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^{13}C NMR Characterization of Guaiacyl, Guaiacyl/Syringyl and Syringyl Dehydrogenation Polymers

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Summary

Dehydrogenation polymers (DHPs) were prepared from coniferyl alcohol (CA), sinapyl alcohol (SA), and a mixture of coniferyl and sinapyl alcohol. The polymers were fully acetylated and their carbon NMR spectra were compared. Comparison of the ^{13}C NMR spectra of the DHPs with those of authentic tri- and tetralignols facilitated the assignment of about 85 % of the 117 reported signals. Most of the unassigned signals were those from syringyl units. Major differences between the DHP prepared from CA and a pine milled wood lignin were: a relative deficiency of β-O-4 linkages and a predominance of b–5, β-β, and CA end units in the DHP. In contrast. there was no significant quantity of SA end units in a DHP prepared from SA. A major conclusion of the study confirmed that DHPs prepared from CA are relatively poor models of natural lignins isolated from gymnosperms.

Introduction

Dehydrogenation polymers (DHPs) of *p*-coumaryl, coniferyl, or sinapyl alcohols are frequently used to model the complex lignin structure. A homopolymer prepared from *p*-coumaryl alcohol models a hypothetical methoxyl-free lignin composed only of *p*-hydroxyphenyl units and is generally designated as "H-DHP". One prepared from coniferyl alcohol models a lignin composed only of guaiacyl units and is designated as "G-DHP". A G-DHP is intended to represent the lignin that is found in softwoods. A homopolymer from sinapyl alcohol models a hypothetical lignin composed only of syringyl units and is designated as "S-DHP". Polymerization of various mixtures of the three monomeric alcohols leads to heteropolymers. These polymers are given appropriate designations such as "GS-DHP" or "HGS-DHP", and model the types of lignins found in hardwoods. grasses. and legumes (Nimz *et al* 1981).

One advantage of DHPs over natural lignins is that the former are free of carbohydrates and extraneous wood components which complicate interpretation of experimental results. For example. lignins isolated from most softwoods are essentially pure guaiacyl lignins (Obst and Landucci 1986). but it is difficult to remove traces of carbohydrates and extraneous components, particularly if they are covalently linked to the lignin polymer. Another advantage of the synthetic lignins is that even though pure H- or S-lignins do not occur naturally, the study of the corresponding DHPs facilitates the interpretation of various functional entities because they are not obscured by the complexities in the natural polymers. For example. lignins from hardwoods and annual plants are composed of all three monomeric units, but woods generally have a very low content of *p*-hydroxyphenyl entities. Thus. the identification and chemical shift assignment of signals due to this minor structural entity in complex lignin spectra is generally very difficult. Finally, another advantage is that specifically labeled DHPs can be easily prepared by polymerizing appropriately labeled *p*-hydroxycinnamyl alcohols. Specifically labeled DHPs facilitate chemical shift assignments and elucidation of the mechanisms of various reactions that the polymer undergoes. DHPs have been labeled with ^{2}H (Gagnaire *et al.* 1970). ^{13}C (Gagnaire and Robert 1977; Lewis *et al.* 1987; Kern *et al.* 1989: Ellwardt *et al.* 1981; Hammel *et al.* 1993), and ^{14}C (Hammel *et al.* 1993). In contrast, *in-situ* labeling of specific sites of the natural polymer is far more tedious and time consuming as it requires the feeding of labeled precursors to the growing plant (Freudenberg and Neish 1968; Terashima *et al.* 1977; Tomimura *et al.* 1980; Xie and Terashima 1991, 1993; Lewis *et al.* 1988).

The major disadvantage of DHPs as lignin models is their lack of qualitative and quantitative integrity. Typically, DHPs have far greater contents of β-β (resinol), β–5 (phenylcoumaran) structures and cinnamyl alcohol end groups and a lower content of β-O-4 structures than does a natural isolated lignin such as milled wood lignin (Nimz and Lüdemann 1976; Brunow and Wallin 1981; Terashima *et a1*. 1995, 1996). All of these characteristics were found in DHPs prepared by both the "Zulauf" and "Zutropf" techniques (Tollier *et al.* 1991). contrary to earlier speculations (Lai and Sarkanen 1975). Also. many of the β-O-4 entities in G-DHPs are crosslinked by α-O-4 bonds. These

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linkages have been previously found in DHPs by NMR methods (Nimz and Lüdemann 1976; Gagnaire and Robert 1977; Landucci and Ralph 1996). However, the occurrence of non-cyclic benzyl-aryl ether bonds in lignin have been somewhat controversial and estimates ranging from about 7 % to zero have been reported (Adler 1977; Nimz 1981; Leary and Sawtell 1984). Trimeric and tetrameric entities containing α-O-4 linkages have been isolated from dehydrogenation mixtures (Freudenberg 1966; Landucci 1995) or by silver oxide oxidation of coniferyl alcohol (Quideau and Ralph 1994). The "abnormalities" of linkage distributions in DHPs, relative to natural lignins, can be used to advantage when assigning signals in ^{13}C NMR spectra, because of the greater intensities of signals due to the "minor" functional entities.

In this report we critically examine and compare the ^{13}C NMR spectra of G-DHP, GS-DHP, and S-DHP and make some definite chemical shift assignments associated with GG, GS, and SS entities, free of any complications caused by H units, carbohydrates, or extraneous wood components. Prior to NMR analysis, the DHPs were acetylated to increase chemical shift dispersion and to facilitate the determination of primary, secondary, and phenolic hydroxyl groups bused on the unique chemical shift ranges of corresponding acetyl carbonyl groups. The assignments herein are based entirely on chemical shifts of authentic acetylated trimeric and tetrameric lignin model compounds as it has recently been demonstrated that they are superior predictors of corresponding lignin and DHP chemical shifts than dimeric model compounds (Landucci and Ralph 1997). The NMR solvent used in this study was acetone-d, as it is the most commonly used solvent for acetylated lignins and acetylated DHPs. Although the pioneering studies of Nimz and coworkers provided invaluable guidelines. exact comparisons of literature data was not feasible because of solvent differences and/or that the assignments were predominantly based on the chemical shifts of monomeric and dimeric model compounds (Lüdemann and Nimz 1973, 1974; Nimz and Lüdemann 1976;: Nimz *et al.* 1981). With the exception of some partial NMR data (9/1 acetone/water) of underivatized α-O-4 linked oligomers (Nimz and Lüdemann 1974), complete data from trimeric and tetrameric model compounds containing more than one β-O-4 linkage or one β-O-4 linkage along with a β-5 or β-β linkage were not yet available. This. along with resolution limitations of early instruments were perhaps why only 40-50 chemical shift assignments were made.

Experimental

Preparation of DHPs

The DHPs were prepared by the "Zutropf" (Freudenberg 1956) method from coniferyl and sinapyl alcohols. Coniferyl alcohol was prepared from vanillin and monoethyl malonate (Kirk and Brunow 1988), and sinapyl alcohol was synthesized from syringaldehyde and monoethyl malonate (Freudenberg and Hübner 1952). The precursors were polymerized using horseradish peroxidase (Sigma Type VI) and H_2O_2, as previously described (Kirk and Brunow 1988). Vanillyl alcohol (2% wt/wt of precursor) was added to all reactions as a water-soluble initiator for polymerization. The GS DHP was prepared from a 3:1 mixture of sinapyl and coniferyl alcohols. All DHPs were purified by gel permeation chromatography on Sephadex LH-20 in *N,N*-dimethylformamide (Hammel *et al.* 1993), and the excluded fraction with molecular weight greater than approximately 1000 (hexamers and above) was used for NMR analysis. Acetylation of the DHPs was accomplished by treating them with 1/1 acetic anhydride/pyridine for at least 6 hr at room temperature.

Preparation of lignin model compounds (1-11 in Fig. 2)

Compounds **1, 11** and **12** were prepared as described (Landucci and Ralph 1997). Compound **2** was a minor product isolated from a complex mixture of small-scale exploratory reactions of sinapyl alcohol with transition metal oxidants such as those previously described (Landucci 1995). Compounds **3-7** were obtained by the reaction of coniferyl alcohol with cupric acetate as described (Landucci *et al.* 1995). Compounds **8-10** were obtained by the reaction of coniferyl alcohol with silver oxide as described (Quideau and Ralph 1994).

NMR experiments

The ^{13}C NMR data for the DHPs were obtained with a Bruker WM250 spectrometer on 180–200mg samples in 2.5ml of acetone-d_6. A 10 mm fixed frequency probe tuned to 62.9 MHz was used. Overnight acquisitions consisting of approximately 50,000 l6K free induction decays (FIDs) were generally required for sufficient signal/noise. Line broadening of 4-6Hz was applied to the FIDs prior to Fourier transformation. Quantitative ^{13}C NMR spectra were obtained and processed similar to techniques previously described (Landucci 1985), but lower precisions (± 10%) were estimated because of sample limitations. For the model compounds (10-30mg in 0.5 ml acetone-d_6) a Bruker DPX-250 or AMX-360 spectrometer fitted with a 5mm quadranuclear probe was used. All chemical shifts were relative to tetramethylsilane (TMS) as internal standard (δ = 0ppm). which corresponded to a central solvent signal of 29.83ppm. Chemical shifts of the model compounds were verified by comparison with authentic compounds in an NMR database (Ralph *et al.* 1993) and by utilizing a variety of standard 1D and 2D NMR experiments.

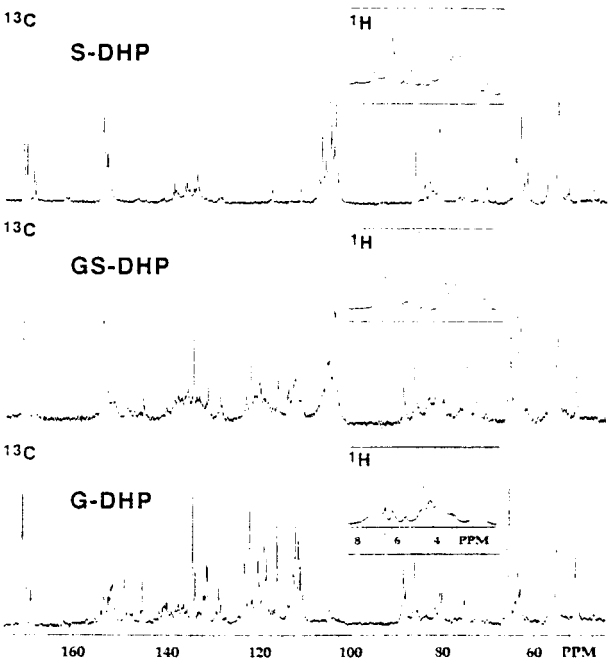

Fig. 1. ^{1}H and ^{13}C NMR spectra of acetyiuted G-, GS-, and S-DHP In acetone-d_6.

Table 1. Terminology of abbreviated structural entities

G = guaiacyl ring
S = syringyl ring
a = α-O-4 linkage
b = β-O-4 linkage
c = β–5 (phenylcoumaran)
r = β-β (resinol)
CA = coniferyl alcohol end unit
SA = sinapyl alcohol end unit
e = *erythro*
t = *threo*

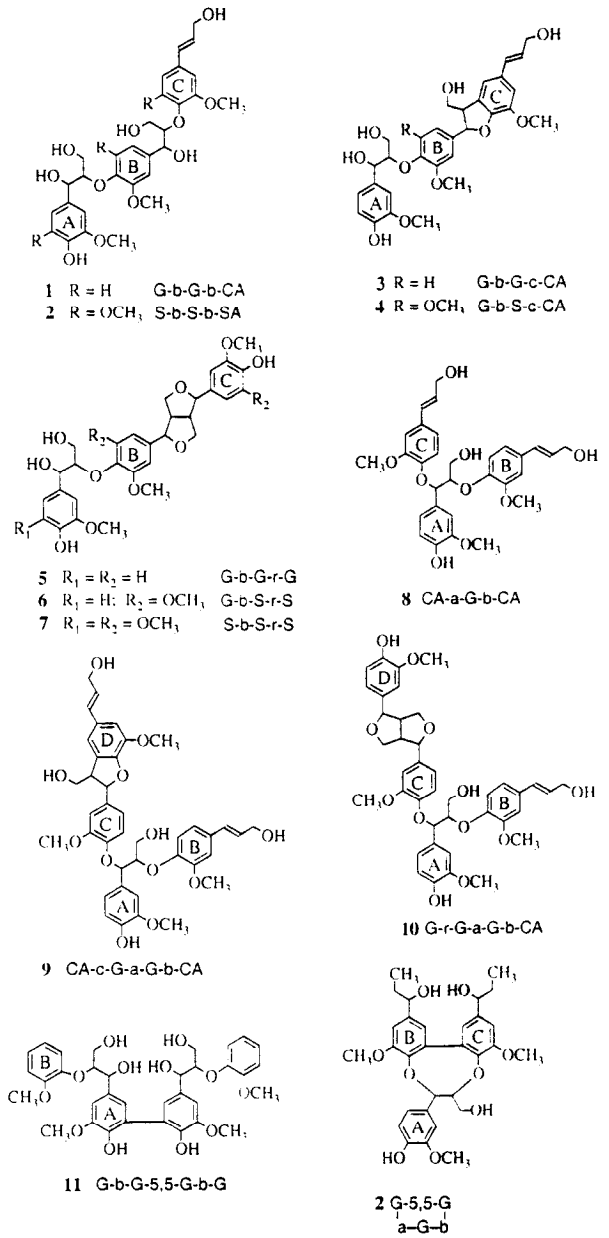

Fig. 2. Lignin model compounds used to assign signals in the DHP spectra (acetyl groups not shown).

Results and Discussion

^{1}H and ^{13}C NMR spectra of acetylated G-DHP, GS-DHP, and S-DHP are shown in Figure 1. The broad and relatively featureless appearance of ^{1}H NMR spectra of DHPs are similar to corresponding spectra of MWLs and are indicative of their polymeric nature (Brunow and Lundquist 1980). In contrast, a ^{1}H NMR spectrum that contains relatively sharp lines is typical of a mixture of low molecular weight oligolignols such as dimers to pentamers (Landucci and Ralph 1996). The signals in the carbon NMR spectra can be divided into three main types: those from carboxyls of acetyl groups (168-171 ppm); those from aromatic carbons, which can be divided further into quaternary (125-160ppm) and methine carbons (110-125 ppm); and those from side-chain carbons (50-90ppm). Exceptions to this classification are signals of aliphatic carbons in the aromatic region attributed to α (134-135ppm) and β (122-124ppm) of unsaturated coniferyl alcohol end groups and the very strong signals at 56-57ppm due to methoxyl groups. For simplicity, signals outside of the illustrated region. such as those from acetyl methyls (20-21ppm) and non-acetyl carbonyl carbons (190-200ppm). are not shown. but they are included in the chemical shift (CS) listings (Tables 2 and 5). Structural entities in the tables and text are abbreviated as illustrated in Table 1. Specific compounds shown in Figure 2 are indicated in Tables 3-5 by their bold face numbers. If a match (± 0.1ppm) to one of the specific compounds is not found, then only a general structure type for a given DHP CS is indicated. Capitalized letters preceding the carbon atom position (e.g. A2,6; B5; C1; Dα) refer to specific rings in the structures and the relative intensities (e.g., vw = very weak, mw = medium to weak, ms = medium to strong, vs = very strong) are based upon quantitative spectra of the DHPs (Fig. 8), which will be discussed later. Intensity measurements of signals in qualitative spectra are generally not meaningful because of relaxation differences among different carbon types.

The CS assignments reported herein were made on the basis of authenticated compounds in the Forest Products Laboratory/Dairy Forage Research Center (FPL/DFRC) NMR database (Ralph *et al.* 1996). The compounds referred to in Tables 3-5 are shown in Figure 2, and their chemical shifts (with exception of acetyl carboxyls) are listed in Table 6.

Carbonyl region

All of the carbonyl resonances that were observed in the DHP spectra are listed in Table 2 and spectra of the acetyl carboxyls are shown in Figure 3. Assignment of the acetyl carboxyls was relatively straightforward by comparison of the DHPs. Also, the CSs were consistent with those of simple lignin models. The main feature in this comparison is the low intensity of signal 7 in the G-DHP, which is attributed to acetyl carboxyl on the benzylic position. The main reason for this is the relatively high content of β–5 and β-β structures. neither of which have a secondary acetyl group.

Table 2. ^{13}C NMR data of carbonyl carbons in acetylated DHPs

Signal	CS	G-DHP	GS-DHP	S-DHP	Assignment
1	198.8	–	–	w	αC = O
2	195.0	–	–	w	αC = O
3	193.8	m w	–	–	γ; coniferaldehyde C = O
4	170.9	s	m s	–	Ac C = O, primary, GG and SG β–5
5	170.7	s	s	s	Ac C = O, primary, G & S α-O-4, β-O-4
6	170.5	w	m w	m	?
7	170.0	m	m s	m s	Ac C = O, secondary, S & G
8	169.0	m	w	–	Ac C = O, G phenolic
9	168.5	–	m w	m	Ac C = O, S phenolic
10	168.2	–	w	m w	Ac C = O, S phenolic with α C = O

Table 3. ^{13}C NMR data of aromatic quaternary carbons in acetylated DHPs[a]

					Assignment	
Signal	CS	G-DHP	GS-DHP	S-DHP	General	Specific
11	154.1	–	–	m	etherified S-b-S	C3,5/**2**
12	154.0	–	m s	s	etherified S-r-S	B3,5/**7**
13	153.8	–	s	v s	etherified S-b-S	B3,5/**2**
14	153.7	–	–	s	etherified S-rings	
15	153.6	m	–	–	?	
16	153.2	–	m	m	free phenolic S-r-S	C3,5/**7**
17	152.9	–	m	m	free phenolic S-b-S	A3,5/**7**
18	152.7	m	v w	–	?	
19	152.5	m s	v w	–	free phenolic G-c-G	A3/G-c-CA[b]
20	152.3	m	v w	–	free phenolic G-r-G	C3/**5**
21	152.2	m s	w	–	free & etherified G-b-G	A3, B4/**1**
22	152.0	m s	w	–	etherified G-b-G	C3/**1**
23	151.8	m s	w	–	α-O-4 units	B3/**8,9,10**
24	150.2	m w	–	–	α, coniferaldehyde units	
25	149.2	s	–	–	c-CA units	C4/**3**
26	148.3	m w	–	–	?	
27	145.3	s	v w	–	c-CA units	C3/**3**
28	141.7	m w	v w	–	free phenolic G-r-G	D1/**10**
29	141.4	–	–	v w	free phenolic S-r-S	C1/**7**
30	141.0	m w	w	–	1, free phenolic G-c-G	
31	140.8	m w	w	–	free phenolic G-b-G	A4/**1**
32	140.6	m w	w	–	free phenolic α-O-4	A4/**8**
33	140.3	m w	w	–	4, free phenolic G-r-G	
34	140.0	m w	w	–	free phenolic G-r-G	D4/**10**
35	138.8	–	–	w	etherified S-r-S	B1/**7**
36	138.4	w	–	–	biphenyl structures	A4/**11**
37	137.7	m w	v w	–	free phenolic α-O-4	A1/**10**
38	137.1	w	v w	–	etherified G-c-G	B1/**3**
39	136.6	w	w	m	free phenolic G-b-units	A1/**3**
40	136.2	–	m	s	etherified -b-S units	B4/**4**
41	135.8	w	–	–	biphenyl structures	A1/**11**
42	135.6	–	–	w	etherified S-r-S	B4/**7**
43	134.6	s	w	–	c-CA sidechain	Cα/**3**
44	134.4	–	–	w	b-SA sidechains	Cα/**2**
45	134.1	m	–	–	b-CA sidechains	Cα/**1**
46	133.9	–	–	m s	etherified S-b-S	B1/**2**
47	132.4	m w	v w	–	α-O-4 units	B1/**8**
48	132.0	w	v w	–	biphenyl structures	A5/**11**
49	131.7	m	w	–	c-CA units	B1/G-c-CA[b]
50	131.5	m s	m w	–	c-CA unit	C1/**3**
51	129.3	–	–	v w	free phenolic S-b-S	A4/**7**
52	129.0	m	w	–	c-CA unit	C5/**3**

[a]With exception of signals 43–45, which are from protonated carbons. [b]A dilignol described previously (Landucci *et al.* 1995)

Table 4. ^{13}C NMR data of aromatic protonated carbons in acetylated DHPs

Signal	CS	G-DHP	GS-DHP	S-DHP	Assignment	
					General	Specific
53	123.4	m	w	–	b-CA sidechain	Cβ/**1**
54	123.3	m	w	–	free phenolic G-b-G	A5/**1,3**
55	122.3	s	w	–	c-CA sidechain	Cβ/**3**
56	122.2	m s	m w	–	c-CA sidechain	Dβ/**9**
57	121.6	w	–	–	biphenyl structures	A6/**11**
58	120.5	m	w	–	free phenolic G-b-G	A6/**1**
59	120.2	m	w	–	?	
60	119.1	m	w	–	etherified b-CA unit	C5/**1**
61	118.6	m	w	–	free phenolic G-r-units	C6/**5**
62	117.6	–	–	v w	G units from vanillyl alc.	
63	116.3	m s	w	–	etherified c-CA units	C6/**3**
64	112.7	m	w	–	free phenolic G-b-G	A2/**3**
65	112.2	m s	w	–	c-CA unit	C2/**3**
66	111.5	m	w	–	etherified G-c-G	B2/**3**
67	111.4	–	–	w	G units from vanillyl alc.	
68	111.0	m	v w	–	free phenolic G-r-units	C2/**5**
69	110.6	m w	–	–	?	
70	107.3	–	w	w	2,6; S-ring with α C = O	
71	106.7	–	m	s	2,6; S-ring with α C = O	
72	105.9	–	m	m	?	
73	105.0	–	s	v s	etherified SA units	C2,6/**2**
74	104.6	–	m s	s	S-b- & S-r-units	A,B2,6/**2**
75	104.1	–	m	m	?	
76	103.9	–	m s	–	etherified S-c-G units	B2,6/**4**
77	103.7	–	m s	s	etherified S-r-S units	B2,6/**6,7**
78	103.2	–	w	m w	free phenolic S-r-S units	C2,6/**6,7**

Table 5. ^{13}C NMR data of side-chain carbons in acetylated DHPs

Signal	CS	G-DHP	GS-DHP	S-DHP	Assignment	
					General	Specific
79	88.9	–	v w	–	etherified S-c-G units	Bα/**4**
80	88.6	m	v w	–	etherified G-c-G units	Bα/**3**
81	88.1	w	–	–	A α; free phenolic G-c-G	
82	86.9	–	–	m w	α; free phenolic S-r-S	
83	86.5	–	m	m s	etherified S-r-S	Bα/**7**
84	86.1	m	w	–	free phenolic G-r-G units	B,Cα/**5**
85	84.4	–	w	m	unknown SS unit	
86	83.3	v w	m	m	?	
87	81.7	w	?	?	free phenolic α-O-4	Aβ/**9,10**
88	81.3	–	m s	s	free phenolic G-b-S/S-b-S	Aβ/**6,7**
89	80.6	m w	w	–	free phenolic α-O-4	Aα/**9**
90	80.2	m w	w	–	G-b-G units	Aβ,Bβ/**1**
91	77.5	–	–	m w	?	
92	75.4	m w	m	m s	α; G-b-G(t) & S-b-S(t)	
93	74.5	m w	–	–	G-b-G(*e*)	Aα/**1**
94	72.5	m	m	m	γ; G-r-G, G-r-S, S-r-S	Cγ,Dγ/**10**
95	71.0	–	w	w	unknown CH_2	
96	66.0	m s	w	–	G-c-G	Bγ/**3**
97	65.5	v s	m w	–	CA sidechain	Cγ/**3**
98	64.5	–	m	m	?	
99	64.6	–	m	m	?	
100	64.1	m w	s	?	?	
101	63.6	m	m s	s	γ;G-a-G, S-b-S	Aγ/**8**
102	63.1	m w	w	–	G-b-G	Aγ/**3**
103	62.7	–	w	m w	?	
104	62.0	–	w	m w	?	

Table 5 continued on page 6

Table 5 continued from page 5

Signal	CS	G-DHP	GS-DHP	S-DHP	Assignment	
					General	Specific
105	61.3	–	w	–	?	
106	56.3–.5	v s	v s	v s	Methoxyl	
107	55.3–.5	m	m s	s	β: G-r-G, G-r-S, S-r-S	Bβ/6
108	53.1	–	w	m w	?	
109	51.4	m	w	w	β: -c-G units	
110	51.1	m	w	?	β: -c-G units	Cβ/9
111	47.9	–	w	w	?	
112	20.9	s	s	s	Acetyl CH_3; primary OH	
113	20.8	s	s	s	Acetyl CH_3; primary OH	
114	20.7	s	m	–	Acetyl CH_3; benzylic OH	
115	20.6	m s	s	s	Acetyl CH_3; benzylic OH	
116	20.5	m s	m	m	Acetyl CH_3; benzylic OH	
117	20.3	–	m	s	Acetyl CH_3; phenolic OH, S	

Table 6. ^{13}C NMR data of lignin model compounds[a]

Carbon	Compound											
	1	2	3	4	5	6	7	8	9	10	11	12
Aα	74.5	75.4	74.5	75.0	74.5	75.1	75.3	80.9	80.7	80.9	74.6	85.3
Aβ	80.2	81.4	80.3	81.4	80.3	81.3	81.3	81.7	81.6	81.7	80.3	83.4
Aγ	63.0	63.4	63.0	63.3	63.0	63.3	63.4	63.6	63.5	63.6	63.0	64.3
A1	136.6	136.6	136.6	137.1	136.7	137.1	136.7	137.5	137.5	137.7	135.9	138.1
A2	112.8	104.7	112.7	112.3	112.7	112.2	104.7	112.7	112.7	112.8	111.9	112.8
A3	152.1	153.0	152.1	152.0	152.3	152.0	153.0	152.1	152.1	152.1	152.5	152.1
A4	140.8	129.4	140.8	140.6	141.1	140.5	129.3	140.7	140.6	140.6	138.4	140.8
A5	123.3	153.0	123.3	123.3	123.3	123.3	153.0	123.3	123.2	123.3	131.7	123.5
A6	120.4	104.7	120.5	120.0	120.5	120.0	104.7	120.5	120.5	120.5	121.6	120.7
Bα	74.6	75.4	88.5	88.9	86.2	86.5	86.5	134.2	134.2	134.2	–	77.5
Bβ	80.3	81.6	51.2	51.3	55.3	55.3	55.3	123.2	123.1	123.1	–	31.3
Bγ	63.2	63.4	66.0	66.0	72.5	72.7	72.7	65.4	65.4	65.4	–	10.4
B1	136.5	134.1	137.1	138.0	136.7	138.9	138.8	132.3	132.2	132.3	124.2	138.6[b]
B2	113.1	104.7	111.5	103.9	111.6	103.7	103.7	111.3	111.2	111.3	113.7	111.0[c]
B3	151.6	153.9	152.0	154.2	151.9	154.0	154.0	151.9	151.8	151.9	152.1	153.3[b]
B4	152.2	136.6	148.1	136.1	147.5	135.5	135.6	148.7	148.6	148.7	148.2	146.9[b]
B5	118.9	153.9	119.4	154.2	119.0	154.0	154.0	119.2	119.1	119.1	120.1	133.4
B6	120.5	104.7	119.1	103.9	119.4	103.7	103.7	120.5	120.5	120.5	121.6	119.5[b]
Cα	134.1	134.4	134.7	134.7	86.2	86.5	86.6	134.2	88.6	86.3	–	77.5
Cβ	123.3	124.1	122.2	122.3	55.4	55.4	55.4	123.0	51.0	55.1	–	31.3
Cγ	65.3	65.3	65.5	65.5	72.6	72.7	72.7	65.4	65.8	72.4	–	10.4
C1	132.6	133.3	131.5	131.6	141.9	141.4	141.5	131.7	135.9	136.9	–	138.7
C2	111.3	105.0	112.2	112.2	111.1	103.2	103.2	111.6	111.4	111.5	–	111.0[c]
C3	151.9	154.2	145.4	145.4	152.3	153.2	153.2	151.4	151.3	151.3	–	153.5
C4	148.4	136.5	149.3	149.3	140.1	128.9	128.9	148.2	148.0	147.4	–	147.2
C5	119.1	154.2	129.0	129.1	123.7	153.2	153.2	117.4	117.3	117.3	–	133.4
C6	120.4	105.0	116.3	116.3	118.6	103.2	103.2	120.5	119.1	118.9	–	120.1
Dα	–	–	–	–	–	–	–	–	134.7	86.2	–	–
Dβ	–	–	–	–	–	–	–	–	122.1	55.4	–	–
Dγ	–	–	–	–	–	–	–	–	65.5	72.5	–	–
D1	–	–	–	–	–	–	–	–	131.4	141.8	–	–
D2	–	–	–	–	–	–	–	–	112.0	111.1	–	–
D3	–	–	–	–	–	–	–	–	145.3	152.2	–	–
D4	–	–	–	–	–	–	–	–	149.2	140.1	–	–
D5	–	–	–	–	–	–	–	–	129.0	123.4	–	–
D6	–	–	–	–	–	–	–	–	116.2	118.6	–	–

[a] Chemical shifts are of fully acetylated compounds in acetone-d_6 and referred to TMS as internal standard. [b] Value may be interchanged with corresponding value in C-ring. [c] Center of a cluster of signals due to mixture of isomers.

Aromatic region

In principle, the S-DHP should be much simpler because polymerization is more limited due to the absence of free 5-positions on the aromatic ring. This is confirmed by comparison of the aromatic regions of the three DHPs (Fig. 4, Tables 3 and 4). Note that all of the protonated aromatic carbons are concentrated in a narrow cluster of signals at 103-107ppm, with the exception of signals 62 and 67, which arise from the incorporation of vanillyl alcohol (used as initiator) in the polymer.

The corresponding spectrum of the G-DHP (Fig. 4) is much more complex because of the greater diversity of structural types. Prominent features (other than the presence of β–5 structures) of the G-DHP include the presence of very strong signals due to the a and β carbons of unsaturated coniferyl alcohol end groups on both β-O-4 and β–5 entities. The a-carbon signals are easily revealed by a CH-only DEPT experiment as they occur in the midst of a background of quaternary carbon signals. A partial DEPT spectrum of the G-DHP along with a conventional qualitative ^{13}C spectrum are illustrated in Figure 5. Signals 43 and 45 are due to the a unsaturated carbon in β–5 and β-O-4 entities, respectively. The GS-DHP contains a smaller amount of α-β unsaturation (signals 43 and 45) than does the G-DHP, as seen in Figure 4. A DEPT spectrum of the GS-DHP (not shown) did not reveal signal 45, indicating that the unsaturation occurred only on β–5 entities. Corresponding structures with sinapyl alcohol end units are present only as a trace in the S-DHP (signal 44) as supported by the identification of the analogous carbon in an authentic sample of the syringyl trilignol 2 (Fig. 2).

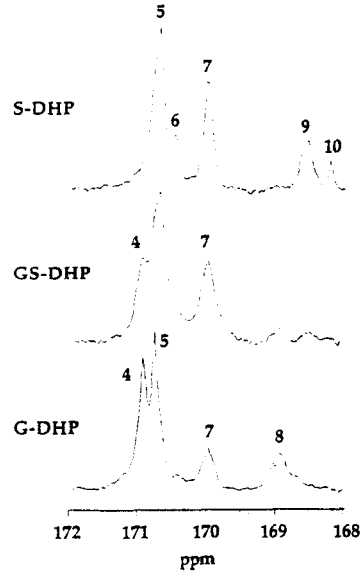

Fig. 3. Acetyl carboxyl regions of the DHP spectra.

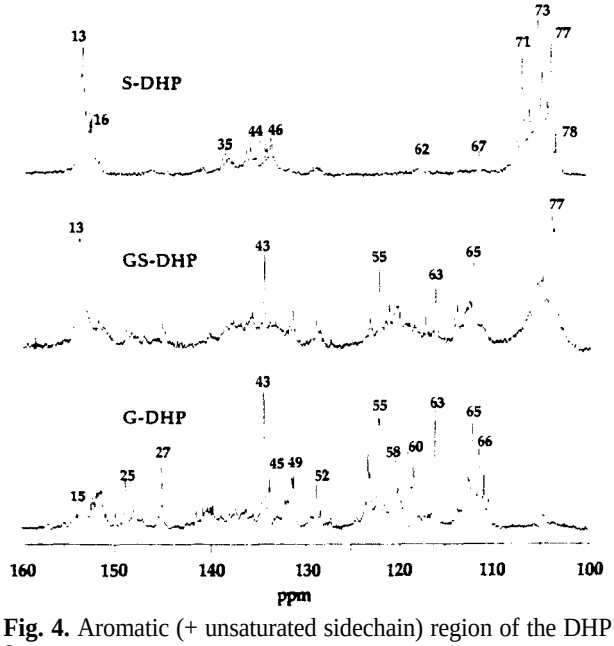

Fig. 4. Aromatic (+ unsaturated sidechain) region of the DHP Spectra.

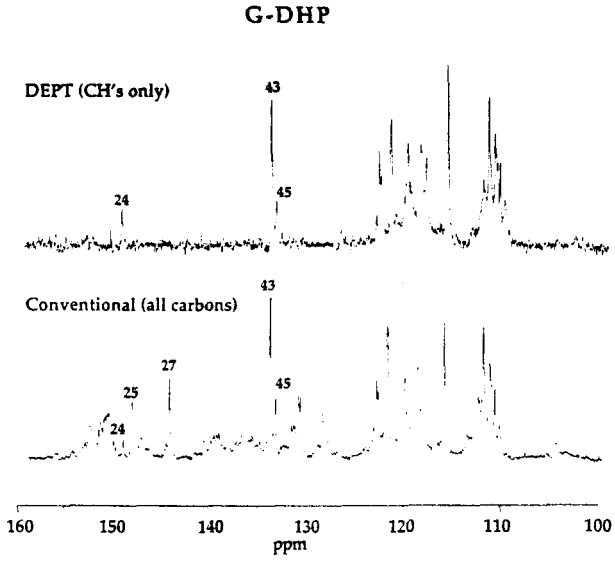

Fig. 5. Comparison of a conventional noise decoupled ^{13}C NMR spectrum of G-DHP with a DEFT spectrum (CH signals only).

Side-chain region

The side-chain region is the most revealing in terms of the various interunit linkages present and their relative abundances. In DHPs, assignment is relatively straightforward because of the relative simplicity of the polymer structure as compared with that of natural lignins and the absence of carbohydrate residues and extraneous wood substances. Expansions of this region are shown in Figure 6 and corresponding CS assignments are listed in Table 5. All of the signals in the side-chain region of the G-DHP were assigned to four structural types: β–5. β-O-4, β-β and α-O-4, in descending order of abundance. It is assumed that all side chains with α-O-4 linkages also contain a β-O-4 linkage as these linkages are the result of quinone methide reactions of β-O-4 dimeric entities (Freudenberg 1966), but the α-O-4/β-O-4 trimeric entities are designated only as "α-O-4" for simplicity. All except two of the side-chain

signals in the G-DHP were assigned and were consistent with corresponding signals of lignin model compounds. The two unassigned signals are one (#86) at 83.3ppm due to a methine carbon and one (#100) at 64.1ppm due to a methylene carbon. In contrast, numerous signals in the S-DHP (and GS-DHP) were not assigned due to the absence of lignin models with corresponding chemical shifts. However, among the medium to strong signals, all except two (#98, 64.5ppm; #99, 64.6ppm) are assigned. These two signals are presumably due to S-S units as they are weaker in the GS-DHP and absent in the G-DHP. It is of interest to note that of the ten unknown signals in the S-DHP, half of them correspond to unassigned signals in a ^{13}C NMR spectrum of lignin from the annual plant kenaf, which has a very high syringyl content (Ralph 1996).

Semi-quantification (qualitative spectrum) of the four linkage types of the G-DHP was reported in a previous study concerning biomimetic preparation of DHPs (Landucci 1995). Assuming no other linkages were present, the values of β–5, β-O-4, b-b and α-O-4 were 44%. 31 %, 18 %, and 7 %, respectively. Corresponding values from an inverse-gated spectrum of the same DHP were 44 %, 31 %, 15 %, and 10%. Clearly, from a small spectral range of similar carbon types, strict quantitation is not always necessary. Precisions based on replicate analysis in either case are estimated to be about ± 1-2 % (absolute). Again, it must be noted that "α-O-4" refers to the trimeric β-O-4/α-O-4 entity, so the actual abundance of C9 units containing a β-O-4 link is 31 + 7(10) = 38(41) %. Also, the excess amount of CA end groups found in G-DHPs does not effect the calculation because they, by definition, are not linkages but may be linked *via* a β–5 or β-O-4 to another C9 unit. The values quoted above for β-5, β-O-4, β-β and α-O-4 linkages in G-DHP contrast with the corresponding literature values (obtained by chemical methods) of a softwood lignin of 10, 48, and 2 and 7 %, respectively (Adler 1977). As noted in the introduction, there is disagreement in the literature on the abundance of α-O-4 entities in natural lignin, so the value of 7 % in softwood lignin may be too high.

The α-O-4 linkage appears to be a main crosslink in G-DHPs in contrast with natural lignins, which presumably are crosslinked primarily by 5-5 linkages (Drumond *et al.* 1989). The rarity of 5-5 linked entities in the DHP is consistent with thioacidolysis studies (Lapierre 1993) in which only traces of 5-5 are typically found in guaiacyl DHPs. A maximum value of 5-5 entities in the G-DHP calculated from a quantitative ^{13}C NMR spectrum (not shown), based upon chemical shifts consistent with compound **11** (Fig. 2), was estimated to be no larger than 1-2 %. An earlier speculation claiming that 5-5 biphenyl entities were more abundant in a DHP than in spruce MWL was based on a chemical shift assignment of an inappropriate model compound (Robert and Brunow 1984). The signal in question (acetylated guaiacyl DHP in acetone-d_6) was at 131.7ppm. and is assigned in this study to carbon-l of CA end units (Table 3). Compounds **11** and **12** in Figure 2 represent free phenolic 5-5 and fully etherified 5-5 entities. respectively. The novel cyclic etherified dibenzodioxocin **12** is analogous to one previously reported (Brunow *et al.* 1995). No clear evidence of the existence of this structure type in the DHPs was obtained. Most of the signals of **12** would occur in crowded regions, but the complete absence of signals between 145.7 and 147.4, where B4 and C4 of **12** should be seen, was inconsistent with **12.**

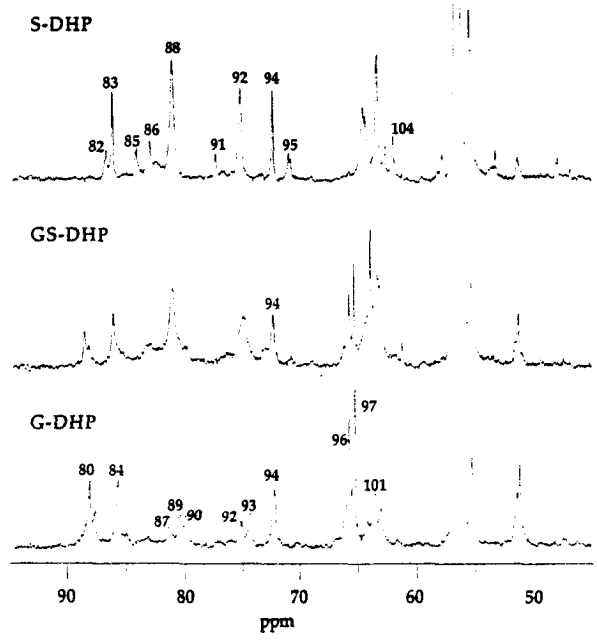

Fig. 6. Aliphatic region of the DHP spectra.

A significant difference between the MWL and the DHP, which is not apparent from linkage analysis is the presence of a large abundance of CA end groups in the latter (Nimz and Lüdemann 1976). In this study a value of about 19 % has been estimated from quantitative NMR in reasonable agreement with an earlier estimation of about 16% in a guaiacyl DHP (Gagnaire *et al.* 1971). In contrast, as seen in Figure 7 which compares a pine MWL and a G-DHP, there appears to be no evidence for the presence of the CA end groups in the MWL. This is especially clear from the DEPT spectrum of the MWL because signals 43 and 45 (134.6 and 134.1 ppm) due to the a carbons of CA end groups are absent. However, a weak signal corresponding to signal 24 (150.2 ppm) in the G-DHP was present in the MWL spectrum. This signal was assigned to the a-carbon of G-c-coniferaldehyde end units. In addition, a larger signal cluster at 153.3 ppm (not numbered) was also present. which was not detected (as a protonated carbon) in the DHPs. This signal may be due to G-b-coniferaldehyde end units, but the lack of suitable model compounds prevented confirmation. It seems reasonable that G-c-coniferaldehyde units are observed in the β–5 rich G-DHP (signal #24) whereas in the β-O-4 rich MWL, G-b-coniferaldehyde units predominate over the corresponding β–5 structures.

Quantitative NMR spectroscopy

The small quantities (< 200mg) of DHPs prevented quantitative analysis of the precision that was reported pre-

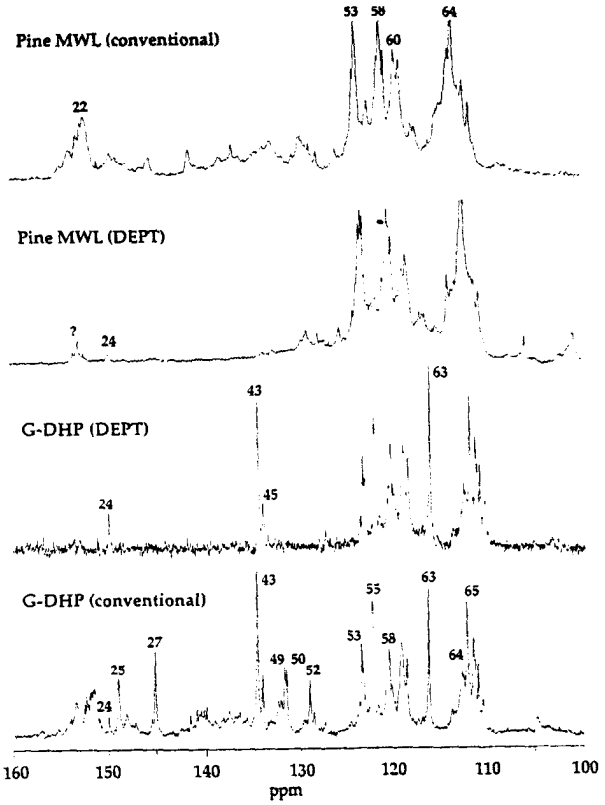

Fig. 7. Comparison of a conventional and DEPT spectrum of pine MWL with the analogous spectra of G-DHP.

viously (Landucci 1985). However. it is informative to compare the quantitative spectra along with integrals of carbon regions as shown in Figure 8. To obtain the integral values for the G-DHP it was assumed that the aromatic region contained 6.00 carbons plus a correction value (0.4) calculated for the presence of two unsaturated carbons (α and β) due to about one CA end group for every 6 C9 units (~ 17 %). All of the other integrals in the spectrum are based upon these assumptions. It can be seen from the Figure that the sum of the aromatic and aliphatic regions total 10.0 carbons, which is expected considering one methoxyl/C9 unit.

With the S-DHP, it was assumed that there were no unsaturated aliphatic signals in the aromatic region, so this region was set to 6.00 carbons. Adding the aromatic and aliphatic region in this case gives a value of 10.5 carbons. whereas a theoretical value of 11.0 would be expected for two methoxyls/C9 unit. The lower S/N in this spectrum may have been one reason for the discrepancy.

For the GS-DHP, it was estimated that a lesser amount of aliphatic unsaturation (about 1/2 that of the G-DHP) was present in the aromatic region. Therefore, a value of 6.20 was assigned to the aromatic region. Adding the aromatic and aliphatic region with this DHP gave a value of 10.7 carbons. This indicates a value of 10.7 - 9.0 = 1.7 methoxyl per C9 unit.

Quantification of the sidechain carbons (for linkage abundances) of the GS-DHP and S-DHP with the technique that was previously used with the G-DHP (Landucci 1995) could not be done because of the presence of unassigned signals in this region. Efforts are currently underway to prepare additional syringyl and guaiacyl/syringyl trimeric and tetrameric model compounds to allow identification of remaining unassigned signals and to facilitate quantification of the GS- and S-DHPs.

Conclusions

Detailed comparisons of ^{13}C NMR spectra of acetylated G-DHP, GS-DHP, and S-DHP with corresponding data from authentic acetylated trimeric and tetrameric lignin model compounds in acetone-d_6 facilitated the identification of many previously unassigned signals. Increased sophistication of the model compounds along with high resolution ^{13}C NMR spectra allowed CS assignments with a precision of ± 0.1 ppm. Therefore, assignment of a specific DHP signal was considered valid only if its CS was within ± 0.1 ppm from that of a corresponding model compound signal. A few general assignments that did not meet this criteria were considered tentative. The data from this study are a considerable improvement over existing NMR literature in which variances of 1 ppm or more for a specific carbon in a DHP or lignin are common. It was confirmed that G-DHP has very different characteristics than does guaiacyl lignin (MWL) isolated from pine wood. As reported previously, there were abnormally high contents of β–5, β-β, and coniferyl alcohol entities in the G-DHP and abnormally low contents of β-O-4 relative to natural lignins.

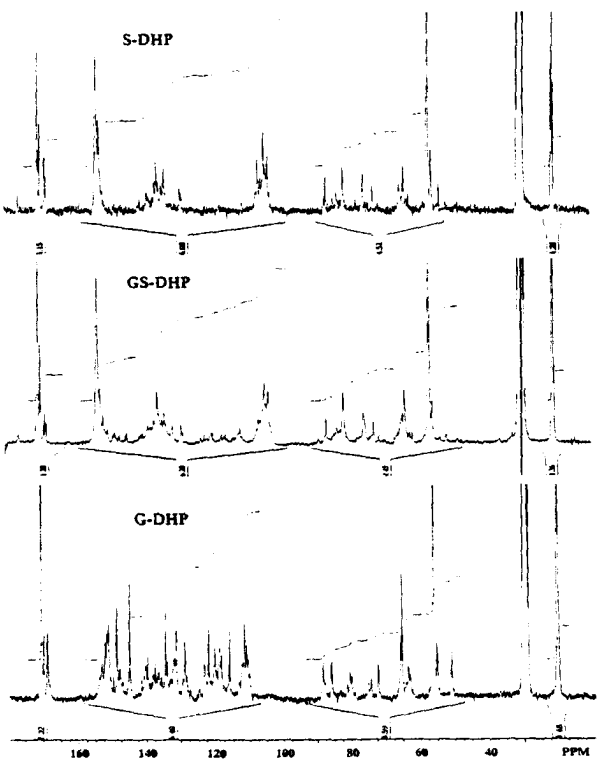

Fig. 8. Quantitative ^{13}C NMR spectra of acetylated DHPs

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