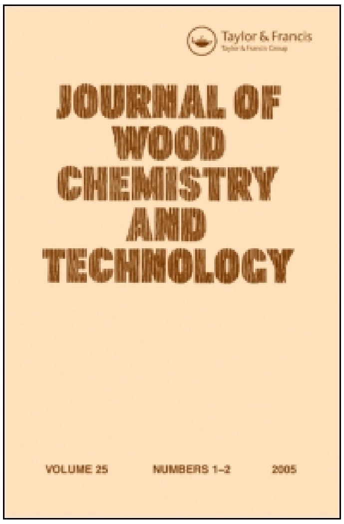

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Lawrence L. Landucci[a]; Susana Luque[b]; Sally Ralph[a]
[a] USDA Forest Service Forest Products Laboratory One Gifford Pinchot Drive Madison, Wisconsin [b] Depto. Ingenieria Quimica, Universidad de Oviedo, Oviedo, Spain

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REACTION OF *p*-HYDROXYCINNAMYL ALCOHOLS WITH TRANSITION METAL SALTS. 2. PREPARATION OF GUAIACYL/SYRINGYL DI-, TRI-, AND TETRALIGNOLS

Lawrence L. Landucci*, Susana Luque#, and Sally Ralph
USDA Forest Service Forest Products Laboratory
One Gifford Pinchot Drive
Madison, Wisconsin 53705-2398

ABSTRACT

Treatment of coniferyl alcohol (CA), sinapyl alcohol (SA), and their mixtures with copper or manganese salts yielded nine dilignols, five trilignols, and one tetralignol. The lignols were composed of guaiacyl (G) or CA units and syringyl (S) or SA units linked together by β-O-4 (b), β-5 (c for coumaran), β–β (r for resinol), and/or a novel γ–β (gb) linkage. None of the isolated lignols contained 5-5′ (biphenyl), 4–O–5, or β-1 linkages. Spectral analysis (^{1}H NMR, ^{13}C NMR, MS) and comparison with authentic compounds confirmed the structures of the dilignols as G-b-CA, G-b-SA, S-b-CA, S-b-SA, G-c-CA, S-c-CA, G-r-G, G-r-S, and S-r-S; the trilignols as G-b-S-r-S, G-b-S-c-CA, S–b-S-r-S, and G-c-G-gb-G; and the only tetralignol as G-b-S-r-S-b-G.

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* Author to whom correspondence may be addressed.

\# Present address: Depto. Ingenieria Quimica, Universidad de Oviedo, 33071 Oviedo, Spain.

INTRODUCTION

The first paper of this series described the oligomerization and polymerization of coniferyl alcohol (CA) with salts of iron, cobalt, and manganese.[1] In that work a variety of products, ranging from dilignols to polylignols (DHPs), was obtained. Although the initial reaction step in oxidation of CA by metal salts is presumably the same as that in the H_2O_2/peroxidase system (phenoxy radical formation), the additional flexibility and lack of enzyme-imposed restraints on reaction conditions resulted in a broad array of products and linkage distributions.

In the present work we extend the technique to oxidation of sinapyl alcohol (SA) and to CA/SA mixtures and concentrate on obtaining all of the possible guaiacyl (G) and syringyl (S) dilignols with β-O-4, β-5, or β-β linkages. These are required for a solid foundation in the interpretation of NMR spectra of higher oligolignols and as building blocks in the synthesis of tri- and tetralignols.

RESULTS AND DISCUSSION

Metal Systems

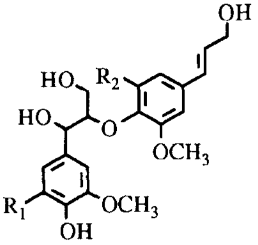
The metal systems used in this study were selected primarily because they could be optimized for relatively high yields of dilignols (Table 1). However, a few higher oligolignols were also obtained in the course of this study.

Dilignols

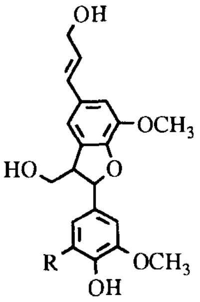
The dilignols obtained in this study are identified by number or by abbreviated letter designations as follows: G = guaiacyl ring, S = syringyl ring, CA = terminal coniferyl alcohol group, SA = terminal sinapyl alcohol group, b = β-O-4 linkage, c = β-5 (coumaran) linkage, r =

TABLE 1.
Metal Systems.

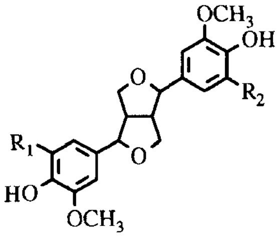
Metal Salt	Medium
$Cu(OAc)_2$	borate buffer, pH 9
$Mn(OAc)_3$	pyridine
$K_3Mn(CN)_6$	0.1 M NaOH



	R_1	R_2	
1	H	H	G-b-CA
2	H	CH_3O	G-b-SA
3	CH_3O	H	S-b-CA
4	CH_3O	CH_3O	S-b-SA



	R	
5	H	G-c-CA
6	CH_3O	S-c-CA



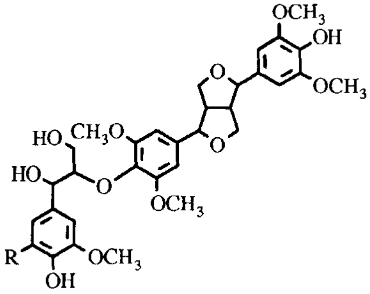
	R1	**R2**	
7	H	H	G-r-G
8	H	CH_3O	G-r-S
9	CH_3O	CH_3O	S-r-S

FIGURE 1. Dilignols

β–β (resinol) linkage, and gb = γ–β linkage. Only the underivatized products are shown, although in many cases, separations and isolations were performed with fully acetylated reaction mixtures. These nine compounds represent all of the possible combinations of G and S rings linked by β-O-4, β-5, or β–β bonds. Dilignols **1**, **5**, and **7** were first found among dehydrogenation products of CA[2] and later synthesized or found in mixtures resulting from the oxidation of CA with metal salts.[1,3,4] The resinols **7**, **8**, and **9** have been isolated from plant material and/or have been synthesized.[5-7] To our knowledge the remaining dilignols, **2**, **3**, **4**, and **6**, have not been reported in the literature.

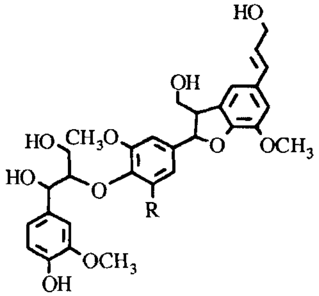
Higher Oligolignols

Trilignols isolated in this study are shown in Figure 2. The β–O–4/β–β trilignols **10** and **11** were previously isolated from the water hydrolysis products from Yachidamo (*Fraxinus mandshurica var. japonica*) wood.[8,9] Trilignols **10** and **11** were more recently found in the methanol extract of *Buddleja davidii*. along with the β-O-4/β-5 trilignol **13**.[10] Trilignol **10** has also been synthesized with 25% overall yield in five steps from **9**.[11] The β-O-4/β-5 trilignol **12** has not been reported, but an isomer with the linkages reversed (G-c-G-b-CA) has been synthesized in six steps (29%) from methyl ferulate.[12] Trilignol **14** has not been reported in the literature. Compound **14** is unusual in that it contains a novel β–γ linkage, which does not occur in DHPs prepared by conventional techniques or in natural lignins. Dilignols containing the β–γ linkage have been previously reported.[1,13] The only tetralignol (**15**) isolated in this study is shown in Figure 3. This compound was previously synthesized from **9** in a manner analogous to the synthesis of the trilignol **10**.[11] All of the isolated compounds were characterized by ^1H and ^{13}C NMR spectroscopy and most were identified by comparison with spectra of authentic compounds in the FPL/DFRC (Forest Products Laboratory/Dairy Forage Research Center)



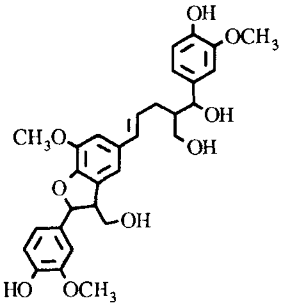
10 R = H G-b-S-r-S

11 R = CH_3O S-b-S-r-S



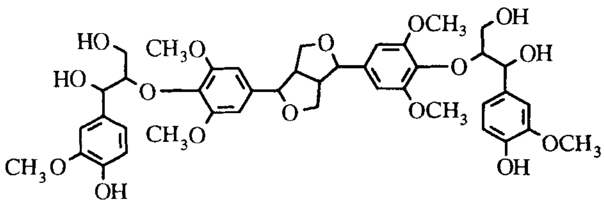
12 R = H G-b-G-c-CA

13 R = CH_3O G-b-S-c-CA



14 G-c-G-gb-G

FIGURE 2. Trilignols



15 G-b-S-r-S-b-G

FIGURE 3. Tetralignol

NMR database.[14] Those not yet in the database were fully assigned and authenticated by mass spectral analysis and the usual array of one- and two-dimensional NMR experiments.

Copper Acetate/Borate Buffer

Treatment of CA, SA, or CA/SA mixtures with $Cu(OAc)_2$ in borate buffer (pH 9.2) gave a mixture of products ranging from monolignols to lignols of five or more C_9 units. This determination was based on gel chromatograms of acetylated reaction mixtures and will be described later in this report. Components in unacetylated reaction mixtures were separated by chromatography on thick-layer silica gel plates. Typical product distributions in CA, CA/SA, and SA experiments are shown in Table 2.

Relative Rf values of the products, following three successive developments (5% CH_3OH/CH_2Cl_2), are shown in Figure 4. In some cases products could not be separated because of similar Rf values. Other solvents and solvent mixtures were tested without success. No products containing a terminal SA group were isolated. This, along with the observation that $SA/Cu(OAc)_2$ reactions generally gave many more bands (of 1-4 mg each for a 100 mg scale) on the silica gel plate than corresponding CA or CA/SA reactions, suggested that the underivatized SA functionality was not stable under the chromatographic conditions.

The crude reaction mixtures were acetylated prior to chromatography to avoid the instability problem. In addition, to simplify the separation of individual components, a preliminary separation of molecular weight (MW) fractions was accomplished with a preparative scale polystyrene gel column with a MW cutoff of about 6000 Da. A typical MW profile of acetylated products from a $CA/Cu(OAc)_2$ reaction is shown in Figure 5. Upon elution with chloroform, five fractions, A-E, were collected. Fraction A had the highest MW and was composed of lignols of five or more C_9 units.

TABLE 2.
Product Yields from CA, CA/SA, and SA Dehydropolymerization with $Cu(OAc)_2$

Products[a]	Yield (%) CA	CA/SA	SA
Monolignols	3	4	18
Dilignols			
G-b-CA	15[b]	---	---
G(S)-b-CA	---	9	---
G-c-CA	---	2	---
G-r-G	8	---	---
G(S)-r-G(S)	---	9	---
S-r-S	---	---	23
Trilignols			
G-b-S-r-S	---	11	---
S-b-S-r-S	---	---	19
G-b-G-c-CA	18	---	---
G-b-S-c-CA	---	4	---
G-c-G-gb-G	8	---	---
Tetralignol			
G-b-S-r-S-b-G	---	8	---
Oligolignols (DP>4)[c]	14	18	12
Unidentified[d]	8	5	5
Total recovery	74	70	77

[a] G = guaiacyl ring, S = syringyl ring, CA = coniferyl alcohol end unit, SA = sinapyl alcohol end unit, b = β-O-4 linkage, c = β-5 linkage, r = β–β linkage, gb = γ–β linkage; [b] About one-third of the β-O-4 dimer was acetylated in the γ position; [c] DP = degree of polymerization; [d] mixtures of tri- or tetralignols.

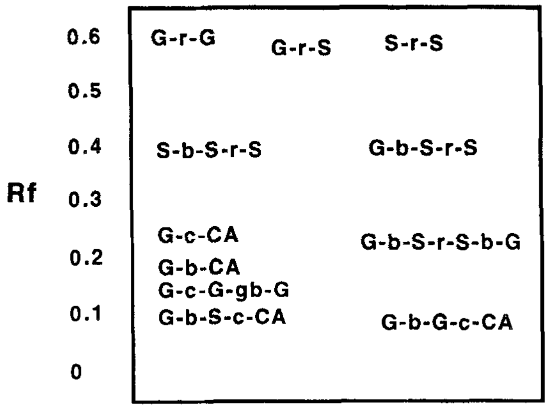


FIGURE 4. Relative Rf values of isolated products. Thick-layer silica gel plates were developed three times with 5% CH_3OH/CH_2Cl_2.

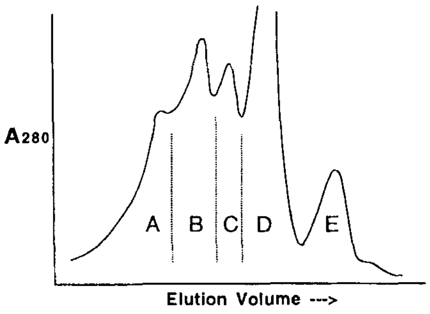


FIGURE 5. Typical molecular weight profile of acetylated products from $CA/Cu(OAc)_2$ reaction.

Fraction B was composed of tetralignols; fraction C, trilignols; fraction D, dilignols; and the lowest MW fraction, E, was composed of monolignols. The total weight recovery of ethyl acetate soluble products from CA reactions ranged from 76 – 85%. With the $CA/SA/Cu(OAc)_2$ reaction, only 53% yield was obtained. This low yield can be explained by a larger amount of insoluble residue observed

TABLE 3.
Effect of Stoichiometry on Lignol Distribution (%)

Mole Ratio	Mono-lignols	Di-lignols	Tri-lignols	Tetra-lignols	Oligo-lignols[a]
CA : Cu salt					
1 : 0.7	11	55	15	10	9
1 : 1	4	41	16	25	14
1 : 1.3	6	50	11	21	12
1 : 1.6	5	55	12	19	10
1 : 2	6	48	12	22	12
CA : SA : Cu salt					
1 : 1 : 2.3	8	33	36	19	4

[a]Degree of polymerization > 4.

in the CA/SA reaction mixture. The oligolignols (Fraction A) and monolignols (Fraction E) were not investigated further in this study. The dilignols (Fraction D) were the main focus as they are the building blocks for the higher oligolignols, and NMR data for the S and G/S dilignols are generally unavailable from the literature.

Optimization of the dilignol yield was attempted by varying the stoichiometry of the CA/$Cu(OAc)_2$ reactions. The dilignol yield varied between 41% and 55% of the total isolated products with no clear correlation with changes in stoichiometry from a 1:0.7 to a 1:2 CA:$Cu(OAc)_2$ mole ratio (Table 3). When a mixture of CA and SA was used, the yield of trilignols increased considerably at the expense of the dilignol fraction. This can be explained by the facile formation of trilignols **10** and **11** containing the S-r-S moiety. This moiety is also present in the tetralignol **15**.

Examination by ^1H and ^{13}C NMR spectroscopy and comparisons with spectra of authentic compounds confirmed that the dilignol fractions from CA reactions were mixtures of **1**, **5**, and **7**. The

TABLE 4.
Product Distribution in CA and CA/SA Dilignol Fractions from $Cu(OAc)_2$ Reactions[a].

Dilignol	CA	CA/SA
1 (G-b-CA)	33[b]	8
2 (G-b-SA)	----	2
3 (S-b-CA)	----	1
4 (S-b-SA)	----	2
5 (G-c-CA)	8	5
6 (S-c-CA)	----	4
7 (G-r-G)	9	nd[c]
8 (G-r-S)	----	7
9 (S-r-S)	----	2

[a]Values are wt% of total acetylated products from ethyl acetate soluble material; [b]present as a 88:12 *erythro:threo* mixture; [c]nd = not detected.

corresponding fraction in the CA/SA reaction was composed of all of the dilignols shown in Figure 1 except for **7**, which was not detected. Quantitation of the side chain region of ^{13}C NMR spectra of the dilignol fractions was done by a technique similar to that described in Part 1 of this series.[1] The results of the quantitation are given in Table 4. Because of the scarcity of G/S dilignols (particularly **2** and **3**), the $Cu(OAc)_2$/borate system was unsuitable for the preparation of sufficient quantities of these important entities for detailed NMR analyses. In contrast, relatively large amounts of trilignols and a tetralignol were isolated from the copper system. For example, examination of fraction C of the CA reaction products by ^1H and ^{13}C NMR spectroscopy indicated that the major component was the trilignol **14** containing the β–γ linkage. Quantitative formation of the dilignol G-bg-G by the treatment of CA with potassium

TABLE 5.
Estimated Product Distribution in CA and CA/SA Trilignol and Tetralignol Fractions[a]

Product	CA	CA/SA
10 (G-b-S-r-S)	----	29
13 (G-b-S-c-CA)	----	7
14 (G-c-G-gb-CA)	7	nd[b]
15 (G-b-S-r-S-b-G)	----	10
Unidentified	5	5

[a]Values are wt% of total acetylated products from ethyl acetate soluble material; [b]not detected.

cyanocobaltate(III)/borate was reported in Part 1 of this series.[1] Interestingly, no trace of this novel linkage was found in the products from CA/SA mixtures. The major trilignol component from the CA/SA/$Cu(OAc)_2$ reaction was **10**. Other components in trilignol and tetralignol fractions remain to be identified and will be the subject of another report in this series. The only tetramer thus far identified is **15**, which is the principal component in the tetramer fraction and the most abundant product obtained from the treatment of CA/SA with $Cu(OAc)_2$. The corresponding tetramer fraction from a CA/$Cu(OAc)_2$ reaction was considerably more complex, as the ^{13}C NMR spectrum indicated the presence of β–O-4, β-5, and β–γ linkages. No β–β linkages were detected in this fraction. Estimated amounts of the trilignols and the tetralignol, obtained by quantitation of ^{13}C NMR spectra, are summarized in Table 5. The scarcity of S/G dilignols and the occurrence of the "unnatural" β–γ linkage in products from copper reactions prompted a search for more suitable metal systems.

Manganese (III) Systems

Comparison of lignol distribution of two manganese(III) systems with the copper acetate system is shown in Table 6. This comparison is

TABLE 6.
Lignol Distribution in CA/SA Reactions with Metal Salts[a].

	$Mn(OAc)_3$[b]	$K_3Mn(CN)_6$[c]	$Cu(OAc)_2$[d]
Monolignols	12[e]	15[e]	8
Dilignols	57	30	33
Trilignols	11	14	36
Tetralignols	11	22	19
Oligolignols (DP>4)[f]	9	19	4

[a]Values are % of total acetylated products; [b]in pyridine; [c]in 0.1 M NaOH; [d]in 0.05 M borate buffer (pH = 9.2); [e]almost pure CA; [f]DP = degree of polymerization.

not intended to reflect strictly the nature of the metal salts as oxidizing agents because different reaction media were used with the three salts. At this point in the study the main impetus was to find a suitable system from which the two β-O-4 dilignols, **2** and **3**, could be generated in yields sufficient for characterization.

In part 1 of this series we reported that manganese acetate (in acetic acid) reacted with CA to give a polylignol (DHP) with a high (55%) β–O–4 content.[1] This, in combination with the large dilignol fraction for manganese acetate shown in Table 6, makes this system an ideal candidate for the production of **2** and **3**. Integration of the α- and β-carbon region of ^{13}C NMR spectra of dilignol fractions revealed the product distributions shown in Table 7. There was no more than a trace of the GS or SS β-O-4 dilignols in the product mixture from the CA/SA/cyanomanganate reaction. Most of the S C_9 units are present in β–β dilignols, a small amount in β-5 dilignols, and the rest in higher oligolignols. The actual oxidant in the cyanomanganate system is possibly Mn_2O_3 or MnO_2, the former resulting from hydrolysis of the cyanomanganate salt and the latter from disproportionation.[15,16] In

TABLE 7.
Product Distribution in CA/SA Dilignol Fractions[a].

Dilignol	$Mn(OAc)_3$[b]	$K_3Mn(CN)_6$[c]	$Cu(OAc)_2$[d]
1 (G-b-CA)	6	6	8
2 (G-b-SA)	11	nd[e]	2
3 (S-b-CA)	11	nd	1
4 (S-b-SA)	19	nd	2
5 (G-c-CA)	5	7	5
6 (S-c-CA)	2	2	4
7 (G-r-G)	nd	nd	nd
8 (G-r-S)	2	4	7
9 (S-r-S)	1	11	2

[a]Values are wt% of total acetylated products from ethyl acetate soluble material; [b]in pyridine; [c]in 0.1 M NaOH; [d]in 0.05 M borate buffer (pH = 9.2); [e]not detected.

the copper system, as also shown previously in Table 4, only a small amount of **2** and **3** was found.

An observation that is not apparent from Table 7 is the difference in stereochemistry of G-b-CA in the three systems. This is illustrated by the partial ^{13}C NMR spectra in Figure 6, which compares the α-carbon region of the three systems. In part 1 of this series it was reported that CA reacted with the $K_3Mn(CN)_6$/NaOH system to form β-O-4 linkages with an approximately 50:50 *erythro*:*threo* (*e*:*t*) ratio. As shown in Figure 6 (middle spectrum), this was also true with a mixture of CA and SA. In Table 4 it was noted that with the copper system, the G-b-CA from CA reactions was present as a 88:12 *e*:*t* mixture. Similarly, with the $Mn(OAc)_3$/pyridine system a 82:18 *e*:*t* G-b-CA mixture was obtained in a CA reaction (not shown). Because the α-carbon of *threo* G-b-CA is almost coincident with the α-carbon of S-b-SA, the former is

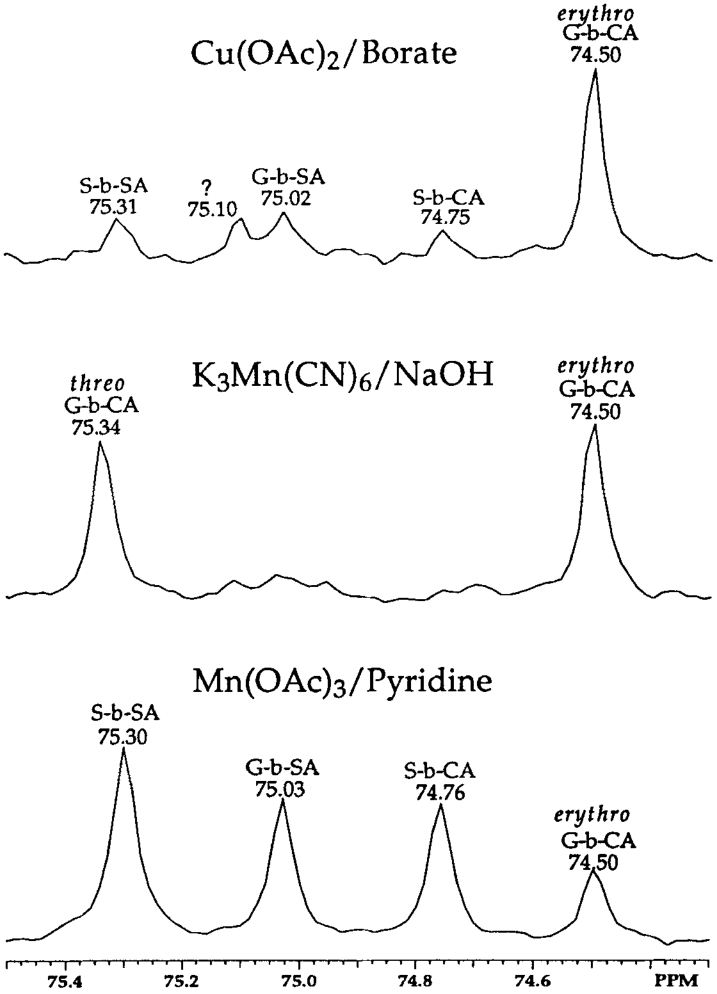


FIGURE 6. α–carbon region of β-O-4 dilignols from CA/SA reactions.

masked by the latter in the top and bottom spectra in Figure 6. However, because of the scarcity of *threo* isomers with the $Cu(OAc)_2$ and $Mn(OAc)_3$ systems, there was not an appreciable error in the assumption that the signal at 75.30 ppm is due principally to S-b-SA. The absence of S-b-SA in the cyanomanganate reaction mixture was confirmed by the absence of the signal due to the β-carbon of the linkage at 81.6 ppm (not shown). There was no evidence from the NMR spectra that dilignols other than G-b-CA were present in more than one isomeric form, which was presumably *erythro*.

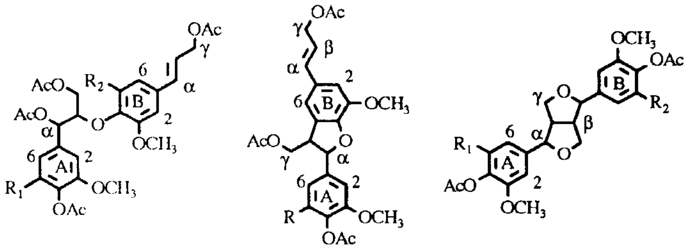
^{13}C NMR Chemical Shift Assignments

Chemical shift assignments of the aromatic and side chain carbons of all of the acetylated dilignols isolated in this study are listed in Table 8. Complete NMR data of the higher oligomers will be included in Part 3 in this series. The assignments of compounds not yet in the FPL/DFRC database[13] were made by comparison with authentic compounds of similar structure. Questionable assignments were confirmed by two-dimensional NMR techniques. Complete NMR data of compounds **1-15** can be obtained upon request.

CONCLUSIONS

The main focus of this study, production of the nine possible G/S dilignol structures that contain the β–O–4, β–5, or β–β linkage, was accomplished by the reaction of CA/SA mixtures with copper and manganese salts. Five G/S trilignols and a tetralignol were also isolated and characterized. NMR characterization of the dilignols provides a firm foundation for subsequent studies involving the production and NMR characterization of higher oligolignols.

TABLE 8.
Chemical Shifts of Acetylated Dilignols.[a]



	1^b	2	3	4	5	6	7	8	9
Aα	74.5	75.0	74.8	75.3	88.4	88.8	86.3	86.2	86.5
Aβ	80.3	81.5	80.2	81.6	51.4	51.4	55.4	55.4	55.4
Aγ	63.0	63.2	63.1	63.3	66.0	66.0	72.6	72.6	72.7
A1	136.6	136.4	136.1	136.6	141.0	140.5	140.2	140.1	141.4
A2	112.7	112.2	105.2	104.5	111.2	103.5	111.2	111.1	103.2
A3	152.1	152.0	153.1	153.0	152.5	153.4	152.3	152.3	153.2
A4	140.8	140.5	129.8	129.3	140.8	129.5	141.8	141.8	128.8
A5	123.4	123.3	153.1	153.0	123.8	153.4	123.5	123.5	153.2
A6	120.4	119.9	105.2	104.5	118.7	103.5	118.6	118.6	103.2
Bα	134.1	134.3	134.1	134.4	134.7	134.7	86.3	86.5	86.5
Bβ	123.4	124.1	123.4	124.1	122.4	122.4	55.4	55.4	55.4
Bγ	65.3	65.3	65.4	65.2	65.5	65.5	72.6	72.7	72.7
B1	132.6	133.4	132.6	133.3	131.7	131.7	140.2	128.9	128.8
B2	111.4	104.6	111.4	104.6	112.4	112.4	111.2	103.3	103.2
B3	152.0	154.2	151.9	154.2	145.4	145.4	152.3	153.2	153.2
B4	148.2	137.1	148.3	136.5	149.3	149.3	141.8	141.4	141.4
B5	119.3	154.2	119.2	154.2	128.9	129.0	118.6	153.2	153.2
B6	120.5	104.6	120.5	104.6	116.4	116.3	123.5	103.3	103.2

[a]Chemical shifts in acetone-d_6 vs tetramethylsilane; [b]predominant isomer, *erythro*

EXPERIMENTAL

Starting materials.

The CA and SA were prepared according to a literature procedure.[17] The Copper(II) acetate monohydrate (98+%) and manganese(III) acetate dihydrate (97%) were obtained from Aldrich Chemical Company. Potassium hexacyanomanganate was obtained from Alfa Inorganics. The 0.05 M borax buffer was prepared from Mallinckrodt AR grade sodium borate ($Na_2B_4O_7 \cdot 10H_2O$).*

Typical oxidation procedures.

Cu(OAc)$_2$/borate buffer

The CA and/or SA (180/210 mg, 1.00 mmol) was dissolved in 0.05 M borate solution (50 ml, 2.50 mmol) at 100°C under a nitrogen atmosphere with magnetic stirring. A solution of the copper acetate (260 mg, 1.30 mmol) in water (3 ml) was then added, resulting in a green suspension. The suspension was stirred for 30 min at 100°C, during which time the color changed to orange. Removal of the insoluble copper salts by filtration left a bright yellow filtrate that was acidified with CH_3COOH or 1M HCl to pH 5. The milky white suspension was extracted with ethyl acetate (5 x 20 ml). The extract was dried over $MgSO_4$ and evaporated under vacuum to give an oil/boric acid mixture. Methanol (5 ml) was added and evaporation was repeated. The methanol evaporation sequence was repeated five times to drive off all of the boric acid as methyl borate. Remaining was a white foamy solid in 75-85% yield. Reaction mixtures were examined by ^1H and ^{13}C NMR spectroscopy and/or acetylated with 1/1 acetic anhydride/pyridine (2-4 h) prior to NMR examination.

Mn(OAc)$_3$/pyridine

A solution consisting of CA (180 mg, 1.00 mmol), SA (210 mg, 1.00 mmol), water (0.18 ml, 10.0 mmols), and pyridine (4 ml) was added to a solution/suspension of $Mn(OAc)_3$ (560 mg, 2.08 mmols) in pyridine (10 ml) under a nitrogen atmosphere with magnetic stirring. The dark mixture was stirred at room temperature for 45 min. Acetic anhydride (6 ml) was added and stirring was continued for 1 h. The resulting dark

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*The use of trade names is for information only and is not intended to be an endorsement by the USDA Forest Products Laboratory.

solution was added to 1% sodium bisulfite solution (200 ml) and the white suspension was extracted with ethyl acetate (5 X 30 ml). The extract was washed with water (25 ml), 0.5 M HCl (3 X 25 ml), water (25 ml), saturated sodium bicarbonate solution (25 ml), and saturated NaCl solution (25 ml) and dried over $MgSO_4$. Evaporation of the solvent under vacuum left a pale yellow foamy acetylated product (387 mg).

$K_3Mn(CN)_6$/NaOH

A solution consisting of CA (180 mg, 1.00 mmol) and SA (210 mg, 1.00 mmol) in 0.1 M NaOH (40 ml, 4.00 mmols) was added to a solution/suspension of $K_3Mn(CN)_6$ (725 mg, 2.21 mmols) in water (15 ml) under a nitrogen atmosphere with magnetic stirring. Stirring at room temperature was continued for 1 h and the manganese salts were removed by filtration. The yellow-orange filtrate was acidified to pH 2 with 1 M HCl and the suspension was extracted with ethyl acetate. The extract was washed with water and saturated NaCl solution and dried over MgSO4. Evaporation of the solvent under vacuum left a dark yellow foam (354 mg, 91% wt recovery). Treatment of the product with 1/1 acetic anhydride/pyridine for 4.5 h yielded 507 mg of acetylated material.

Separation of components in oxidation mixtures.

Unacetylated oxidation mixtures were applied to pre-eluted (1% CH_3COOH in ethyl acetate) Whatman thick-layer preparative silica-gel plates and developed with 5% CH_3OH in CH_2Cl_2. Multiple developments (with thorough drying between developments) were frequently required for band separation. Individual bands were scraped from the plate and the silica gel was extracted with ethyl acetate (5 x 5 ml). See Rf data in Figure 4.

Separation of components in acetylated oxidation mixtures.

Acetylated oxidation mixtures were applied to a 95 x 5-cm Bio-Rad Bio-Bead SX-1 column. The column was eluted with $CHCl_3$, and the eluant was monitored with an ISCO Model UA-5 Absorbance Monitor (280 um). Five fractions were collected (see Figure 5 for typical MW profile). Further separation of acetylated mixtures was accomplished with thick-layer plates as described above, except that the pre-elution solvent was ethyl acetate and the developer was 20-40% ethyl acetate in cyclohexane or 5% CH_3OH in $CHCl_3$.

NMR spectroscopy

The NMR spectra were obtained with a Bruker WM250 spectrometer. Samples were dissolved in acetone-d_6, and TMS was used as internal reference. Typically, the ^{13}C (62.9 MHz) spectra were obtained with the standard Bruker Powgate microprogram. Quantitation of the side chain carbons in oligolignol and DHP preparations were performed as described in Part 1 of this series.[1] Two–dimensional spectra were obtained with a Bruker AMX-360 narrow bore instrument using standard pulse programs and conditions.

MS spectroscopy

Mass spectra were determined with a solid probe on a Finnigan 4510 MS, ev = 70, source temp = 210°C. The ammonia CI spectra were run at 0.5 Torr.

Dilignols (acetates)

1 EI; M^+ 544(15), 323(46), 281(14), 222(88), 221(100), 179(81).
2 EI; M^+ 574(7), 400(12), 358(10), 323(71), 252(78), 222(32), 221(100), 179(82).
3 EI; M^+ 574(17), 353(33), 323(17), 311(36), 293(14), 252(42), 251(90), 222(98), 221(32), 209(100), 208(42), 179(47).
4 EI; M^+ 604(3), 430(5), 353(41), 323(3), 311(21), 293(8), 252(100), 251(53), 209(49).
5 EI; M^+ 484(86), 425(25), 424(28), 383(22), 382(100), 365(12), 323(24), 322(44), 307(12), 291(38), 247(27), 162(31), 161(21).
6 EI; M^+ 514(100), 484(11), 412(49), 382(17), 352(38), 337(14), 321(50), 309 (14), 291(13), 167(23).
7 EI; M^+ 442(6), 400(31), 358(32), 163(23), 151(100), 137(47).
8 EI; M^+ 472(0.2), 430(8), 388(3), 193(7), 182(7), 181(15), 167(12), 163(28), 151(100).
9 EI; M^+ 502(0.2), 460(32), 418(82), 193(30), 182(51), 181(100), 167(96).

Trilignols (acetates)

10 CI; (NH_3, M^+ +18) 800(2): EI; 460(19), 418(52), 323(53), 222(86), 221(100), 181(55): 179(82), 167(50).
11 CI; (NH_3, M^+ +18) 830(12): EI; 460(8), 418(34), 353(29), 311(21), 252(95), 251(68), 209(100), 181(72), 167(58).
12 CI; (NH_3, M^+ +18) 782(19): EI; M^+ 764(0.5), 382(13), 323(12), 281(9), 222(58), 221(100), 179(94).
13 CI; (NH_3, M^+ +18) 812(14): EI; 514(5), 412(7), 352(7), 323(39), 281(10), 222(77), 221(100), 179(65).
14 CI; (NH_3, M^+ +18) 766(100): EI; 323(16), 222(59), 221(28), 179(42), 137(100).

Tetralignol (acetate)

15 EI; (M^+ above scan range), 418(11), 323(14), 252(17), 222(100), 179(49).

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