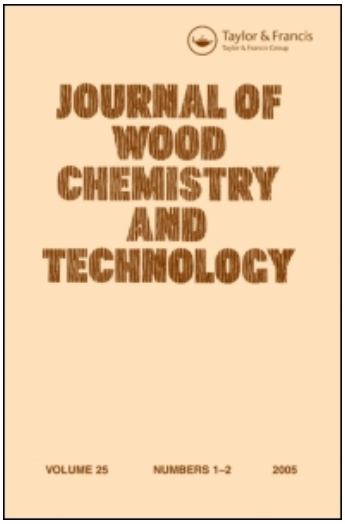

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Reaction of *p*-Hydroxycinnamyl Alcohols with Transition Metal Salts 3. Preparation and NMR Characterization of Improved DHPs

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REACTION OF *p*-HYDROXYCINNAMYL ALCOHOLS WITH TRANSITION METAL SALTS 3. PREPARATION AND NMR CHARACTERIZATION OF IMPROVED DHPS[1,2]

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ABSTRACT

Dehydropolymerization of *p*-hydroxycinnamyl alcohols with manganese(III) acetate in either aqueous acetic acid or pyridine resulted in dehydropolymers (DHPs) that more closely approximate the structure of natural lignins than do DHPs produced by enzymic techniques. The ^{13}C NMR spectrum of a "biomimetic" guaiacyl-DHP (G-DHP) from coniferyl alcohol was very similar to that of a lignin isolated from spruce wood, unlike a corresponding spectrum of a G–DHP prepared by a conventional enzymic technique. A similar comparison between a biomimetic guaiacyl/syringyl DHP (GS-DHP), a conventional GS-DHP, and a natural lignin from elm wood also confirmed a closer resemblance between the biomimetic DHP and the natural lignin. Likewise, a biomimetic S-DHP prepared from sinapyl alcohol was remarkably similar to a high syringyl fraction of lignin isolated from birch wood. The flexibility of the biomimetic approach is

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illustrated by the preparation of a DHP that is enriched in coniferaldehyde entities and one that is almost devoid of free-phenolic entities.

INTRODUCTION

Recently, it has been shown that a non-enzymic approach to the synthesis of dehydropolymers (DHPs) of *p*-hydroxycinnamyl alcohols can result in polymers that have a closer resemblance to corresponding milled wood lignins (MWL) than do DHPs produced with enzymes.[3] The well-known chemistry of transition metal one-electron oxidants was utilized to generate phenoxy radicals from *p*-hydroxycinnamyl alcohols, corresponding to the action of the conventional peroxidase/hydrogen peroxide system.[4,5] This approach is much more flexible in that the reaction conditions are not constrained within the temperature and pH limits required by the enzyme. Consequently, there is more control over the linkage type, linkage abundance, and molecular weight of the products.

In this report, the ^{13}C NMR spectra of "biomimetic" guaiacyl (G), guaiacyl/syringyl (G/S), and syringyl (S) DHPs are compared with spectra of enzyme-produced DHPs and with spectra of MWLs isolated from representative softwoods and hardwoods. Rather than critiquing the entire spectra in detail, only the important similarities and differences between the materials are addressed. Based on these comparisons, the biomimetic DHPs are clearly the best models for MWLs produced thus far. These improved models are expected to facilitate characterization of the more complex natural lignins.

Prior to NMR analysis, the DHPs and MWLs were fully acetylated to increase chemical shift dispersion and to facilitate the determination of primary, secondary, and phenolic hydroxyl groups based on the

unique chemical shift ranges of corresponding acetyl carbonyl groups. The chemical shift assignments were made on the basis of a series of trimeric and tetrameric guaiacyl model compounds.[6] Also, all of the DHPs and MWLs were fractionated on a polystyrene gel column and only the highest molecular weight fractions were examined. It should be noted that the α–hydroxyl group in the sidechains of all β-O-4 entities produced by manganese acetate reactions were acetylated.

RESULTS AND DISCUSSION

Comparison of Natural G-Lignins

Prior to attempts to mimic the structure of a guaiacyl MWL, lignins from several natural sources were examined. The partial ^{13}C NMR spectra of selected G-MWLs isolated from three different genera, *Ginkgo, Pinus, and Picea,* are illustrated in Figure 1. The remarkable similarity of this aromatic or "fingerprint" region clearly illustrates the uniformity amongst guaiacyl MWLs. Thus, the task to mimic a guaiacyl MWL is simplified in that there is only one target structure. The only differences observed between numerous isolated guaiacyl MWLs were in the aliphatic region and were mainly due to signals from carbohydrates.

Evaluation of G-DHPs and Comparison with Spruce MWL

Aromatic and unsaturated region (100-160 ppm)

Partial ^{13}C NMR spectra of the aromatic and unsaturated region of two biomimetic G-DHPs (DHP-1 and DHP-2), an enzyme-produced G–DHP (DHP-3), and a MWL from spruce are compared in Figure 2.

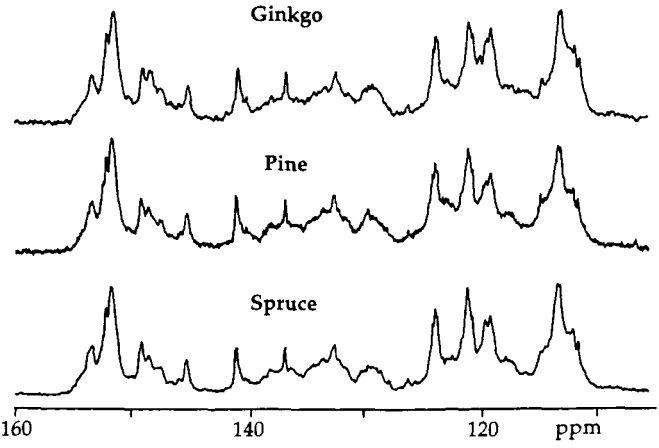

FIGURE 1. ^{13}C NMR spectra of aromatic region of guaiacyl MWLs.

Both of the biomimetic DHPs were obtained by dehydropolymerization of coniferyl alcohol with manganese triacetate. However, DHP-1 was formed in pyridine and DHP-2 was formed in aqueous acetic acid media. The enzyme-produced DHP-3 was from a conventional "Zutropf" enzymic dehydropolymerization of coniferyl alcohol.[4,5] A DHP from the enzymic dehydropolymerization of coniferin, rather than coniferyl alcohol, was found to give a similar structure, but with a slightly better ratio of β–O–4, β–5, and β–β linkages relative to natural lignin.[7]

It is clear from the partial spectra in Figure 2 that the DHPs most closely resembling the MWLs are the two biomimetic DHPs. The prominent signals in the enzymic preparation (DHP-3), which are much weaker in the other spectra, are due to the typically high contents of β-5 entities in DHPs prepared by conventional methods. The corresponding signals in DHP-1 and DHP-2 are similar in magnitude to those in the MWL. However, prominent signals that are common in

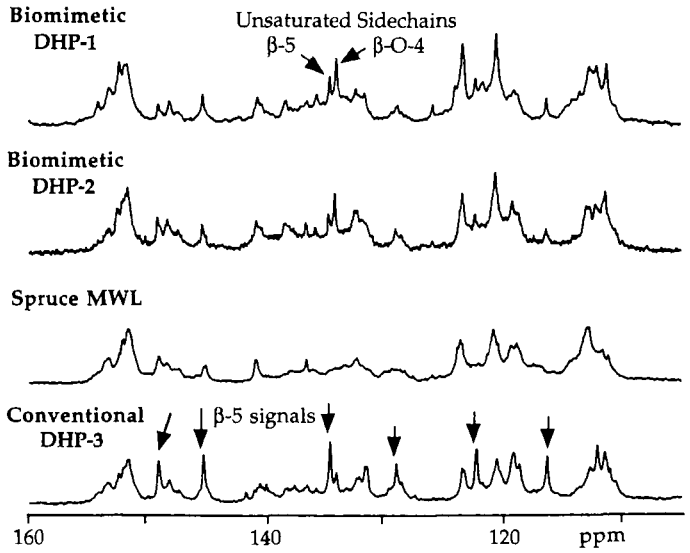

FIGURE 2. Aromatic and unsaturated regions of ^{13}C NMR spectra of DHPs and spruce MWL. Arrows indicate some signals from β-5 structures.

all the DHPs are those due to unsaturated sidechains on β–O–4 and β-5 dimeric entities. Corresponding signals generally are not detected in the spectra of MWLs.

Sidechain region (50-90 ppm)

The sidechain regions of the the DHPs and natural lignin are illustrated in Figure 3. This is the most informative region in terms of the relative abundance of the β–O–4, β–5, β–β, and α–O–4 linkages in the polymer. The chemical shift assignments corresponding to this region are listed in Table 1. The two main differences between the enzymic DHP and the biomimetic DHPs are the ratio of the β-O-4, β–5, and β–β linkages and the relatively high abundance of α–O–4 linkages

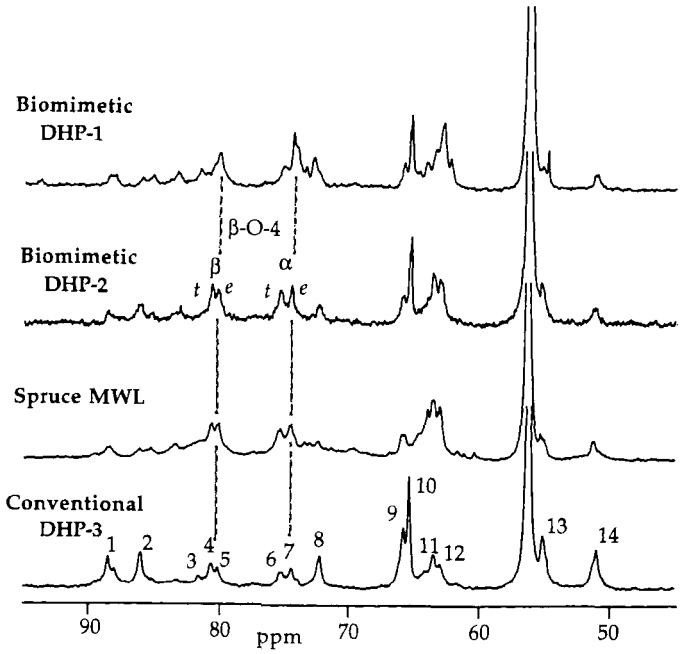

FIGURE 3. ^{13}C NMR spectra of sidechain region of DHPs and spruce MWL.

(signal 3 and part of signals 4 and 11) in the former. In a previous publication,[8] we reported that the presence of α–O–4 linkages is indicative of quinone methide reactions that occur subsequent to radical coupling. With the exception of the unsaturated sidechain methylene signal (signal 10), the spectra of the biomimetic DHPs are clearly very similar to that of the natural lignin. Interestingly, the *erythro/threo* ratio of the β-O-4 linkages of two DHPs and the MWL is similar (about 1/1), whereas the biomimetic DHP prepared in pyridine appears to be predominantly *erythro*.

TABLE 1
Signal Assignments of Aliphatic Region
Chemical shifts (CS) of acetylated material are referenced to the central signal in acetone-d_6 (29.83 ppm) and tetramethylsilane (0 ppm).

Peak	CS	Assignment
1	88.6	α of β–5 structures
2	86.1	α of β–β structures
3	81.7	β of α–O–4 structures
4	80.6	β(*threo*) of β-O-4 structures (plus α of α-O-4)
5	80.2	β(*erythro*) of β-O-4 structures
6	75.3	α(*threo*) of β-O-4 structures
7	74.5	α(*erythro*) of β-O-4 structures
8	72.4	γ of β–β structures
9	65.9	γ of β–5 structures
10	65.4	γ of coniferyl alcohol sidechain
11	63.6	γ of α–O–4 & β-O-4 structures
12	63.1	γ of β-O-4 structures
13	55.3	β of β–β structures
14	51.3	β of β–5 structures

Acetate carbonyl region

The acetate carbonyl region of the ^{13}C NMR spectra is shown in Figure 4. In the conventional DHP, the typically weak signal due to the acetate functionality on secondary (benzylic) hydroxyls is consistent with the high abundance of β-5, β-β, and α-O-4 entities, none of which contains benzylic hydroxyl groups. In contrast, the relative abundance of primary, secondary, and phenolic acetate carbonyls in DHP-1 and DHP-2 is similar to the MWL. A small signal overlapping the normal phenolic acetate in DHP-1, and to a smaller extent in DHP-2 and the MWL, was assigned to phenolic acetates on 5-5′ linked C9 units. This assignment was based on the equivalency of the phenolic acetate chemical shifts with those of corresponding 5-5′ model compounds

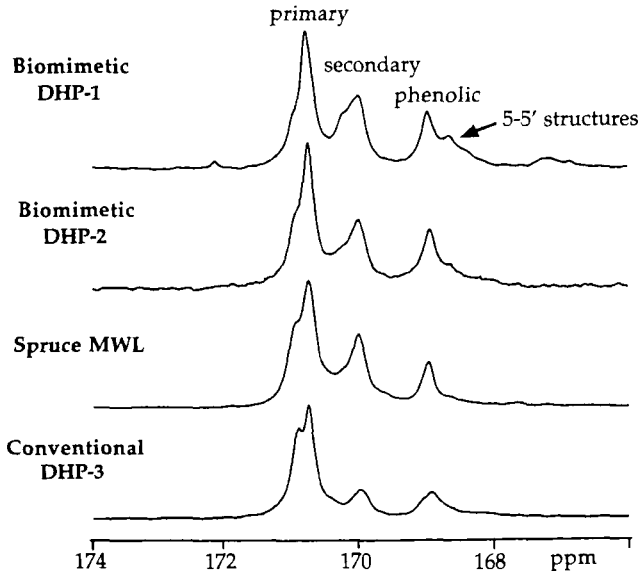

FIGURE 4. ^{13}C NMR spectra of acetyl carbonyl region of DHPs and spruce MWL.

(169 and 247) in the NMR database of lignin and cell wall model compounds.[9]

Introduction of Aldehydes into G-DHPs

The flexibility of the biomimetic approach allows easy introduction of miscellaneous functionalities into DHPs when it is necessary to obtain a more representative model of a particular lignin. For example, the introduction of the aldehyde functionality is illustrated in Figure 5. A typical biomimetic DHP formed from coniferyl alcohol is shown in the top control spectrum. The next

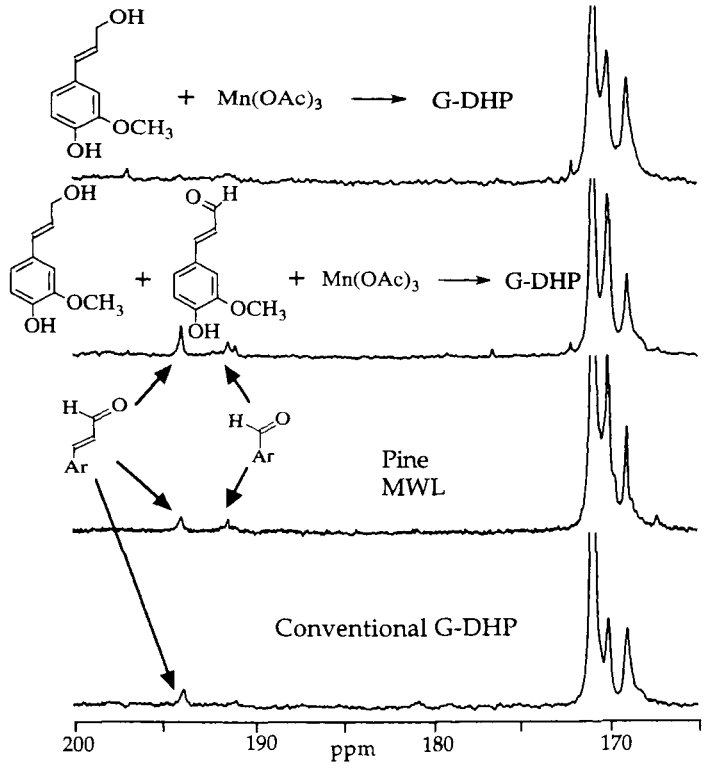

FIGURE 5. Carbonyl region of DHPs and pine MWL.

spectrum is the DHP that results when about 10% of coniferaldehyde is added to the reaction mixture. As seen in the figure, this spectrum closely resembles the corresponding spectrum of pine MWL. The weaker signals assigned to vanillyl groups presumably arise from oxidation of unsaturated sidechains. The last spectrum is a conventionally prepared G-DHP that contains only one aldehyde signal. A comprehensive study detailing the role of the aldehyde function in DHPs prepared by enzymic techniques has recently been reported.[10]

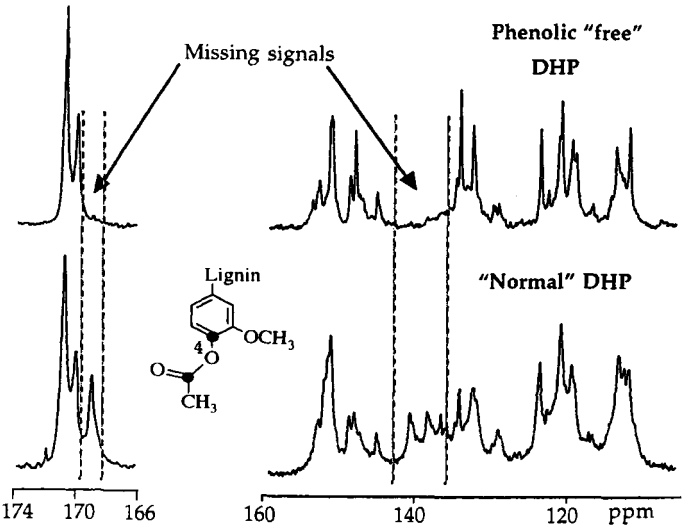

FIGURE 6. An unusual biomimetic G-DHP almost devoid of free phenolic units compared with a more typical biomimetic G-DHP.

An Unusual G-DHP

When relatively dilute solutions of coniferyl alcohol and manganese acetate were mixed over a period of several hours, a rather unusual G-DHP was obtained that had only traces of phenolic C9 units. Partial ^{13}C NMR spectra of the acetate carbonyl region and the aromatic region are shown in Figure 6 along with corresponding spectra of a more typical biomimetic G-DHP. In addition to the disappearance of the phenolic acetate signals at about 169 ppm, a cluster of signals around 140 ppm is absent. These signals are due to carbon 4 of acetylated C9 phenolic units. When the phenolic hydroxyl is etherified, these signals shift downfield to >150 ppm. The absence of the phenolic acetate signal, along with the absence of signals in the 135-145

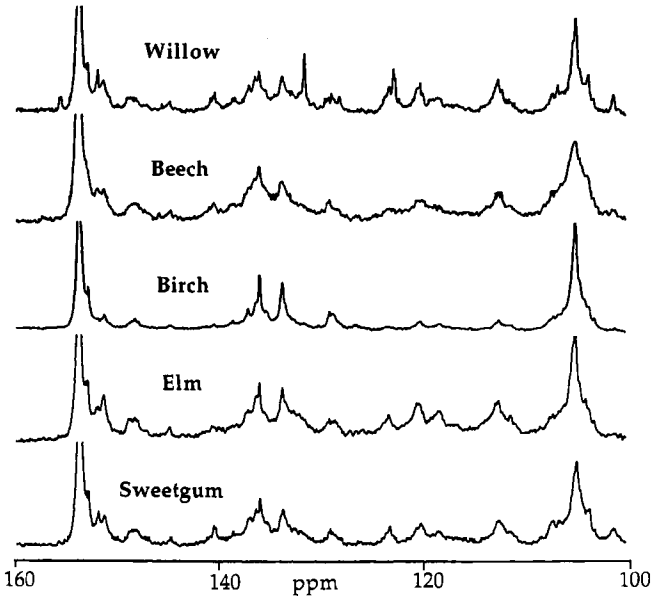

FIGURE 7. ^{13}C NMR spectra of aromatic region of GS MWLs.

ppm range, is consistent with the formation of 5–O–4 linkages. Another explanation could be extensive 5-5′ condensation followed by etherification to form dioxocin structures, analogous to model compounds previously prepared by Brunow and coworkers.[11]

Comparison of Natural GS-Lignins

In contrast to the similarity of G-lignins, GS-lignins are frequently very dissimilar. For example, the ^{13}C NMR spectra (aromatic region) of GS lignins isolated from willow, beech, birch, elm, and sweetgum are shown in Figure 7. It is clear from the variability of GS-lignins that it would be more difficult to prepare an analogous GS-DHP. A reasonable approximation can usually be obtained by adjusting the

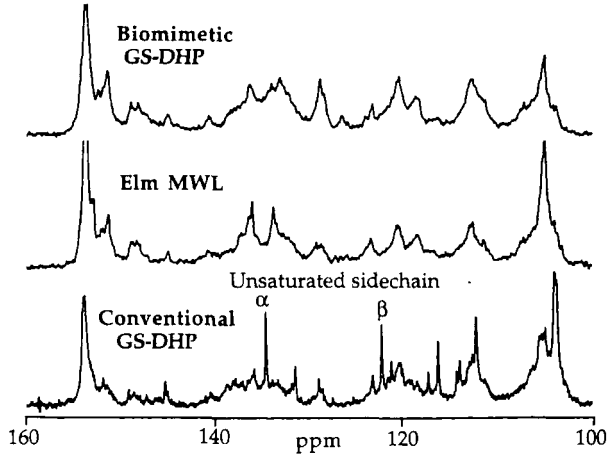

FIGURE 8. ^{13}C NMR spectra of aromatic region of GS-DHPs and elm MWL.

coniferyl alcohol/sinapyl alcohol ratio. However, in the case of hardwood lignins that contain "unique" entities, such as the 4-hydroxybenzoate structures in willow lignin,[12] other means to incorporate such structures must be used.

Evaluation of GS-DHPs and Comparison with Elm MWL

In Figure 8 is a comparison of the aromatic region of a biomimetic GS-DHP with that of an elm MWL and a conventionally prepared GS-DHP. With the exception of some clear differences in the G/S ratio of the biomimetic DHP and MWL, they are much more similar to each other than to the conventional GS-DHP. As with the G-DHP, the predominance of β-5 and β-β structures and the corresponding deficiency of β-O-4 structures in the conventional DHP render it less suitable as a lignin model. This is more clearly seen in the aliphatic

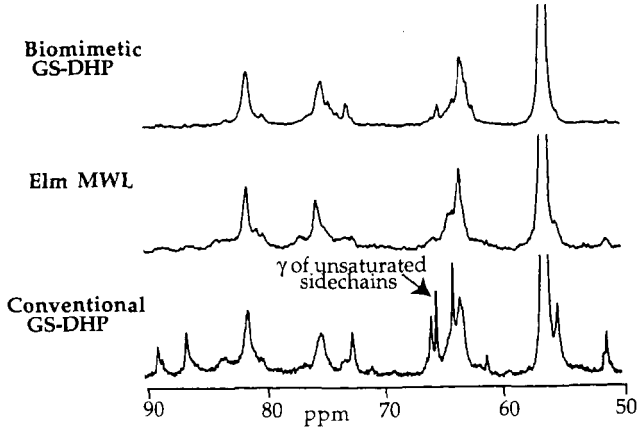

FIGURE 9. ^{13}C spectra of aliphatic region of GS-DHPs and elm MWL.

region of the NMR spectrum, as illustrated in Figure 9. Also, as with the conventional G-DHP, the predominance of unsaturated sidechains in the conventional GS-DHP is evident in both Figures 8 and 9. Interestingly, the biomimetic GS-DHP appears to be devoid of these entities, unlike the corresponding G-DHPs (Figures 2-3).

Evaluation of S-DHPs and Comparison with Birch MWL

Aromatic and acetyl carbonyl regions

Syringyl lignins and S-DHPs are inherently much simpler than their G or GS counterparts because of the limited options available for linking C9 units. In Figure 10 (aromatic region) and Figure 11 (acetyl C=O region), a biomimetic S–DHP and a conventionally prepared S–DHP are compared with a birch MWL with a very high syringyl content. A notable difference between the biomimetic DHP and the

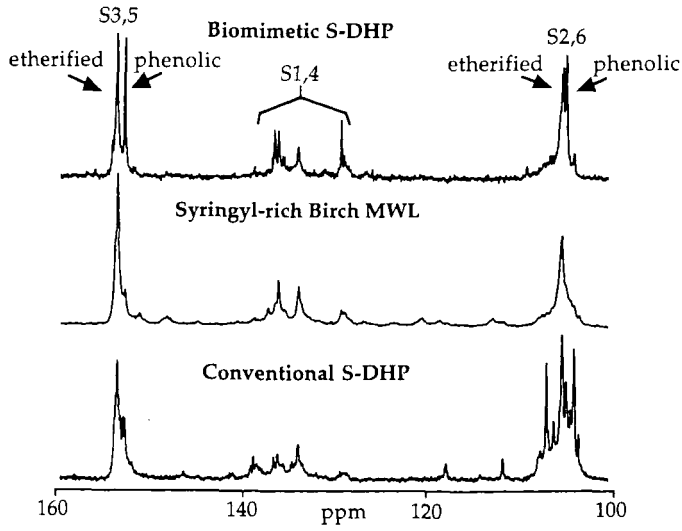

FIGURE 10. ^{13}C NMR spectra of aromatic region of S-DHPs and syringyl-rich birch MWL.

MWL occurs with the signals assigned to the 2,6 and 3,5 carbons on the S units. In the MWL, there is only one major signal in each of these regions corresponding to etherified C9 units, whereas in the biomimetic DHP, there are pairs of signals indicating an appreciable content of phenolic C9 units. This is consistent with the signals due to acetyl carbonyl groups as shown in Figure 11. The biomimetic DHP clearly has a much higher phenolic content than does the MWL. The corresponding spectrum of the conventional S-DHP is considerably more complicated, particularly in the 2,6 region (Figure 10). This is partly due to the high content of β–β structures, but the origin of several other signals in this spectrum remains unknown.

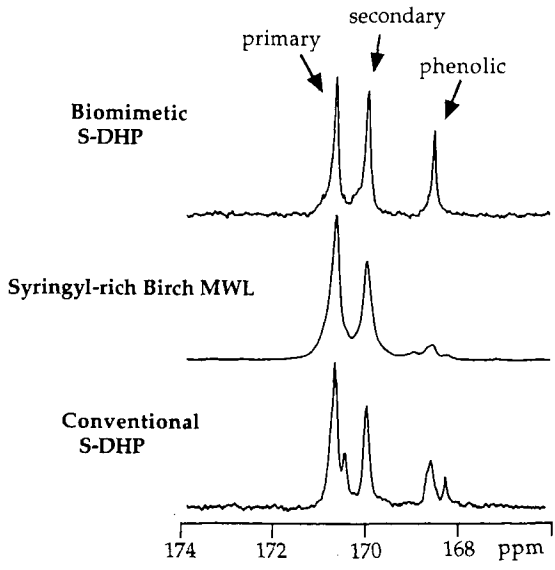

FIGURE 11. ^{13}C NMR spectra of acetyl carbonyl region of S-DHPs and syringyl-rich birch MWL.

Aliphatic region

The aliphatic region, as shown in Figure 12, is particularly interesting because it would indicate that the biomimetic S-DHP is essentially a "pure" β-O-4 polymer. In addition, comparison of the chemical shifts of the single sharp signals for the α, β, and γ sidechain carbons with authentic dimeric and trimeric lignols[9] indicates that only the *erythro* isomers are present. Similarly, the birch MWL is very high in β-O-4 content and predominantly *erythro* in nature. In contrast, the relatively complex sidechain region of the conventional S-DHP has considerable contributions from β–β structures, as well as several signals of unknown origin.

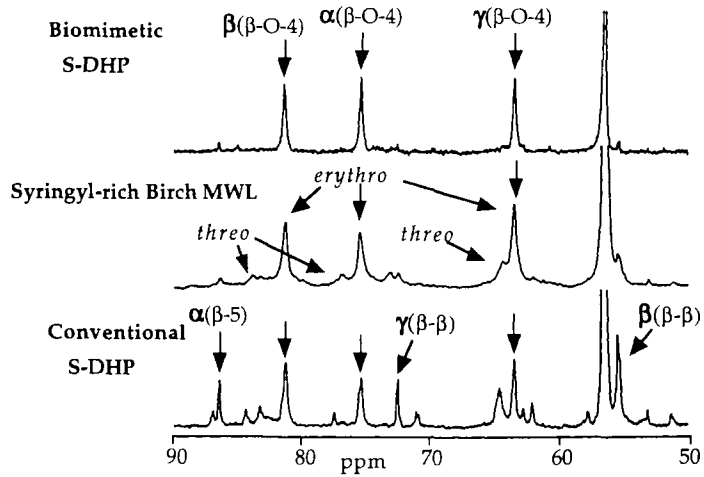

FIGURE 12. ^{13}C NMR spectra of aliphatic region of S-DHPs and syringyl-rich birch MWL.

The simplicity of the sidechain region of the biomimetic S–DHP, which is a high molecular weight fraction, does not appear to be consistent with the relatively high phenolic content, as indicated in Figure 11. That is, for a long chain of C9 units connected by β-O-4 linkages the phenolic content would be much lower, as it is in the birch MWL. High phenolic content could be explained in part by crosslinks between relatively short linear β-O-4 chains, but there is no clear evidence that such crosslinks are present in the biomimetic S-DHP. Research aimed at clarifying this situation is in progress.

SUMMARY

"Biomimetic" guaiacyl DHPs prepared by the dehydropolymerization of coniferyl alcohol with manganese triacetate had a greater

resemblance to natural lignins isolated from wood than did conventional G-DHPs prepared by enzymic techniques. A small amount of coniferaldehyde was incorporated into the biomimetic G-DHP to further fine-tune the structure for a closer resemblance to a pine MWL. Guaiacyl/syringyl DHPs prepared by the same biomimetic technique were better models of lignins isolated from hardwoods than were conventionally prepared DHPs. A pure biomimetic syringyl DHP closely resembled a fraction of lignin isolated from birch wood that was predominantly syringyl in nature. However, an unusually high phenolic content in the biomimetic S-DHP was inconsistent with the relatively low phenolic content in the corresponding lignin. The reason for this discrepancy is not yet known.

A rather unusual G-DHP was prepared that was almost devoid of phenolic entities. Presumably, extensive cross linking by 5-O-4 bonds could lead to such a DHP.

EXPERIMENTAL

Starting Materials

The coniferyl alcohol and sinapyl alcohol were prepared according to a literature procedure.[13] The $Mn(OAc)_3 \cdot 2H_2O$ was obtained from the Aldrich Chemical Co. The conventional enzyme DHPs were the same samples used in a previous study[6] and were produced by the "Zutropf" method.[4,5] The ginkgo (*Ginkgo biloba*), pine (*Pinus taeda*), spruce (*Picea mariana*), beech (*Fagus silvatica*), birch (*Betula papyrifera*), elm (*Ulmus americana*), sweetgum (*Liquidambar styraciflua*), and willow (Salix alba) acetylated MWLs were obtained from previous studies[12,14,15] and were applied on a 96 x 5.1 cm column of Bio-Rad Bio-Beads S-X3 and eluted with methylene chloride to obtain the highest molecular weight (MW) fraction. The high MW

fractions that were essentially excluded from the column amounted to 79%-91% of the total applied material.

Preparation of Biomimetic DHPs

G-DHP (DHP-1)

A solution of $Mn(OAc)_3 \cdot 2H_2O$ in pyridine was prepared by heating a suspension of the salt (552 mg, 2.00 mmol) in pyridine (18 mL) in a steam bath. A N_2 flow through the suspension provided the necessary agitation. Following dissolution of the solid, the solution was allowed to return to room temperature and was then filtered through a glass wool plug directly into a 25-mL graduated cylinder. The small amount of residue (< 5 mg) was washed with pyridine to bring the solution volume up to 20 mL. A solution of coniferyl alcohol (180 mg, 1.00 mmol) in pyridine (10 mL) was placed in another graduated cylinder. Over a period of 22 h, the two solutions were added, by means of peristaltic pumps, to a central reaction flask (immersed in a 50°C oil bath) that contained pyridine (5 mL) and water (50 µL). Magnetic stirring was utilized throughout the addition.

At the end of the addition, stirring was continued at 50°C, during which time a N_2 flow was maintained above the solution in the vented reaction flask to remove some of the solvent. When the volume was reduced to about 10 mL (required ~ 1 h), the dark opaque solution was removed from the oil bath, acetic anhydride (3 mL) was added, and stirring (at room temp) was continued for 3.5 h. Following acetylation, the solution was added, with stirring, to water (100 mL).

The resulting cream-colored suspension was acidified to pH 1-2 with conc HCl (~5 mL) and was followed by extraction with ethyl acetate (5 x 25 mL). The organic amber-colored solution was washed with brine, dried over anhydrous magnesium sulfate, and evaporated under vacuum on a rotary evaporator. The residual oil was azeotroped several times with toluene to remove residual acetic acid and pyridine and the toluene was removed by azeotroping with acetone. The residual amber oil (219 mg) was then applied to a 96 x 5.1 cm column of Bio-Rad Bio-Beads S-X3, which was eluted with methylene chloride to obtain the high molecular weight (excluded) fraction (110 mg, 54% of total).

G-DHP (DHP-2)

A solution of coniferyl alcohol (180 mg, 1.00 mmol) in 20% acetic acid/water (10 mL) and a solution of $Mn(OAc)_3 \cdot 2H_2O$ (402 mg, 1.50 mmol) in acetic acid (10 mL) were simultaneously added (peristaltic pump) over a 4.5 h period to 60% acetic acid/water (5 mL). After one additional hour, a few milligrams of solid sodium bisulfite were added to destroy excess Mn(III) and ice water (120 mL) was added. The resulting suspension was filtered and the solid was washed with water and freeze-dried, resulting in a cream-colored solid (114 mg, 63%). The product was then acetylated with 1/1 acetic anhydride/pyridine and applied on an 80 x 5.2 cm column of Bio-Rad Bio-Beads S-X1. The column was eluted with methylene chloride and the high molecular weight fraction (70% of the total material) was collected.

G-DHP (phenol "free")

Solutions of $Mn(OAc)_3 \cdot 2H_2O$ (536 mg, 2.00 mmol) in acetic acid (200 mL) and coniferyl alcohol (180 mg, 1.00 mmol) in acetic acid (200 mL) were added, at room temperature, over a period of 6 h to a reaction vessel containing acetic acid (50 mL). The solution was then concentrated down to about 10 mL by vacuum distillation and the concentrate was stirred into water (100 mL). The resulting white suspension was filtered, and the residue was washed with water and then freeze-dried. The white solid (191 mg, of α–acetylated product) that was obtained was fully acetylated to give a pale yellow solid (225 mg). Chromatography of the acetylated material, as described above, gave a high molecular weight fraction (86% of total).

G-DHP with incorporated coniferaldehyde

A solution of $Mn(OAc)_3 \cdot 2H_2O$ (536 mg, 2.00 mmol) in acetic acid (200 mL) was added over a 6 h period to a solution of coniferyl alcohol (180 mg, 1.00 mmol) and coniferaldehyde (29 mg, 0.16 mmol) in acetic acid (15 mL) at room temperature. Workup as described in the previous preparation of DHP-2 yielded a cream-colored solid (164 mg of α-acetylated product), which was acetylated and chromatographed as described above to give 125 mg (58% of total acetylated material) of DHP.

GS-DHP and S-DHP

These DHPs was prepared using the same procedure used for DHP–1. The only differences were as follows. In the case of GS–DHP, a mixture of coniferyl alcohol (81 mg, 0.45 mmol) and sinapyl alcohol (74 mg, 0.35 mmol) was used along with 1.75 mmol of manganese salt; for S–DHP, sinapyl alcohol (210 mg, 1.00 mmol) was used along with 1.00 mmol of the manganese salt. Following fractionation on Bio-Beads S-X3, the yield of acetylated GS–DHP was 125 mg (65% of total acetylated material) and that of acetylated S–DHP was 110 mg (45% of total acetylated material).

^{13}C NMR Spectroscopy

The NMR data were obtained at ambient temperature with a Bruker DPX-250 spectrometer (62.9 MHz ^{13}C) with acetone-d_6 solutions. All chemical shifts are given in δ ppm and are referenced to the centerline of the solvent at 29.83 ppm, which is based on tetramethylsilane (δ = 0.0). Generally 30,000 to 35,000 transients were collected (using standard proton-decoupled qualitative conditions) over a 15 hr period with a sample of 100-150 mg in 0.4 mL of solvent.

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