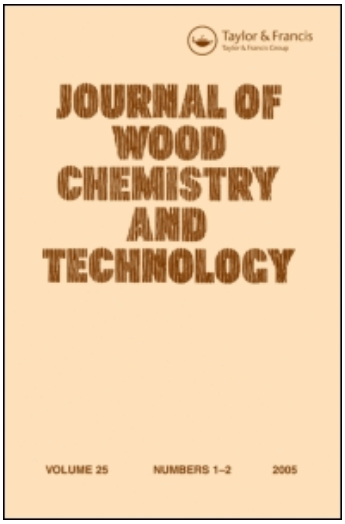

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CARBON-13 NMR SPECTRA OF LIGNINS, 9.[1] SPIN-LATTICE RELAXATION TIMES (T_1) AND DETERMINATION OF INTERUNIT LINKAGES IN THREE HARDWOOD LIGNINS (ALNUS GLUTINOSA, CORYLUS AVELLANUS AND ACER PSEUDOPLATANUS)

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ABSTRACT

The spin-lattice relaxation times (T_1) of the twelve most abundant carbon atoms in acetylated milled wood lignin (MWL) from hazelnut (Corylus avellanus) have been determined by the progressive saturation pulse Fourier transform (PSFT) technique. T_1 values below 0.5 s have been found for the aliphatic carbon atoms in the C_γ side chains, while substituted aromatic, carbonyl and CH_3 carbon atoms in methoxy and acetyl groups have T_1 values ranging between 1 and 3 s. From these results a quantitative determination of interunit linkages in lignin may be possible from carbon-13 spectra with pulse interval times of at least 3 s.

Furthermore, the carbon-13 NMR spectra of three acetylated hardwood MWLs, from black alder, hazelnut and mountain maple, have been recorded at 90.7 MHz. Five interunit linkages, β-O-4, α,β--bis-O-4, β-1, β-β and β-5, have been traced in each lignin and their frequences qualitatively compared with each other.

INTRODUCTION

For a macromolecule like lignin, having a non-regular, cross-linked structure with some ten different kinds of interunit linkages, carbon-13 NMR spectroscopy, in comparison with proton NMR, offers four basic advantages: A fivefold wider absorption range at a given magnetic field, narrower line widths, due to longer spin-spin relaxation times (T_2), lack of homonuclear coupling

and higher sensitivity of carbon-13 nuclei towards substitution patterns, leading to a much higher degree of structural information.

However, a main drawback in routine carbon-13 NMR spectra of lignins has been that the signal areas are not proportional to the carbon concentrations, which is the case in proton NMR spectra. Due to the low gyromagnetic ratio and natural abundancy of carbon-13 nuclei, up to 100,000 spectra had to be accumulated in previous carbon-13 NMR spectra of lignins[4,5] for obtaining reasonable signal/noise (S/N) ratios at 25.2 MHz. This means that, at a total recording time of at the most 24 hours, pulse repetition times of less than 1 s had to be applied. At such short pulse repetition times carbon-13 nuclei cannot relax quantitatively to their Boltzmann distributions, so that their S/N ratios become dependent on their spin-lattice relaxation times (T_1). In order to eliminate this influence of the T_1 values on the S/N ratios, pulse repetition times of at least 5 times T_1 have to be chosen. Knowledge of the T_1 values is therefore necessary for the choice of appropriate pulse repetition times, if a quantitative evaluation of the carbon-13 spectra is intended.

Depending on the fact that different influences contribute to the relaxation of carbon-13 nuclei, their T_1 values fall in a broad range. As spin-lattice relaxation occurs mainly by dipole-dipole (DD) interaction, carbon atoms with directly bound hydrogen atoms have shorter relaxation times than quaternary carbon atoms. In small molecules T_1 values of carbon-13 nuclei range between about 1 and 100 s, which means that at repetition times of 5 T_1 only a few hundred spectra can be gathered within 24 hours. However, T_1 values also depend on the molecular mobility in the sample solution, characterized by the molecular correlation time τ_c, which is the average time of reorientation for a molecule in solution. τ_c of small molecules is in the order of ps, increasing with the viscosity of the solution and the size of the molecules. DD interaction with surrounding fluctuating magnetic fields ("lattice") reaches a maximum, when τ_c is reciprocal to the Larmor

frequency of the carbon-13 nuclei which, at a magnetic field of 2.3 tesla (T), is about 25 MHz. This means that T_1 decreases with decreasing mobility of the molecules in solution, approaching its lowest value at τ_c of about 10^{-8} s. Furthermore, it has been found by ESR spectroscopy[6] that lignins contain unpaired electrons at concentrations of some 10^{17} spins/g. Due to their strong dipole moments, unpaired electrons participate strongly in DD interaction with carbon-13 nuclei, causing a significant reduction in their T_1 values. We may therefore expect much shorter spin-lattice relaxation times for the carbon atoms in lignins than for those in smaller molecules.

Another important fact in pursuing quantitative carbon-13 NMR spectroscopy of lignins is the recent accessibility of NMR spectrometers with higher magnetic fields, which improve not only the resolution of the signals but also their intensities. With such instruments, the number of accumulated spectra (scans) for getting appropriate S/N ratios can be significantly reduced and longer pulse-repetition times can be used.

The first quantitative determination of β-O-4 linkages and total hydroxyl contents in lignins by carbon-13 NMR spectroscopy has been carried out recently by D. Robert and D. Gagnaire[7], using pulse-delay times of 11 s at 62.9 MHz. According to these authors[8], the T_1 values, measured by the inversion-recovery method, ranged between 0.1 and 2.8 s.

In the present paper we are dealing with the determination of the T_1 values of the carbon atoms in acetylated hazelnut MWL by the progressive saturation pulse-Fourier transform (PSFT) technique. Furthermore, the carbon-13 NMR spectra of three acetylated hardwood MWLs, from hazelnut, black alder and mountain maple, have been recorded at 90.7 MHz.

EXPERIMENTAL

Milled wood lignins (MWLs) were obtained from hazelnut (Corylus avellanus), black alder (Alnus glutinosa) and mountain maple (Acer pseudoplatanus) according to the Björkman procedure[9]

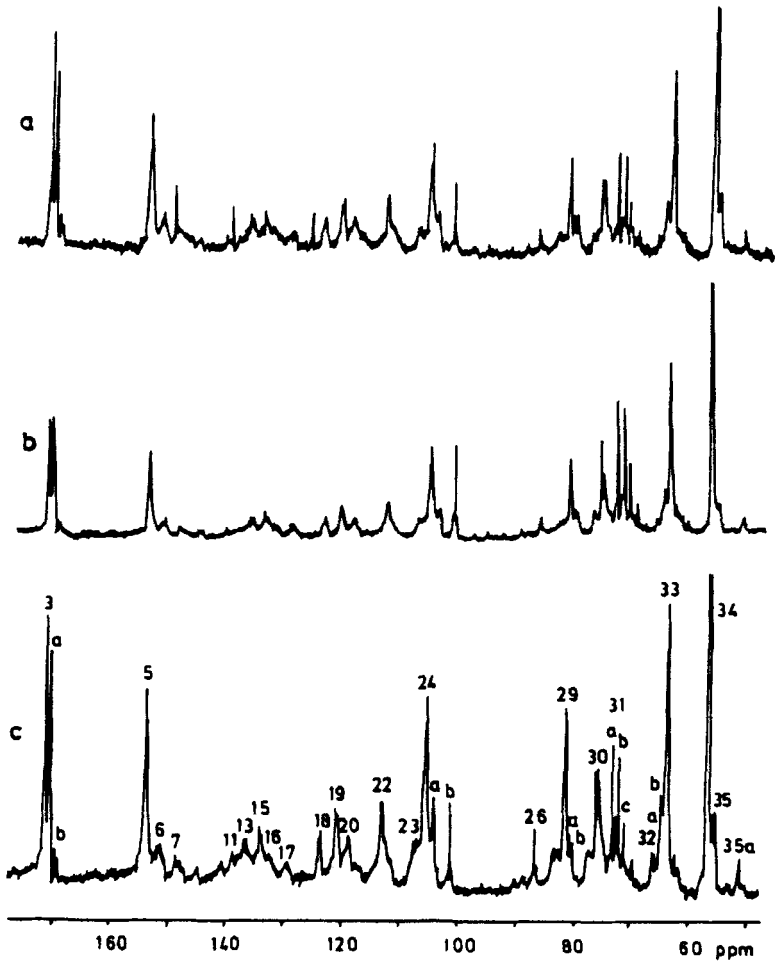


FIGURE 1. Proton Noise Decoupled 90.7 MHz-^{13}C-PFT-NMR Spectra of Acetylated MWLs from Black Alder (Alnus glutinosa) (a), Hazelnut (Corylus avellanus) (b) and Mountain Maple (Acer pseudoplatanus) (c). Internal Standard: TMS

and subsequently acetylated with pyridine/acetic anhydride. Samples of acetylated lignins, ranging between 210 and 350 mg, were dissolved in 3 ml acetone-d_6/D_2O (9:1) each, and the solutions clarified by centrifugation. T_1 values were determined according to the progressive saturaturation pulse Fourier trans-

form (PSFT) technique[10]. 5000 scans each were accumulated with a Bruker WH 360 spectrometer at a 90°, τ... sequence, with τ = 0.51, 1.01, 1.51, 2.01, 3.01, and 8.41 s. Total recording time for each spectrum varied between 0.7 and 11.7 hours. In order to get $T_1 \gg T_2$, powergated decoupling of protons was applied with 12H decoupling power during pulse-delay and 6H during data aquisition, at an aquisition time of 0.41 s. T_1 values were obtained as the reciprocal slope of a semilogarithmic plot of $(I_\infty - I_\tau)$ versus τ, where I_∞ is the signal intensity at τ = 8.41 s. Carbon-13 NMR spectra of the acetylated MWLs, shown in Fig. 1, were obtained at pulse-intervals (repetition times) of 1.5 s and the number of scans was about 20,000 each, under otherwise identical conditions.

RESULTS AND DISCUSSION

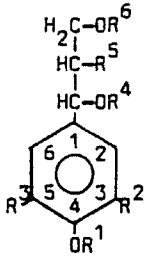
Spin-Lattice Relaxation Times (T_1)

The T_1 values of the carbon atoms, corresponding to the fifteen most prominent signals in the carbon-13 spectrum of hazelnut

TABLE 1

Spin-Lattice Relaxation Times (T_1) of Carbon Atoms in Acetylated MWL from Hazelnut (for Notation of Carbon Atoms See Scheme 1)

Carbon Atom	ppm	Sign.No.	T_1 (s)
carbonyl-C in prim. acetyl groups	171.1	3	3.0
carbonyl-C in sec. acetyl groups	170.4	3a	2.1
Se-3/5	153.4	5	2.0
Se-1(α-OAc)	133.6	15	<0.5
Ge-6	120.2	19	<0.5
G-2(α-OAc)	112.7	22	<0.5
Se-2/6	105.3	24	<0.5
C-1 in xylan	101.0	24b	<0.5
C-β in β-O-4	81.4	29	<0.5
C-α in β-O-4	75.2	30	<0.5
C-4 in xylan	73.1	31a	1.0
C-3 in xylan	71.8	31b	0.5
C-γ in β-O-4	63.2	33	<0.5
OCH_3	56.4	34	1.02
CH_3 in acetyl	20.6	40	2.1



C_9 unit in lignin

G (guaiacyl): $R^2 = OCH_3$, $R^3 = H$

S (syringyl): $R^2 = R^3 = OCH_3$

H (p-hydroxy-phenyl): $R^2 = R^3 = H$

a (acetylated): $R^1 = COCH_3$; e (etherified): $R^1 = R$

α-OAc: $R^4 = COCH_3$; α-OR: $R^4 = R$

β-O-4: $R^4 = R^6 = COCH_3$, $R^5 = O\text{-}4'$

α,β-bis-O-4: $R^4 = C\text{-}4'$, $R^5 = O\text{-}4''$, $R^6 = COCH_3$

β-1: $R^4 = R^6 = COCH_3$, $R^5 = C\text{-}1'$

β-5: $R^4 = C\text{-}4'$, $R^5 = C\text{-}5'$, $R^6 = COCH_3$

β-β: $R^4 = C\text{-}\gamma'$, $R^5 = C\text{-}\beta'$, $R^6 = C\text{-}\alpha'$

Scheme 1: Notation of Carbon Atoms in C_9 Units of Lignins

MWL acetate are listed in Table 1. As T_1 values smaller than 0.5 s cannot exactly be determined by the PSFT method, they are indicated in Table 1 by $T_1 < 0.5$ s. The notation of carbon atoms in Tables 1-3 is carried out according to common nomenclature in lignin chemistry[11], as indicated in Scheme 1.

Table 1 shows that carbon atoms in lignin directly linked to hydrogen atoms have relaxation times shorter than 0.5 s with only two exceptions, the methyl carbon atoms in methoxy and acetyl groups, appearing at 56.4 and 20.6 ppm, respectively. This can be attributed to the higher mobility of the carbon atoms in terminal methyl groups, causing a less effective DD interaction with the "lattice". The same applies for the signals at 71.8 and 73.1 ppm, which are assigned to carbon atoms 3 and 4 in oligomeric xylans, having a higher molecular mobility, due to their lower molecular weight.

Quaternary carbon atoms in substituted aromatic rings, e.g. Se-3/5, or in carbonyl groups have T_1 values longer than 0.5 s. An exception is Se-1, giving a signal at 133.6 ppm, which again can be explained by assuming reduced mobility, due to their preferred rotation of the syringyl residue about the C-1-C-4 axis (anisotropic molecular mobility).

As the aliphatic carbon atoms in the C_3 side chains of the C_9 units in lignin have T_1 values below 0.5 s, pulse repetition times of only 3 s are required for the quantitative determination of interunit linkages in lignin. This means that some 20.000 spectra can be gathered within 20 hours. Taking into account the elimination of the nuclear Overhauser effect (NOE) on the S/N ratio by gated decoupling[7], about 500 mg of a lignin sample are needed for the quantitative determination of the main interunit linkages in lignin with a 360 MHz spectrometer. This amount has to be increased to about 2 g, if the quantitative determination of quaternary aromatic and carbonyl carbon atoms is intended.

Carbon-13 Spectra of Acetylated MWLs from Black Alder, Hazelnut and Maple

The three spectra, shown in Fig. 1, are only of qualitative character, due to the fact that they were recorded from only some 200 mg samples at pulse interval times of less than 5 T_1 (s. EXPERIMENTAL). In comparison to earlier hardwood lignin spectra[1,5], a higher resolution of signals is achieved at a carbon-13 resonance of 90.7 MHz (360 MHz for protons). The assignment of signals is given in Table 2.[1,4,5]

In Fig. 1, carbonyl carbon atoms give the signals 3, 3a and 3b for primary, secondary and phenolic acetyl groups, respectively. Very interestingly, signal 3b is split by about 0.3 ppm, which may be attributed to different chemical vicinities for acetyl groups in acetylated guaiacyl and syringyl units. However, quantitative values of these groups can only be obtained from carbon-13 spectra recorded at pulse intervals of about 15 s, requiring some 2 g samples for about 5000 scans (cf.[7]).

The ratio of acetylated to etherified guaiacyl residues (Ga/Ge) can be approximately estimated from the areas of signals 18 and 19, because these signals each correspond to only one carbon atom, 5 and 6, respectively, in acetylated or etherified guaiacyl residues (cf. Table 2). This ratio is about 1:3 for alder

TABLE 2

ppm Values, Relative Areas and Assignments of Signals in Figure 1

Sign. No.	ppm	Rel. Signal Areas Alder	Hazelnut	Maple	Assignment[+]
3	171.3–170.9	1.24	0.97	0.92	primary acetoxy–CO
3a	170.9–170.2	1.22	0.98	0.83	secondary acetoxy–CO
3b	169.7–169.2	0.05			phenolic acetoxy–CO
5	154.0–152.8	1.43	0.89	1.16	S–3/5, Ga–3(α–OR)
6	151.8–151.3	0.19			Ga–3(α–OAc), Ge–4
7	149.4	0.10			Ge–3(α–OR)
8	149.0–148.0				Ge–3(α–OAc)
11	139.6	0.06			Ga–4, Ga–1(α–OR)
13	136.4	0.08			Se–4,Sa–1,Ga–1,all(α–OAc)
14	136.1–135.9	0.13			Ge–1(α–OR), Se–1(α–OR)
15	133.8–133.4	0.30	0.02		Se–1(α–OAc)
16	132.6–131.8				Ge–1(α–OAc)
17	129.3–128.6				Sa–4, C–1' in B–5, H–2/6
18	123.6–123.2	0.19		0.57	Ga–5
19	120.6–119.9	0.56	0.18	1.67	Ge–6
20	118.5–118.2	0.31		0.72	Ga–6, Ge–5(α–OAc)
22	113.0–112.5	0.67	0.23	0.81	G–2(α–OAc)
22a	111.8–111.5	0.24			G–2(α–OR)
23	107.0–106.0	0.04		0.80	Sa–2/6
24	105.9–104.6	1.42	1.16	3.00	Se–2/6
24a	104.3–103.6	0.41	0.03	0.27	
24b	101.1–100.9	0.21	0.21	0.13	C–1 in xylan
26	86.4	0.03		0.07	C–α in B–B
28	83.0–81.8				C–α/β in α,B–bis–O–4
29	81.6–81.2	0.91	0.62	0.82	C–B in B–O–S
29a	80.5–80.4	0.26	0.08		C–B in B–O–G
29b	80.1–79.9	0.18	0.04		
30a	78.0–77.0				
30b	76.1–75.8	0.24	0.46	0.37	C–α in B–1
30	75.6–74.3	1.10	0.77	0.54	C–α in B–O–4
31a	73.3–73.0	0.38	0.74	0.26	C–4 in xylan
31	72.4	0.10	0.23	0.17	C–γ in B–B
31b	71.8	0.35	0.50	0.25	C–3 in xylan
31c	70.8	0.31	0.20	0.08	C–2 in xylan
32	66.1				C–γ in B–5
32a	64.7	0.09	0.12		C–γ in B–1
32b	64.3	0.48	0.40	0.46	C–γ in α,B–bis–O–4
33	63.4–62.9	2.01	1.71	1.45	C–γ in B–O–4
34	56.4	6.00	4.23	4.80	OCH_3
35	55.5–55.1	0.80	0.17	0.36	C–B in B–B
35a	51.1	0.06			C–B in B–5 and B–1
40	21.1–20.1	6.33	4.89	4.72	CH_3 in acetyl

[+] For notation of carbon atoms see Scheme 1

and maple, corresponding to 25% free phenolic groups in the non-acetylated MWLs, which constitutes a reasonable value. The reliability of this number must, however, be cautioned by the fact that the spectra were recorded at pulse repetition times lower than 5 T_1, at low signal intensities and no elemination of the NOE.

Similarly, acetylated and etherified syringyl units (Sa and Se) are indicated by signals 23 and 24. In this case, however, signal 23 is exceptionally broad and the assignment of signal 24a is still unknown.

The ratio of syringyl to guaiacyl units (S/G) in hardwood lignins is best reflected by signals 23 plus 24, assigned to carbon atoms 2 and 6 in syringyl units (S-2/6), and signals 22 plus 22a, caused by carbon atom 2 in guaiacyl units (G-2). Recently, D. Robert and D. Gagnaire[12] have determined the S/G ratio in hardwood lignins from the splitting of the methoxy signal in the carbon-13 NMR spectra. However, we were unable to find the same splitting in our spectra. On the other hand, methoxy carbon atoms show longer T_1 values than unsubstituted aromatic carbon atoms (cf. Table 1), requiring longer pulserepetition times for quantitative analysis.

The signals between 51.1 and 86.4 ppm in Fig. 1, assigned to the aliphatic carbon atoms in the C_3 side chains, indicate five interunit linkages in the three hardwood lignins (cf. Table 3). According to their signal areas, shown in Table 3, β-O-4 units constitute by far the most abundant interunit linkages. In accordance with earlier results[1], the predominance of β-O-4 structures is particularly high in hardwood lignins and less significant in softwood lignins. D. Robert and D. Gagnaire[7] found 0.59-0.65 β-O-4 structures per C_9 unit in birch lignin by quantitative carbon-13 NMR spectroscopy.

Discrete signals are also given by β-β as well as β-5 structures, though the signals of the latter are too weak for a determination of their areas. They are indicated in Table 3 by + signs, while minus signs (-) indicate the abscence of a signal.

TABLE 3

Relative Signal Areas in Fig. 1 of Carbon Atoms α, β and γ For the Five Most Abundant Interunit Linkages in Lignin

Interunit Linkage	C	ppm	Sign. No.	Integrated Signal Areas Alder	Hazelnut	Maple
β-O-4	α	74.3-75.6	30	1.10	0.77	0.54
	β	79.9-81.6	29	1.36	0.74	0.82
	γ	62.9-63.4	33	2.01	1.71	1.45
α,β-bis-O-4	α β	80.7	29	see C-β in β-O-4		
	γ	64.3	32b	0.48	0.40	0.46
β-1	α	75.8-76.1	30b	0.24	0.46	0.37
	β	51.1-51.2	35a	0.06	+	+
	γ	64.7	32a	0.09	0.12	+
β-β	α	86.4-86.6	26	0.03	+	0.07
	β	55.0-55.7	35	0.80	0.17	0.36
	γ	72.4	31	0.10	0.23	0.17
β-5	α	88.6	25	-	-	-
	β	51.1	35a	see C-β in β-1		
	γ	66.1	32	+	+	+

In α,β-bis-O-4 linkages carbon atoms α and β give the same signal at 80.7 ppm overlapping with the signal of carbon atom β in β-O-5 linkages (cf. Table 2). However, C-γ gives rise to a discrete signal (32b) at 64.3 ppm. The possibility that this signal may also be assigned to carbon atom 5 in acetylated xylan can be ruled out by the fact that it appears too in the carbon-13 spectra of acetylated spruce lignin and DHP with comparable intensities.

Similarly, carbon atom β in β-1 structures gives the same signal as C-β in β-5 structures (35a), while the signal of carbon atom γ (32a) overlaps with the signal of carbon atom γ in α,β-bis-O-4 linkages. Only carbon atom α in β-1 structures gives a discrete signal (30b) at 75.8-76.1 ppm.

From Table 3 it can be seen that β-O-4, β-1 and β-β structures are indicated by three discrete signals each, while α,β--bis-O-4 as well as β-5 structures give only one. Due to the

above mentioned reasons, the relative signal areas do not quantitatively reflect the frequences of the corresponding interunit linkages. However, it may be concluded that these frequences decrease in the following order: β-O-4>α,β-bis-O-4>β-1>β-β>β-5.

CONCLUSIONS

The conclusions that can be drawn from the relatively short T_1 values of the carbon atoms in lignin (cf. Table 1) are that they lead to broader signals, due to shorter spin-spin relaxation times (T_2). Taking into account the complex lignin structure, spectrometers with high magnetic fields should improve the resolution of the carbon-13 spectra of lignins significantly. On the other hand, low T_1 values allow a quantitative determination of interunit linkages in lignins by carbon-13 NMR spectroscopy. For this purpose, about 500 mg lignin are required at 90.7 MHz, while at least 2 g are necessary for a quantitative determination of quaternary carbon atoms in carbonyl and substituted aromatic groups. This shows that carbon-13 NMR spectroscopy offers an important analytical tool not only for structural research and comparison of various lignins, but also for tracing lignin reactions, e.g. during technical wood pulping and bleaching procedures.

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