

What Factors Control Dimerization of Coniferyl Alcohol?¹⁾

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Summary

Data suggest that the dimerization of coniferyl alcohol is not under thermodynamic control. In this study, molecular dynamics calculations were used to estimate the effect of the solvent environment. In water, the coniferyl alcohol radicals were forced to associate by the formation of a solvent cage. In glycerol, the solvent cage effect appeared to be absent. These results suggest that in water, the product distribution of the dimers will be modified by interactions with the solvent. The computed results are consistent with experimental observations of coniferyl alcohol reactions in various solvents.

Introduction

Since the foundational studies of Freudenberg (1964) coniferyl alcohol has become the building block of choice for the synthesis of lignin-like models. A justification for its use is the observation that coniferin, the coniferyl alcohol glycoside, can be used by higher plants to form lignin (Kratzl *et al.* 1957; Billek 1959; Freudenberg 1959; Terashima *et al.* 1993). By careful adjustment of reaction conditions, several researchers have synthesized polymers that exhibit many chemical and physical properties of natural lignin (Higuchi 1990). The fundamental difference between natural and synthetic lignin appears to be in the ratios of the various linkages (Lapierre 1993; Nimz 1995). Many factors that affect these ratios have been identified experimentally, including radical production rate, presence of carbohydrates, identity of the solvent, ionic strength, and pH. Since a large number of factors seem to have an impact on the course of the reaction, it appears that intrinsic properties of the molecules alone (for example, heat of formation, steric repulsion, electron distribution) are insufficient for determining the product distribution. To sort out the underlying molecular details of the coupling reactions, it is necessary to look beyond the reactants to the molecular environment. Specifically, where in the reaction path does the environment play a deciding role?

The following analysis is restricted to the initial dimerization of coniferyl alcohol radicals. Although understanding the formation of the polymer is the ultimate goal, the current focus is on determining the factors that control the formation of various dimeric products.

Previous Work

Figure 1 shows the generally accepted reaction scheme that leads to the formation of the major dimeric products from coniferyl alcohol. Each of these products is formed via an initial radical-radical coupling followed by either a

rearrangement or a nucleophilic addition of water. The nomenclature for these products is derived from the sites of initial radical-radical coupling. For example, the β - β dimer is formed by coupling between the β carbons of both molecules. A critical question is the relative rate and reversibility of the various reactions shown in Figure 1. For example, if the initial coupling reactions are reversible and the subsequent rearrangements are slow, then one would expect the product distribution to be thermodynamically controlled. However, if the initial coupling is fast and irreversible, then the relative orientation of the two molecules during their initial encounter will determine the product distribution, and thus the reaction can be considered under kinetic control

Determining the relative rates in the reaction network shown in Figure 1 has been the subject of many experimental and theoretical studies. An early hypothesis was that the linkage ratios should be related to spin density at the various positions on the molecule. Resonance stabilization principles, which have been applied with some success to other aromatic systems, suggest that radical character should exist at the β position of the side chain, at the phenolic oxygen, and at the 1, 3, and 5 positions on the aromatic ring. The conclusion that spin density was the determining factor was reasonable because, as expected, the dominant linkages observed were the β - β , β -O-4, β -5, and 5-5 linkages. Reactions at the 1 and 3 positions on the ar-

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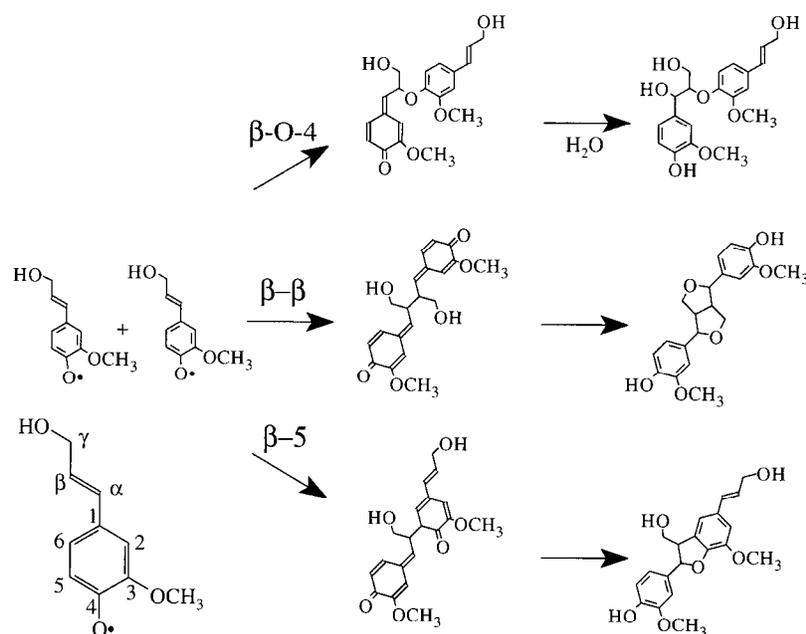


Fig. 1. Reaction network leading to major dimeric products of coniferyl alcohol. Insert shows typical numbering of various carbon atoms.

matic ring were eliminated due to steric hindrance (Martensson and Karlsson 1964). A more sophisticated analysis was used by Elder and Worley (1984) to test this hypothesis. They used semi-empirical methods to calculate the spin densities on all the atoms in the molecule. As expected, the atoms with the most unpaired spin density were the ones predicted by resonance stabilization, but the magnitude of the calculated densities did not correlate with the observed linkage ratios. Recently, Elder and Ede (1995) looked to thermodynamic control as an explanation of the experimental results. Since the coupling reactions must pass through o-complexes, these researchers calculated the heat of formation of both the o-complexes and the final rearranged dilignols. From their calculated heats of formation, one can estimate the relative concentration of the various species if the population follows a Boltzmann distribution. The results of this analysis for 25 °C are shown in Table 1.

The semi-empirical calculations of Elder and Ede (1995) suggest that the β -5 dilignol is more stable than any of the other products by 12 kcal mole⁻¹, so it is predicted to be the only product observed at room temperature. Since all five products have been identified in aqueous reaction mixtures (Tanahashi *et al.* 1976), the heats of formation of the final dilignols do not determine the product distribution. Fur-

thermore, since the heats of formation of the dilignols do not relate to the observed product distribution, it is unlikely that the rearrangements or nucleophilic additions shown in Figure 1 are reversible. The calculated ratios from the σ -complexes are more similar to the experimentally observed product distribution, but these ratios are still incorrect. The typical value for the formation of β -O-4 in water at room temperature is 20 %, the value for β - β is 30%, and the value for β -5 is 50 % (Tanahashi *et al.* 1976). This simple analysis, however, leaves open the question of the reversibility of the initial coupling reaction.

Fortunately, the calculations of Elder and Ede (1995) provide the data required to estimate the activation energy of the homolytic bond cleavage of the initial coupling product of two coniferyl alcohol radicals. From this activation energy, one can estimate the likelihood that the initial coupling reaction is reversible. Since radical-radical coupling reactions exhibit very low activation energies, the heats of formation can be equated to the activation energy of the reverse reactions. In an unrelated study, homolytic bond cleavage reactions yielding two aryl radicals with an enthalpy exceeding 20 kcal mole⁻¹ appeared to be irreversible for temperatures below 50°C (Malhoney and Weiner 1972). Since the initial coniferyl alcohol coupling reactions are exothermic by greater than 65 kcal mole⁻¹ (Elder and Ede 1995), a kinetic model is required to explain the dimeric product distribution.

In this paper, kinetic arguments are used to explain the product distribution of the dimerization of coniferyl alcohol. If initial radical-radical coupling is assumed to be rapid and irreversible, what linkage ratios can be predicted for the dilignol products? Specifically, molecular dynamics were used to test the hypothesis of kinetic control. Following the association of coniferyl alcohol radicals in aqueous solution allowed the associations of these molecules to be described and their reaction products to be predicted.

Table 1. Prediction of product distribution at 25 °C^a

Linkage	o-complex	dilignol
β - β	60.9 %	0 %
β -5	37.5 %	100%
β -O-4	1.5%	0 %
5-5	< 0.004%	0 %
5-O-4	< 0.0006%	0 %

^aBased on Elder and Ede (1995).

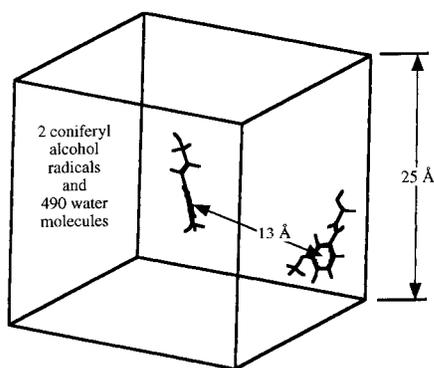


Fig. 2. Starting configuration of MD calculations. Coniferyl alcohol radicals were initially 13 Å apart.

Computational Methods

All molecular dynamics (MD) calculations were done using INSIGHTIII v 2.3 and DISCOVER v 2.95, distributed by Biosym Technologies Inc., San Diego, CA. A general purpose force field, CVFF, was used for all calculations. Partial charges for the atoms of the coniferyl alcohol radicals were adapted from a Mulliken analysis of the orbitals calculated with MOPAC 6.0 using the UHF-AM1 Hamiltonian. The greatest changes in the charges were on the phenolic oxygen atoms as a result of removal of the hydrogen atom. These charges were scaled to be consistent with the CVFF force field. Unfortunately the forces associated with unpaired spin density cannot be treated with this formalism. The partial charges for the water and the glycerol were set at the default values included in the force field parameterization. The calculations were done using the minimum image convention for the periodic boundary conditions. The computational box was cubic with a side dimension of 25 Å. The computational box and the starting configuration are shown in Figure 2. For the calculation with water, 490 water molecules were included to represent the solvent. For this box dimension and number of water molecules, the density was 0.97 g cm⁻³. For the calculation with glycerol, 112 glycerol molecules were included, resulting in a density of 1.25 g cm⁻³. Since electrostatic charges are treated using group contributions, a cutoff of 12.5 Å was used to reduce the amount of calculation without a significant loss in accuracy. The dielectric scaling factor was set to 1.0. All calculations were done in the canonical ensemble: constant number of molecules, volume, and temperature. The temperature for all calculations was 300 K. The calculations were performed on either a Silicon Graphic Iris (SGI) Indigo XS24 R4000 or the National Energy Research Supercomputer Center Cray C90. Calculation of a 600 ps trajectory for water required 288 CPU hours on the SGI or 27 CPU hours on the Cray C90. And the 2600 ps trajectory for glycerol required 1250 CPU hours on the SGI.

Results

Although MD calculations cannot be used to follow the electronic details of a chemical reaction, they can provide insight into the molecular associations that may precede the reactions. In the case of rapid and irreversible reactions, the relative orientation of the two molecules allowed by the solvent environment may completely determine the product distribution. The electrostatic and steric forces that are responsible for the relative orientation of the two reactants are likely well-described by the simple force fields used in MD, and thus MD studies should lead to insight into the kinetic factors that influence the dimerization product distribution.

Simulations in water

The MD calculations for the two coniferyl alcohol radicals in water showed that pure water is likely a poor solvent for these reactants. During the course of two separate simulations (600 ps and 300 ps), the coniferyl alcohol radicals became closely associated. A typical associated structure is illustrated in Figure 3. For long periods (50 ps) during the simulation, the phenyl rings of the two molecules were oriented with the planes of the phenyl rings parallel to each other. This association was also indicated in a plot of the distances between the center of masses (COM) of the two reactants. The COM distance as a function of time is shown in Figure 4. In this figure, the results of two separate simulations are shown on the same axis. During one simulation, the two molecules became associated after only 100 ps. During the other simulation, the two molecules briefly associated at 100 ps and then drifted apart, only to associate again at 300 ps. In both cases, once the COM of the two molecules came within 5 Å of each other, the association continued for the duration of the simulation. The irreversibility of the association, on the time scale of the simulation, may indicate that the forces holding the molecules together are relatively strong.

To quantify the energy change upon association, the 600-ps simulation results were divided into two ensembles: one set of configurations with the COM of coniferyl alcohol radicals closer than 6 Å and the other with the radicals more than 6 Å apart. Comparison of the average potential energies of the two ensembles revealed that the ensemble with the closely associated radicals was lower in energy by

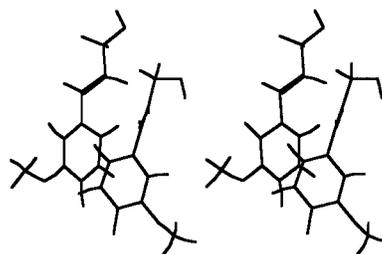


Fig. 3. Stereo image of typical associated complex in water.

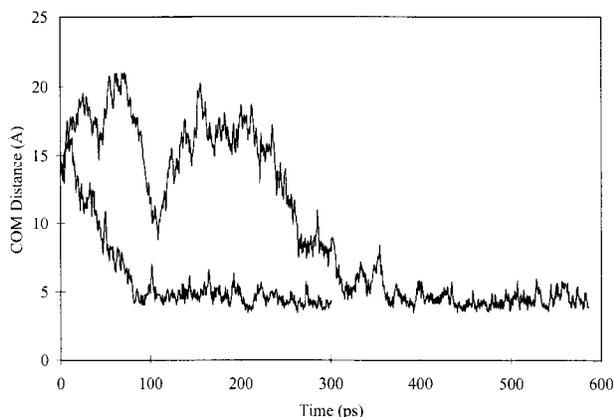


Fig. 4. Plot of distance between center of masses of two radicals during MD simulation in water.

14.2 kcal mole⁻¹. A large part of this energy appeared to be the result of improved hydrogen bonding in the water. Another indication of association can often be found in the tumbling rate of the molecules. Tumbling rate can be estimated from the decay of the vector autocorrelation function (Allen and Tildesley 1987). The molecular vector connecting the 1 and 4 carbons of the phenyl rings of the radicals is an indicator of the orientation of the molecule. An exponential fit to the autocorrelation function calculated from those configurations with the COM closer than 6 Å showed a decay time of 17.6 ps, whereas those configurations further than 6 Å apart had a decay time of 14 ps. Since this difference is not statistically significant at the $p = 0.05$ level, the calculation suggests that the molecules are not strongly bonded to each other; instead, they are held near to each other by the solvent cage.

The analysis of the MD simulations in water suggests that coniferyl alcohol radicals are restricted in their motions by the solvent. The impact of this restriction on the radical-radical coupling reaction is difficult to determine. Intuitively, the reaction products that are most similar to the associated structure (Fig. 3) will be favored. This argument suggests that β - β and β -5 linkages should be most common in poor solvents. To test this hypothesis, the simulation results were further analyzed. For a reaction to occur, the carbon centers with unpaired spin density must move within 4 Å of each other. Thus, the simulation can be analyzed by counting the number of times each carbon of interest is within 4 Å of the reactive site on the other radical. Although this simple method of analyzing the results ignores the effect of relative orientation, it provides a reasonable estimate of the relative occurrence rate of the various linkages allowed by the solvent environment. Occurrence of the three most often observed linkages is shown in Table 2. For reference, this table includes an experimental result and a prediction based on completely random coupling. The random coupling results were predicted using simple statistical arguments. The β - β linkage is less likely because only four relative orientations of the two approaching radicals can form this product, whereas the β -5 and β -O-4 linkages can each be formed from eight possible relative orientations.

The data presented in Table 2 show that completely random coupling does not match the experimentally determined product distribution. Furthermore, analysis of the MD simulation suggests that the association imposed by the molecular environment may affect the fate of the lignols.

Simulations in other solvents

The results of the simulations in water pose a critical question. If the reaction occurs in a "good" solvent for coniferyl alcohol radicals, does the product distribution approach the values suggested for completely random coupling? This question was answered experimentally by Chioccaro *et al.* (1993) and Terashima and Atalla (1995). Chioccaro *et al.* (1993) observed that the amount of β -O-4 dimers formed during the dimerization of coniferyl alcohol increased as the amount of methanol in the solvent increased. Unfortunately, the results of this study are com-

Table 2. Prediction of linkage ratios

Linkage	Simulation (water)	Experiment (water)	Random
β - β	31%	27%	20%
β -5	56%	54%	40%
β -O-4	13%	19%	40%

plicated by the fact that methanol participates in the nucleophilic addition to give a different final dimer than the product observed in water. To avoid this complication Terashima and Atalla (1995) measured product distributions from the dimerization of coniferyl alcohol in various water/diglyme mixtures. Diglyme, a polyether, cannot participate in the nucleophilic addition. Figure 5 shows the product distributions for various water/diglyme mixtures. The results from the water MD calculation and the random coupling prediction are included for comparison. A small addition of diglyme (20%) dramatically increased the production of β -O-4 dimers at the expense of the β -5 and β - β dimers. These shifts in the product distribution brought the experimental observation into closer accord with the prediction based on random coupling. Similar effects were observed for other water/dioxane and water/glycerol mixtures (Terashima and Atalla 1995).

To test the hypothesis that using better solvents in dimerization reactions for coniferyl alcohol radicals results in random coupling, MD simulations of coniferyl alcohol radicals were undertaken in glycerol. Starting configurations for the glycerol simulation were similar to the configuration shown in Figure 2. One significant difference between the simulations in water and glycerol is that the rate of motion was reduced in glycerol. The tumbling time constant increased from 13 ps in water to 44 ps in glycerol. Since the viscosity of glycerol is significantly higher than that of water, a slowing of motion was expected. A necessary implication of this result is that any simulation of glycerol must be continued four to five times longer than an equivalent simulation in water. Thus, the glycerol simulation was continued for 2600 ps. Unfortunately, the coniferyl alcohol radicals did not associate over the course of this simulation.

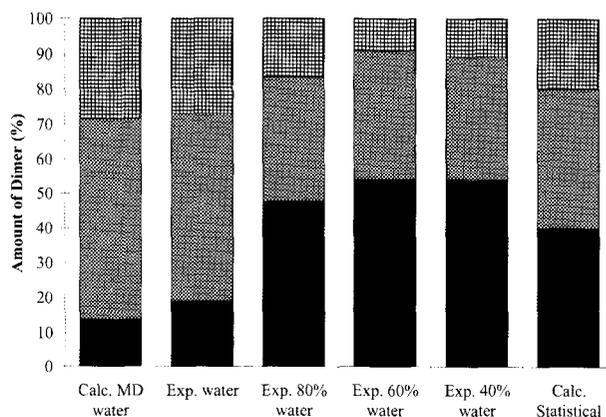


Fig. 5. Product distribution in various solvent mixtures. Symbols: Cross-hatched, β - β ; clear, β -5; shaded, β -O-4.

The radicals did make contact a few times, but they did not spontaneously associate, as in the case of the water simulation. Since only very brief encounters were observed during the course of the simulation, no statistically significant conclusions about the predicted linkage ratios can be drawn from this simulation. On a less quantitative level, however, this simulation does support the conclusion that "good" solvents for coniferyl alcohol may result in linkage ratios more similar to those predicted by random coupling.

Discussion

The MD results, although inconclusive, suggest a mechanism by which solvent environment determines the product distribution of radical-radical coupling reactions. Specifically, coniferyl alcohol radicals are not free to adopt all possible relative orientations in water. This restriction of motion translates to a modification of the product distribution since the outcome is likely determined by the relative orientation of the molecules at the moment the orbitals of two centers of unpaired spin density overlap. In contrast, the relative orientations of the molecules appear to be more free in glycerol, and thus the product distribution is more similar to the distribution predicted from a statistical analysis of the probability of forming each product, assuming an equal likelihood of each relative orientation.

The results of this study offer an explanation for the experimental observation that the polymerization of cinnamyl alcohols in different solvents gives different product distributions (Tanahashi *et al.* 1976). The implications of these results are less clear for understanding wood tissue biosynthesis. These calculations, on a simplified system, have ignored a wide variety of other components present during lignin biosynthesis, such as cellulose, non-cellulosic carbohydrates, enzymes, and ions. For example, previous work showed that coniferyl alcohol monomers associate with model cellulose surfaces (Houtman and Atalla 1995). Furthermore, Davin *et al.* (1997) demonstrated that the dimerization of coniferyl alcohol radicals is stereo-controlled by an auxiliary protein. However, these results do imply that if a nascent cell controlled the environment in which the lignol polymerization occurs, it could likely control the primary structure of the lignin polymer.

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