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# A New Synthesis of (Z)-Coniferyl Alcohol, and Characterization of its Derived Synthetic Lignin

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**ABSTRACT:** The *(Z)*-monolignols (hydroxycinnamyl alcohols) are found along with their predominant *(E)*-isomers in a number of plants. Their role in lignification is unknown. A new synthetic route to *(Z)*-coniferyl alcohol has been developed via Still and Gennari's modification of the Horner-Emmons olefination to produce *(Z)*-unsaturated esters. Although previous studies have shown that *(Z)*-coniferyl alcohol will polymerize by single-electron (radical) processes to produce synthetic lignins, the stereochemistry of units in the resulting structure had not been addressed. NMR analysis of a synthetic lignin from *(Z)*-coniferyl alcohol indicates that unsaturated sidechains in the resulting lignin retain their *(Z)*-geometry. Other structures are altered in their relative proportions but retain the same stereochemistry. Assignment of structures in these synthetic lignins provides the necessary database for more careful examination of real plant lignins.
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## INTRODUCTION

A polymer crucial to all terrestrial plants, lignin derives from simple units but contains significant complexity; interunit **A-E** and end-group **X-Y** structures are among the most prominent features, Figure 1.[1,2] Lignins are produced principally from a dehydrogenative polymerization of one or more of three hydroxycinnamyl alcohols; *p*-coumaryl alcohol (4-hydroxycinnamyl alcohol), coniferyl alcohol (4-hydroxy-3-methoxycinnamyl alcohol), and sinapyl alcohol (3,5-dimethoxy-4-hydroxycinnamyl alcohol).[3-5] Most work has logically focussed on the *(E)*-hydroxycinnamyl alcohols, but the *(Z)*-isomers present some intrigue. The case has been developed in several papers on the subject.[6-9] Basically, *(Z)*-monolignols have been found in *Fagus grandifolia* where they accumulate in the bark along with their glucosides to the apparent exclusion of the *(E)*-isomers. Whether it is therefore only the *(E)*-isomers that polymerize to form lignin, invoking stereoselective reactions, or whether *(Z)*-monolignols accumulate as metabolites that are not involved in lignification are issues raised by these works.

Morelli *et al.*[7] devised a synthesis of *(Z)*-coniferyl alcohol **1** and successfully prepared synthetic lignin from that isomer. It was established that *(Z)*-coniferyl alcohol was capable of free-radical polymerization, as was the traditional *(E)*-isomer. Unfortunately, no serious structural characterization of the product lignin has been forthcoming. One of the key issues remaining unanswered therefore is whether lignification from *(Z)*-isomers results in structural or isomeric differences and whether these can be detected in isolated plant lignins. One of the simplest issues that is not obvious *a priori* is whether unsaturated sidechain endgroups retain the *(Z)*-configuration. Although such end-groups are minor in native lignins, they are rather predominant in synthetic lignins due to the ready ability of monomers to radically couple with other monomers.[10,11] This process is far more rare in the plant, restricted to presumably only initiating reactions; monomers diffusing into the cell wall form radicals which more typically encounter radicals of the growing lignin polymer. It is for this reason that β–O–4-ether and β–5

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units predominate in native lignins — monolignol radicals primarily couple at the β-sidechain position, whereas lignin oligomers or polymers have only 4–O– and 5–positions available.

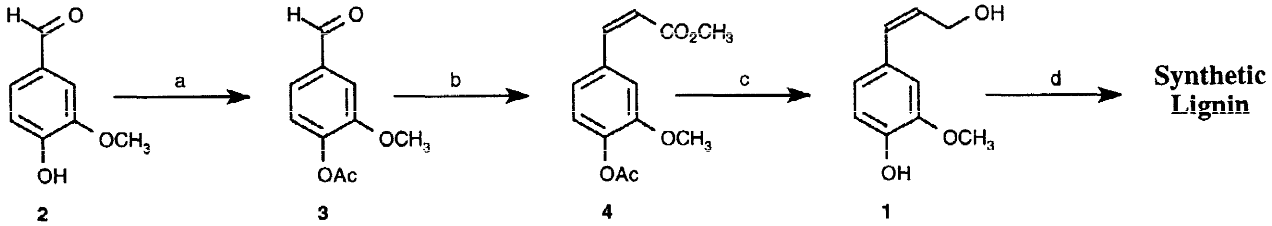
The current study provides a more convenient synthetic route to the *(Z)*-monolignols, and examines structural aspects of the synthetic lignin produced by biomimetic free-radical polymerization of coniferyl alcohol. Features that distinguish product moieties arising from *(Z)*- *vs* *(E)*-monolignols can then be sought in isolated plant lignins to establish whether *(Z)*-monolignols have any (significant) role in lignification.

## DISCUSSION

The key step in our synthesis of *(Z)*-monolignols, Scheme 1, is the almost stereoexclusive conversion of 4-acetoxybenzaldehydes to *(Z)*-4-acetoxycinnamates using methyl bis(trifluoroethyl) phosphonoacetate and $KN(TMS)_2$/18-crown-6 as base.[15,16] The reaction with acetoxybenzaldehyde **3** (as well as with other analogues) proceeded in ~90% yield and produced almost exclusively the methyl *(Z)*-4-acetoxycinnamate **4** providing the temperature was carefully maintained at ~-78 °C during the reaction. Allowing the temperature to raise to even -60 °C (as during our early reactions), produced higher amounts of the *(E)*-4-acetoxycinnamates. *(Z)*-Coniferyl alcohol **1** was prepared in a single step via DIBAL reduction, which effected the required regioselective 1,2-reduction[17] and removed the acetate protecting group.[18] Final *(Z)*-coniferyl alcohol was obtained in ~80% crystalline yield. The overall scheme has fewer total steps than the prior synthesis[7] and produces *(Z)*-monolignols with acceptably low levels of the *(E)*-isomer (<2%).

A synthetic lignin was prepared from *(Z)*-coniferyl alcohol **1** by a procedure similar to those described previously for the *(E)*-isomer,[19] but in lower yield. The polymer was an almost white (very pale beige) solid with similar macroscopic physical properties to the traditional *(E)*-coniferyl alcohol DHP. The molecular weight range, as determined by gel-permeation chromatography on Biobeads SX-1 (exclusion limit 14,000) was lower, containing a smaller proportion of high molecular weight material (DP >20). Whether (Z)-coniferyl alcohol truly polymerizes less efficiently than the (E)-isomer is not known since the production of synthetic lignins is somewhat capricious.

Comparisons by NMR revealed some striking differences between the lignins derived from *(Z)*- and *(E)*-coniferyl alcohol. Firstly, the sidechains of coniferyl alcohol end-units **X** (which retained the original unsaturated sidechain) strongly conserved the *(Z)*-geometry, despite being the products of hydroxycinnamyl alcohol radicals. Equilibration of *(Z)*- and *(E)*-radicals by rotation about the α–β-bond was apparently minor. No *(Z)*-sidechains **Xb** are seen in the *(E)*-coniferyl alcohol lignin, but about 5% *(E)*-sidechains **Xa** result in the *(Z)*-coniferyl alcohol lignin. We assume that the ~5% *(E)*-sidechains **Xa** in the *(Z)*-coniferyl alcohol lignin arose from the ~2% residual *(E)*-coniferyl alcohol contaminant. Phenylcoumaran (8–5-coupled) structures **B** were markedly less prominent in the *(Z)*-coniferyl alcohol polymer, but retained the *trans*-ring stereochemistry.[20] Resinols **C** (8–8-coupled) were of the pinoresinol type (*S/R–R/S*) rather than the alternate *epi*-pinoresinol isomers.[21-23] β-Aryl ether structures **A** had approximately the same *threo:erythro* ratio. α,β-Diaryl ethers **E**, structures scarcely found in isolated lignins[24] but routinely found in synthetic lignins,[11,25] were at similar levels. For unknown reasons, 5–5-coupled units and, in particular, the dibenzodioxocins[26-28] **D** were markedly less significant in the *(Z)*-coniferyl alcohol polymer.



Scheme 1. Synthetic scheme for *(Z)*-coniferyl alcohol **1**. Reagents and conditions: a: Acetic anhydride, pyridine, r.t.; b: bis(2,2,2-trifluoroethyl) (methoxycarbonylmethyl)phosphonate, 18-crown-6, $KN(TMS)_2$, THF, -78 °C; c: DIBAL, toluene, 0 °C; d: peroxidase, $H_2O_2$.

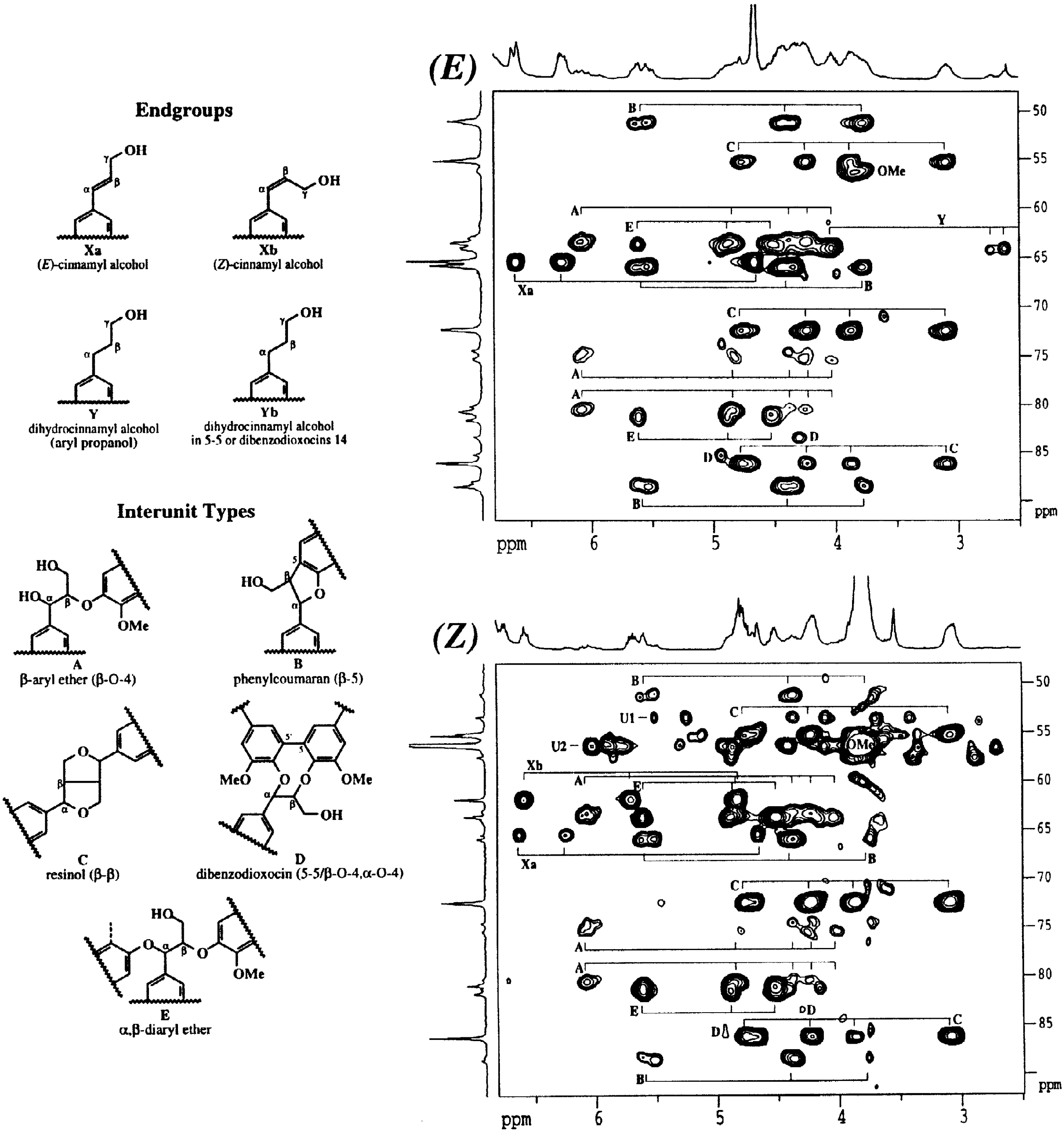


Figure 1. Sidechain region of 2D HMQC-TOCSY[29] spectra of acetylated synthetic lignins prepared from *(E)*- and *(Z)*-coniferyl alcohols. Such spectra correlate a carbon with its attached proton and all protons within the same coupling network as that attached proton. They consequently give redundant information which is particularly valuable is tracing out lignin substructures. Lignin units **A**-**E** (shown in their unacetylated forms) are identified, along with coniferyl alcohol endgroups **Xa**, the *(E)*-cinnamyl alcohol sidechain, and **Xb**, this *(Z)*-sidechain. Contours at carbon frequencies labeled U1 and U2 are currently unassigned. The small amount of dihydroconiferyl alcohol endgroups **Y** in the *(E)*-coniferyl alcohol lignin are from a 2% dihydroconiferyl alcohol impurity in the monomer. The *(E)*-cinnamyl alcohol endgroups **Xa** (~5% of the **Xb** contours) in the *(Z)*-coniferyl alcohol polymer are presumed to arise from the 2% *(E)*-coniferyl alcohol that was in that monomer. A clearer color version of this figure is available from: *http://www.dfrc.wisc.edu/publications/fullpapers.*

The most striking differences regrettably remain unexplained. Unknown structures labeled U1 and U2 (in the carbon domain) contain correlations with protons that should be revealing but are perplexing. U2 looks (in the proton dimension) to be a β-aryl ether **A** with characteristic α-, β-, and γ-protons, but the carbon shift (~56 ppm, under the methoxyl) is unknown. Similarly, for U1, a set of three correlations line up well (in the proton dimension) with phenylcoumarans **B** (β–5), but again the carbon shift (~53 ppm) is unexplained. The possibility that some of the correlation peaks might be attributed to noise from the intense methoxyl was ruled out by running the sample on an NMR instrument equipped with a gradient accessory — the artifact-free gradient HMQC-TOCSY experiment showed the same correlations. We hope that dimers and small oligomers produced from metal reactions or truncated polymerization might help us eventually identify these unusual components.

## CONCLUSIONS

As has been noted previously,[7] *(Z)*-coniferyl alcohol can polymerize via single-electron oxidation to produce a lignin-like polymer. In our case, this polymer was created in lower yield and with a lower molecular weight. All of the interunitary linkages are represented, and appear to be the same isomers that are traditionally observed in synthetic and natural lignins from *(E)*-coniferyl alcohol, although they are present in different proportions. The striking difference comes from the remaining unsaturated sidechains which arise from coupling of monomers at other than their β-positions. These sidechains retain the geometry of the parent coniferyl alcohol. However, such units are minor in real lignins where monomers seldom encounter other monomers. No lignin examined to date reveals authenticated detectable *(Z)*-sidechains. Unknown structures appear to have been produced from *(Z)*-coniferyl alcohol that have similarities to β–ether and β–5 products but unusual carbon shifts. Further work will be required to elucidate their structures and to determine if such structures are found in plant lignins.

## EXPERIMENTAL

### *General*

Reagents were all purchased from Aldrich Chemical Co. NMR spectra were taken on a Bruker AMX-360 instrument fitted with a 5 mm probe with normal geometry (proton coils furthest from the sample) using standard Bruker pulse programs. Samples were in 0.4 mL of acetone-$d_6$, with the central solvent peak as internal reference ($\delta_H$ 2.04, $\delta_C$ 29.80). The carbon/proton designations are based on the standard lignin numbering system (Figure 1). NMR assignments were authenticated by the usual complement of 1D and 2D NMR experiments. Melting points were on an Electrothermal digital m.p. apparatus and are uncorrected. Petroleum ether is the 40-60 °C boiling fraction. Molecular weight profiles of synthetic lignins were on Biobeads SX-1 (exclusion limit 14,000).

### *Methyl 4-O-acetyl-3-methoxy-(Z)-cinnamate 4*

A solution of bis(2,2,2-trifluoroethyl) (methoxycarbonylmethyl)phosphonate (3.3 g, 10.3 mmol), 18-crown-6 (10.9 g, 41.2 mmol) in anhydrous THF was cooled to -78 °C under nitrogen and treated with $KN(TMS)_2$ (20.6 mL, 0.5 M in toluene, 10.3 mmol). Compound **3** (2 g, 10.3 mmol, from acetylation of vanillin **2**) was then added and the resulting mixture was stirred for 30 min. at -78 °C. [Note: if the reaction is kept strictly at low temperature, the selectivity is >99% for the *(Z)*-isomer; if the temperature is allowed to rise during the addition, 5% or more of the *(E)*-isomer can result even at -60 °C]. Satd. $NH_4Cl$ solution was added and the product was extracted into ethyl ether (3 x 50 mL). The organic phase was dried over $MgSO_4$ and the solvents removed to give an oil that was used directly for the following reduction (2.34 g, 90%); $^1$H NMR δ: 2.24 (3H, s, OAc), 3.69 (3H, s, $CO_2CH_3$), 3.83 (3H, s, Ar $OCH_3$), 5.97 (1H, d, $J_{\beta\text{-}\alpha}$ = 12.9 Hz, β), 6.97 (1H, d, $J_{\alpha\text{-}\beta}$ = 12.9 Hz, α), 7.04 (1H, d, $J_{5\text{-}6}$ = 8.2 Hz, H-5), 7.23 (1H, dd, $J_{6\text{-}5}$ = 8.2 Hz, $J_{6\text{-}2}$ = 2.0 Hz, H-6), 7.68 (1H, d, $J_{2\text{-}6}$ = 2.0 Hz, H-2); $^{13}$C NMR δ: 20.5 ($ArOCOCH_3$), 51.6 ($COOCH_3$), 56.2 ($ArOCH_3$), 115.2 (2), 119.9 (β), 123.2 (6), 124.1 (5), 134.4 (1), 141.7 (4), 143.0 (α), 151.7 (3), 167.0 ($ArOCOCH_3$), 168.8 (γ).

### *(Z)-Coniferyl alcohol 1*

Compound **4** (910 mg, 3.64 mmol) in toluene, under nitrogen, was reduced with diisobutylaluminium hydride (24.2 mL, 1.5 M in toluene, 36.4 mmol) as described previously[17] to give a light yellow solid (573 mg, 87%). Crystallization from $CH_2Cl_2$/pet. ether gave **1** as pale yellow spherulites (512 mg, 78%), mp 106.6-107.3 °C with minor appearance change at ~97 °C (lit.[7,12] 104-106 °C); $^1$H NMR δ: 3.81 (1H, t, $J_{HO\text{-}\gamma}$ = 5.4 Hz, γ-OH), 3.84 (3H, s, $OCH_3$), 4.37 (2H, ddd, $J_{\gamma\text{-}\beta}$ = 6.2 Hz, $J_{\gamma\text{-}OH}$ = 5.4 Hz, $J_{\gamma\text{-}\alpha}$ = 1.8 Hz, γ's), 5.70 (1H, dt, $J_{\beta\text{-}\alpha}$ = 11.8 Hz, $J_{\beta\text{-}\gamma}$ = 6.2 Hz, β), 6.37 (1H, dt, $J_{\alpha\text{-}\beta}$ = 11.8 Hz, $J_{\alpha\text{-}\gamma}$ = 1.8 Hz, α), 6.72 (1H, dd, $J_{6\text{-}5}$ = 8.1 Hz, $J_{6\text{-}2}$ = 2.0 Hz, H-6), 6.79 (1H, d, $J_{5\text{-}6}$ = 8.1 Hz, H-5), 6.87 (1H, d, $J_{2\text{-}6}$ = 2.0 Hz, H-2), 7.60 (1H, s, ArOH); $^{13}$C NMR δ: 56.1 ($OCH_3$), 63.4 (γ), 113.2 (2), 115.6 (5), 122.8 (6), 129.7 (1), 130.2 (α), 131.4 (β), 146.7 (4), 147.9 (3) [Note that the α- and β-assignments are reversed from the *(E)*-isomer].

*Synthetic Lignin from (Z)-Coniferyl Alcohol*

First-crop crystals of **1** (443 mg, 2.46 mmol), containing ~2% *(E)*-isomer (from reaction to **4** before we were aware that low temperature must be strictly maintained) were dissolved in acetone (10 mL) and added with stirring to phosphate buffer (200 mL, 0.01 M, pH 6.5, degassed), containing horseradish peroxidase (4.4 mg, 770 units, EC 1.11.1.7, Type II). A second solution containing commercial hydrogen peroxide (286 μL of 30% solution, 2.52 mmol) was prepared in phosphate buffer (200 mL). The two solutions were simultaneously added with stirring, at room temperature, to phosphate buffer (100 mL) containing an aliquot (10 mL) of the coniferyl alcohol solution. The additions were accomplished using a double-channel Masterflex peristaltic pump at the rate of 8 mL/h. The reaction mixture was kept in the dark and, after additions were complete, was left stirring for ~70 h. The resulting pinkish suspension was then filtered through 0.2 μm nylon membrane and thoroughly washed with distilled water. The insoluble DHP polymer was transferred with distilled water and freeze dried to give an amorphous light-beige powder (155 mg, ~35%). The molecular weight was lower than for the *(E)*-coniferyl alcohol-derived lignin. Acetylation using acetic anhydride/pyridine (1:1), followed by vacuum evaporation of solvents, gave the acetylated DHP in essentially quantitative yield.

*Synthetic Lignin from (E)-Coniferyl Alcohol*

The *(E)*-coniferyl alcohol DHP utilized coniferyl alcohol labeled ($O^{12}C^2H_3$) to have the methoxyl group invisible in NMR prepared for another study.[13,14] That monomer was contaminated with ~2% dihydroconiferyl alcohol. The lignin preparation was otherwise similar, starting with 504 mg of coniferyl alcohol and yielding 268 mg of synthetic lignin (~53% yield).

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