins.

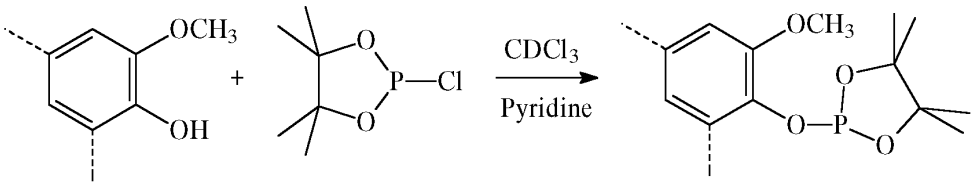

Figure 1. Derivatization of phenolic structures with 2–chloro–4,4,5,5–tetramethyl–1,3,2–dioxaphospholane (TMDP).

Quantitative information gained from this 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.

^{31}P NMR experiments can be carried out in accordance with established literature methods as follows:

1. A solvent solution (50 mL) of 1.6:1 (v/v) of pyridine to deuterated chloroform was prepared.
2. The solvent solution (25 mL) was used to prepare a mixture solution containing 100 mg of cyclohexanol (internal standard) and 90 mg of chromium acetylacetonate (relaxation agent).

3. Previously dried lignin (20-25 mg) was accurately weighed into a 4 mL vial that contained a magnetic spin bar. All lignins were dried under vacuum (30 in. Hg) at 40°C for 24 hours.
4. An aliquot of the solvent solution (400 μl) and an aliquot of the mixture solution (150 μl) were introduced into the vial, (sealed with a Teflon cap) containing the lignin and the spin bar, from two different Hamilton syringes.
5. The mixture in the vial was mixed for a few minutes (approx. 5 min).
6. 2-Chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (70 μL; TMDP or phosphitylating agent) was introduced into the vial. The vial was shaken for approximately 20 seconds before it was transferred to a 5-mm NMR tube to record the NMR spectrum.

Each ^{31}P-NMR acquisition was performed with a 25 s delay between 90° pulses. An inverse gated decoupling pulse sequence was used to obtain quantitative spectra. A minimum of 150-200 transients were acquired for each sample. The acquisitions were performed at room temperature, using a 62 ppm sweep width. (TD = 32,768) and a 4 Hz line broadening. Chemical shifts were calibrated relative to an internal standard, either the cyclohexanol peak signal centered at δ 145.1 ppm or N–hydroxyl phthalimide at δ 150.7 ppm. Integration regions that were used to assign the signals are tabulated in Table 1.

Table 1. Integration regions used for ^{31}P–NMR analysis of TMDP treated SW lignin.

Structure	δ ^{31}P–NMR
1) Aliphatic OH	150.0 – 145.5
IS) Cyclohexanol (Internal standard)	144.7 – 145.5
2) Phenols	136.6 – 144.7
2a) Combined *para*–OH–ϕ and guaiacyl	137.3 – 140.0
2b) C_5 substituted "condensed"	140.0 – 144.7
2b1) β–5	142.8 – 144.7
2b2) 4–O–5	141.7 – 142.8
2b3) 5–5	140.2 – 141.7
2c) Guaiacyl	139.0 – 140.0
2d) Catechol	138.2 – 139.0

2e) *Para*–hydroxy–phenyl	137.3 – 138.2
3) Carboxylic acid OH	133.6 – 136.6
TMDP	176.0
TMDP hydrolysis product	132.2

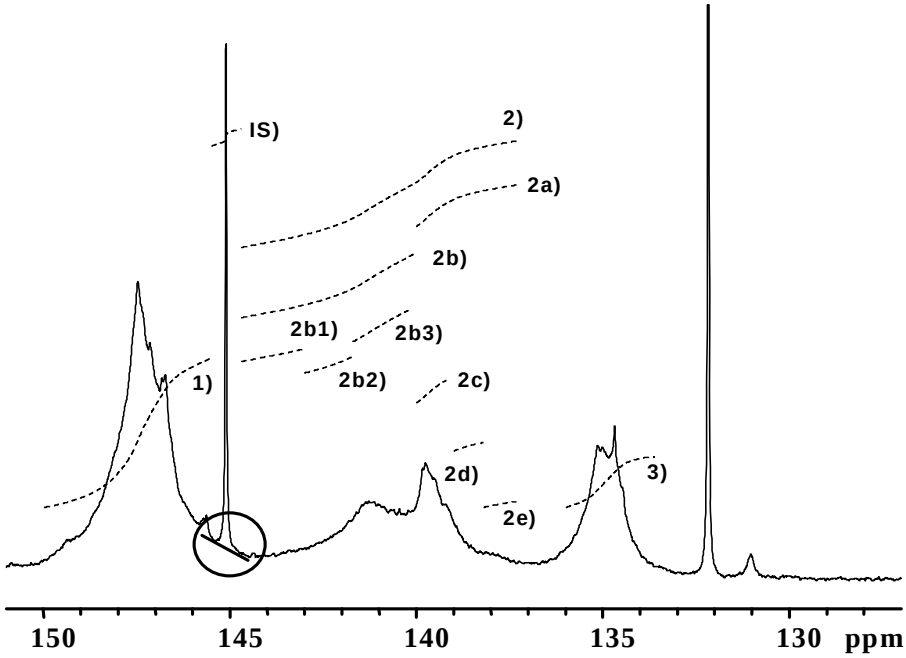

Figure 2. ^{31}P NMR spectrum, with integration regions, of a softwood kraft effluent lignin treated with TMDP

Sample	Aliphatic OH	C5 condensed phenolic OH	5-5'-Biphenyl OH	Syringyl PhOH	C5 noncondensed PhOH	Catechol OH	*p*-hydroxyphenyl OH	Carboxyl OH
Pine Residual Lignin isolated from kraft pulp Pulp Kappa #								
162.8	2.19	0.64	0.34		1.026	0.11	0.07	0.27
129.7	2.19	0.64	0.34		1.026	0.11	0.07	0.27
88.5	2.19	0.64	0.34		1.026	0.11	0.07	0.27
69.2	2.19	0.64	0.34		1.026	0.11	0.07	0.27
52.2	1.63	0.79	0.42		1.066	0.14	0.08	0.32

37.9	1.63	0.79	0.42		1.066	0.14	0.08	0.32
30.5	1.63	0.79	0.42		1.066	0.14	0.08	0.36
28.8	1.63	0.79	0.42		1.066	0.14	0.08	0.39
Sweetgum Residual Lignin isolated from kraft pulp Pulp Kappa #								
29	2.69	0.39		0.72	0.34	.13		.43
23	2.40	0.36		0.63	0.29	.13		.32
14	2.25	0.34		0.61	0.32	.16		.27