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Coupling and Reactions of 5-Hydroxyconiferyl Alcohol in Lignin Formation

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ABSTRACT: The catechol alcohols, caffeyl and 5-hydroxyconiferyl alcohol, may be incorporated into lignin either naturally or through genetic manipulation. Due to the presence of *o*-OH groups, these compounds form benzodioxanes, a departure from the interunit connections found in lignins derived from the cinnamyl alcohols. In nature, lignins composed of caffeyl and 5-hydroxyconiferyl alcohol are linear homopolymers and, as such, may have properties that make them amenable for use in value-added products, such as lignin-based carbon fibers. In the current work, results from density functional theory calculations for the reactions of 5-hydroxyconiferyl alcohol, taking stereochemistry into account, are reported. Dehydrogenation and quinone methide formation are found to be thermodynamically favored for 5-hydroxyconiferyl alcohol, over coniferyl alcohol. The comparative energetics of the rearomatization reactions suggest that the formation of the benzodioxane linkage is under kinetic control. Ring-opening reactions of the benzodioxane groups show that the bond dissociation enthalpy of the *α-O* cleavage reaction is lower than that of the *β-O* reaction. The catechol lignins represent a novel form of the polymer that may offer new opportunities for bioproducts and genetic targets.

KEYWORDS: *5-hydroxyconiferyl alcohol, coniferyl alcohol, lignin, benzodioxane, stereochemistry*

■ INTRODUTION

Conventionally, lignin is defined as a polymer of the cinnamyl alcohols (coniferyl, sinapyl, and *p*-coumaryl alcohol).[1] Over the recent past, however, lignin has been found to exhibit considerable plasticity with respect to its composition. Numerous monomers other than the cinnamyl alcohols have been found naturally and have been introduced through genetic manipulation.[2] Among these are the catechol monolignols, caffeyl alcohol and 5-hydroxyconiferyl alcohol. The properties and potential uses of lignins containing such monomers have recently been described by Nar et al.,[3] who successfully produced carbon fibers based on caffeyl alcohol lignin, the structure of which is described as linear and homopolymeric.[4] The novel structures and properties exhibited by such lignins may present considerable potential for genetic manipulation and the generation of high-value downstream products.

The bulk of the initial work on these compounds was concerned with genetic manipulation by downregulation of the caffeic acid *O*-methyltransferase enzyme, which catalyzes the conversion of 5-hydroxyconiferyl alcohol to sinapyl alcohol in angiosperms.[5−8] As a consequence, sinapyl alcohol production was diminished and 5-hydroxyconiferyl alcohol was incorporated into the lignin polymer. In particular, Vanholme et al.[8] showed that such genetic manipulations resulted in *Arabidopsis* with lignins made up of ~70% 5-hydroxyconiferyl alcohol. Analogous work on softwoods, depressing caffeoyl CoA 3-*O*-methyltransferase, interrupted the conversion of caffeoyl-CoA to feruloyl-CoA, resulting in the incorporation of caffeyl alcohol into the lignin.[9] In the recent literature, lignins composed of homopolymeric caffeyl alcohol and 5-hydroxyconiferyl alcohol have been found to occur naturally.[4,10]

The reactions that account for the incorporation/polymerization of 5-hydroxyconiferyl alcohol are initiated through an enzymatic dehydrogenation of the monomers (Figure 1), coupling to form a quinone methide (Figure 2), and a rearomatization step[11] (Figure 3). With the addition of an *o*-OH group, however, the rearomatization of the catechol-based quinone methides can proceed either through internal trapping

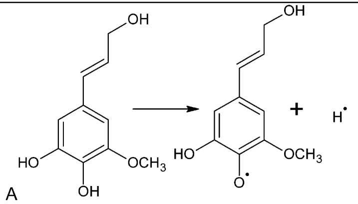
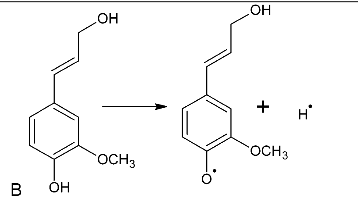
Reaction	Enthalpy of reaction (298 K) kcal/mol
	81.79
	84.11

Figure 1. Dehydrogenation reactions of (A) 5-hydroxyconiferyl alcohol and (B) coniferyl alcohol.

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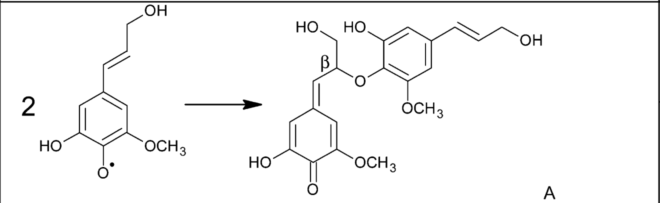
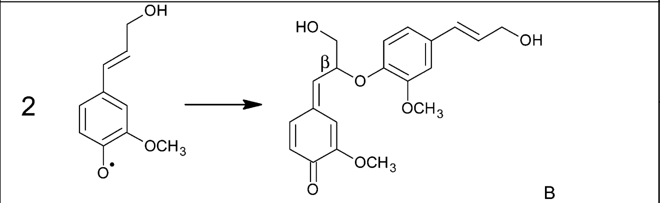
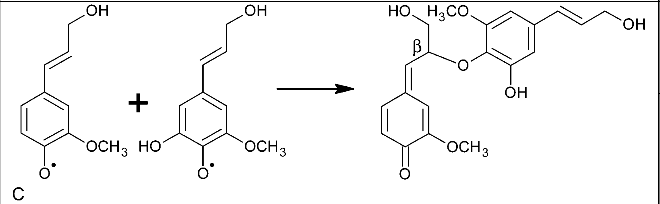
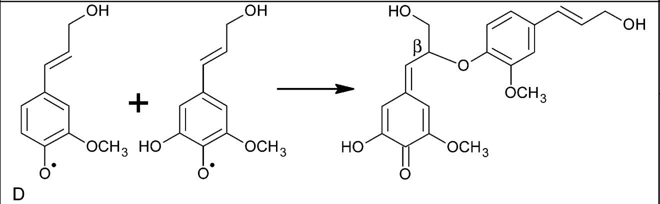
Reaction	product chirality *relative enthalpy* kcal/mol	Enthalpy of reaction (298 K) kcal/mol
	β*R* *0.00*	-46.37
	β*S* *0.42*	-46.79
	β*R* *0.00*	-36.65
	β*S* *0.00*	-36.65
	β*R* *0.26*	-43.32
	β*S* *0.00*	-43.58
	β*R* *0.62*	-43.17
	β*S* *0.00*	-43.79

Figure 2. Quinone methide formation of (A) self-coupled 5-hydroxyconiferyl alcohol, (B) self-coupled coniferyl alcohol, (C) cross-coupling through C-*β* of coniferyl alcohol, and (D) cross-coupling through C-*β* of 5-hydroxyconiferyl alcohol. Relative enthalpy values are based on the lowest energy enantiomer for each quinone methide product.

to form a benzodioxane ring or more conventionally by the nucleophilic addition of water. If the quinone methide is formed by phenoxy attack of the 5-hydroxyconiferyl alcohol radical on the *β*-carbon of either coniferyl alcohol or another 5-hydroxyconiferyl alcohol radical, the internal trapping reaction is proposed to occur. Alternatively if the phenoxy coniferyl alcohol radical reacts with the *β*-carbon of 5-hydroxyconiferyl alcohol, nucleophilic addition of water results, forming the more commonly observed acyclic *β*-*O*-4 linkage.[4]

In the current work, the energetics of self-coupling and cross-coupling of 5-hydroxyconiferyl and coniferyl alcohol are examined using computational chemical methods to determine if there are thermodynamic differences that would favor one reaction over the other. Such calculations have been applied to the more conventional lignin monomers by numerous authors. In previous work, the coupling of monolignols and initial steps in the polymerization of lignin have been examined using density functional theory by Sangha and co-workers.[12,13] Bond dissociation energetics of dilignols have been reported in two large studies,[14,15] and the energetics and mechanisms of pyrolysis reactions have been extensively examined computationally.[16−30] There have also been recent reports on the use of modeling of lignin reactions under acidic conditions.[31,32]

■ MATERIALS AND METHODS

The structures and reactions addressed in the current work are representative of self-coupling and cross-coupling of coniferyl alcohol and 5-hydroxy coniferyl alcohol. The first steps in the process are taken to be the dehydrogenation (Figure 1) and quinone methide formation (Figure 2). Upon quinone methide formation, a chiral center is generated at the *β*-carbon, and both enantiomers were evaluated for each structure.

Next, the rearomatization reactions were considered as shown in Figure 3. Benzodioxanes are formed by self-coupling of 5-hydroxyconiferyl alcohol and cross-coupling with coniferyl alcohol through the *β*-carbon of the latter (Figure 3A,B). The more conventional acyclic *β*-*O*-4 linkages were formed through self-coupling of coniferyl alcohol (Figure 3C), cross-coupling with 5-hydroxyconiferyl alcohol through its *β*-carbon (Figure 3D), and the nucleophilic addition of water. These reactions result in an additional chiral center, such that there are four stereoisomers (*RR*, *SS*, *RS*, and *SR*) with calculations done for each.

Upon dimer formation, the next step in the polymerization process is oxidation by an activated monolignol, according to the mechanism proposed by Hatfield and Vermerris,[33] as shown in Figure 4. For the purposes of the current work, the oxidant is assumed to be the coniferyl alcohol radical. As before, all stereoisomeric forms of the oxidized dilignols are considered.

Lastly, the ring-opening reactions of the benzodioxane products (Figure 5) and the homolytic *β*-*O* cleavage reactions for the acyclic *β*-*O*-4 dimers are reported (Figure 6). Such reactions have been proposed to occur with the thermal degradation of lignin,[23,27,28] which may also be of importance in the formation of carbon fibers from lignin. The products of these reactions were modeled as triplets in accordance with previous calculations.[23,27,28]

It can be seen that the products of these reactions can be geometrically quite flexible, with numerous rotational degrees of

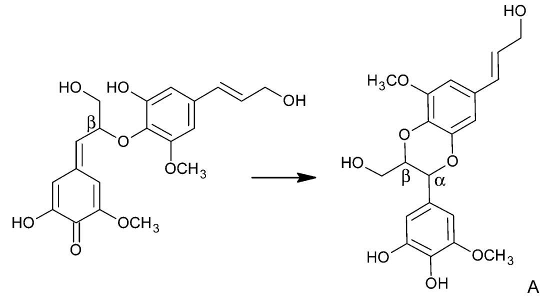
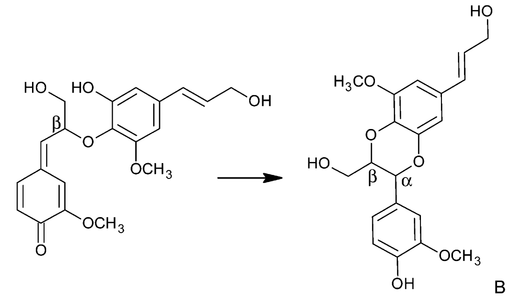
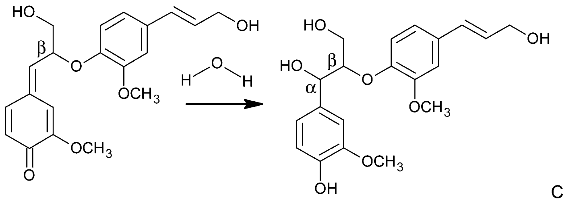
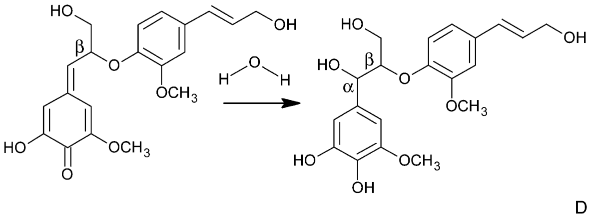
Reaction	reactant chirality	product chirality *relative enthalpy* kcal/mol	Enthaply of reaction (298 K) kcal/mol
	βS	α*R*,β*R* *threo* *1.99*	-18.58
		α*S*,β*R* *erythro* *0.00*	-20.57
	βR	α*S*,β*S* *threo* *1.21*	-18.94
		α*R*,β*S* *erythro* *0.21*	-19.94
	βS	α*R*,β*R* *threo* *1.11*	-19.47
		α*S*,β*R* *erythro* *0.00*	-20.59
	βR	α*S*,β*S* *threo* *1.11*	-19.73
		α*R*,β*S* *0.00*	-20.84
	βS	α*R*,β*R* *threo* *1.81*	-32.65
		α*S*,β*R* *erythro* *3.64*	-30.83
	βR	α*S*,β*S* *threo* *0.00*	-34.46
		α*R*,β*S* *erythro* *4.66*	-29.80
	βS	α*R*,β*R* *threo* *1.05*	-27.08
		α*S*,β*R* *erythro* *4.59*	-23.51
	βR	α*S*,β*S* *threo* *0.00*	-28.57
		α*R*,β*S* *erythro* *3.03*	-25.53

Figure 3. Rearomatization reaction for (A) self-coupled 5-hydroxyconiferyl alcohol benzodioxane, (B) cross-coupled 5-hydroxyconiferyl alcohol and coniferyl alcohol benzodioxane, (C) self-coupled coniferyl alcohol acyclic *β-O*-4, and (D) cross-coupled 5-hydroxyconiferyl alcohol and coniferyl alcohol acyclic *β-O*-4. Relative enthalpy values are based on the lowest energy stereoisomer for each product.

freedom. As such, conformational searching was done for each by the application of a 500 step Monte Carlo search with PM3 optimization, as implemented in Spartan. The lowest 10 conformations from the Monte Carlo search were optimized with M06-2X/6-31+G(d) and the ultrafine integration grid, consisting of 99 radial shells and 590 angular points per shell, using Gaussian09, revision D.01. The lowest energy conformation among this group was selected and further optimized at the M06-2X/6-311++G(d,p) level, again with the ultrafine grid and frequency calculation to confirm the identification of a stationary point and for the determination of enthalpies of reaction at 298 K.

In addition, the ring-opened triplets were modeled by simply deleting the appropriate bond and increasing the interatomic distance to 2.5 Å for the low-energy conformer from the initial density functional theory optimizations. This was followed by M06-2X/6-311++G(d,p) optimization and frequency calculation as before. While a conformational search will more effectively explore the potential energy surface, this approach may be of use in evaluating the initial nature of the reactant.[27,28]

The density functional calculations were done using the resources of the Texas Advanced Computer Center (TACC) and the Alabama Supercomputer Authority.

RESULTS AND DISCUSSION

Figure 1 shows the enthalpies associated with the initial dehydrogenation reaction of both 5-hydroxyconiferyl alcohol and coniferyl alcohol. The reaction is endothermic, with the

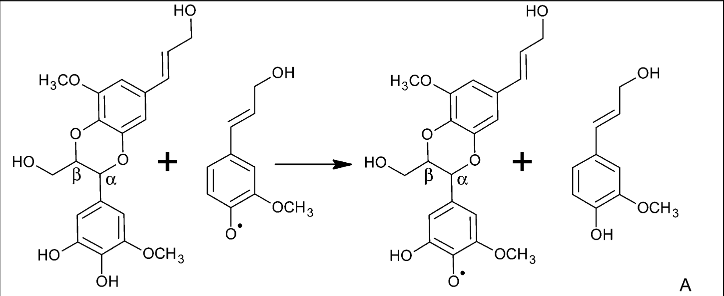
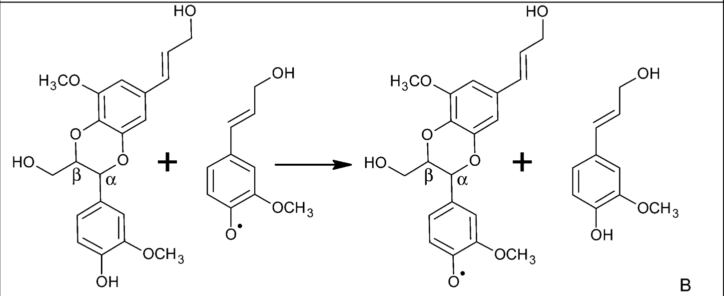
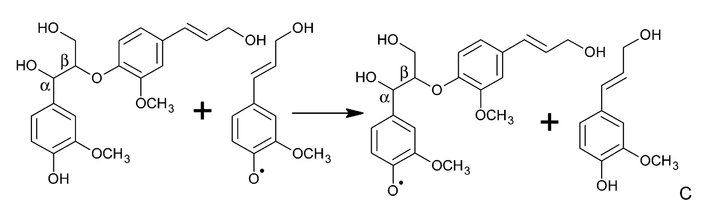
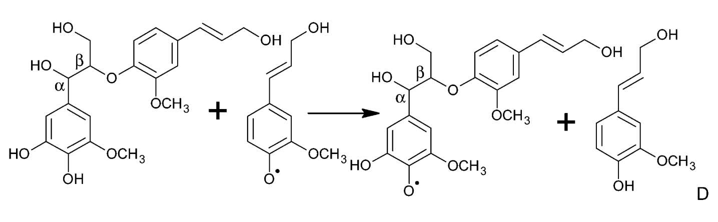
reaction	product chirality *relative enthalpy* kcal/mol	Enthalpy of reaction (298 K) kcal/mol
	α*S*,β*R* *erythro* *0.14*	-3.02
	α*S*,β*S* *threo* *0.63*	-3.75
	α*R*,β*R* *threo* *0.46*	-4.69
	α*R*,β*S* *erythro* *0.00*	-3.38
	α*S*,β*R* *erythro* *0.00*	3.45
	α*S*,β*S* *threo* *0.79*	3.12
	α*R*,β*R* *threo* *0.92*	3.26
	α*R*,β*S* *erythro* *0.00*	3.45
	α*S*,β*R* *erythro* *9.35*	6.46
	α*S*,β*S* *threo* *0.24*	-0.89
	α*R*,β*R* *threo* *0.00*	-2.95
	α*R*,β*S* *erythro* *8.96*	3.16
	α*S*,β*R* *erythro* *8.99*	-5.39
	α*S*,β*S* *threo* *0.00*	-9.79
	α*R*,β*R* *threo* *1.43*	-9.41
	α*R*,β*S* *erythro* *9.39*	-3.42

Figure 4. Hydrogen abstraction by coniferyl alcohol radical from (A) self-coupled 5-hydroxyconiferyl alcohol benzodioxane, (B) cross-coupled 5-hydroxyconiferyl alcohol and coniferyl alcohol benzodioxane, (C) self-coupled coniferyl alcohol acyclic *β*-*O*-4, and (D) cross-coupled 5-hydroxyconiferyl alcohol and coniferyl alcohol acyclic *β*-*O*-4. Relative enthalpy values are based on the lowest energy stereoisomer for each phenoxy radical product.

former being less endothermic than the latter. As such the formation of the 5-hydroxyconiferyl radical would be thermodynamically preferred when both monolignols are present and subjected to enzymatic oxidation. This observation notwithstanding, the importance and potential control of kinetics, enzymatic binding, and possibly radical transfer between open-shell and closed-shell monolignols, as reported for coniferyl and sinapyl alcohols,[11] on this process is not, and cannot be, discounted.

The enthalpies of the exothermic coupling reactions of the radicals to form quinone methides and the relative enthalpies of each enantiomer are shown in Figure 2. The enantiomers of a given quinone methide exhibit very similar but not the identical thermodynamic values that would be expected of mirror images, with errors ranging from 0.00 to 0.62 kcal/mol. This may be accounted for by slight conformational differences between the enantiomeric structures, which are dependent on the conformational search identifying identical internal dihedral angles. Furthermore, the accuracy of density functional calculations has been reported to be ~0.75 kcal/mol,[34]while "chemical accuracy" is defined by Lewars[35] as ±2.4 kcal/mol (±10 kJ/mol), such that the current results are well within these error ranges, and the enantiomers can be considered to be thermodynamically indistinguishable. The self-coupling of 5-hydroxyconiferyl alcohol (Figure 2A) is ~10 kcal/mol more exothermic than the self-coupling of coniferyl alcohol (Figure 2B). The cross-coupling reactions are similar to each other and

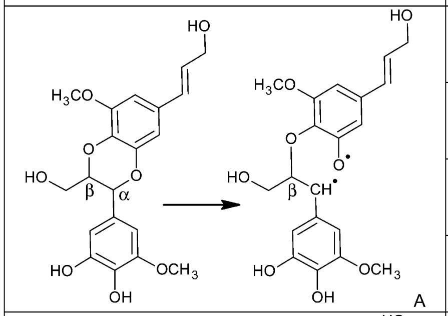
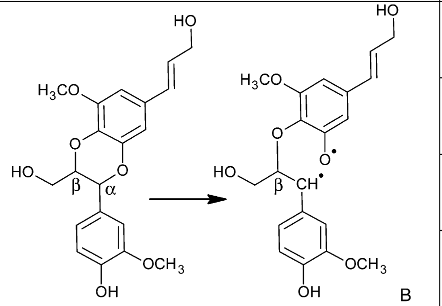
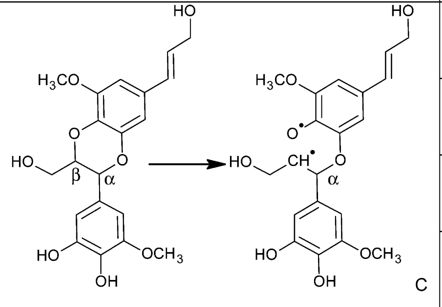
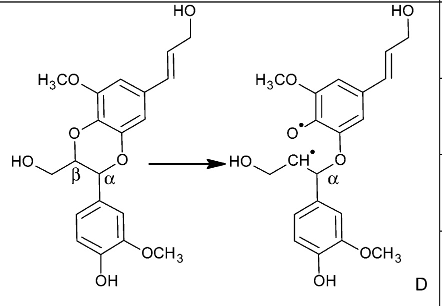
reaction	chirality		Enthalpy of reaction (298 K) kcal/mol
	reactant	product *relative enthalpy* kcal/mol	
	αR,βR *erythro*	β*S* *0.00 (0.00)*	50.50 (50.94)
	αS,βS *threo*	β*R* *0.57 (1.26)*	51.85 (52.97)
	αS,βR *erythro*	β*S*	52.49 (52.93)
	αR,βS *threo*	β*R*	52.84 (53.97)
	αR,βR *erythro*	β*S* *0.00 (0.00)*	50.55 (51.72)
	αS,βS *threo*	β*R* *0.85 (0.00)*	51.41 (51.72)
	αS,βR *erythro*	β*S*	51.67 (52.84)
	αR,βS *threo*	β*R*	52.53 (52.84)
	αR,βR *erythro*	α*S* *0.00 (0.30)*	53.76 (55.98)
	αS,βS *threo*	α*R* *0.00 (0.00)*	54.54 (56.46)
	αS,βR *erythro*	α*S*	55.75 (57.98)
	αR,βS *threo*	α*R*	55.54 (57.46)
	αR,βR *erythro*	α*S* *2.87 (0.00)*	57.81 (58.63)
	αS,βS *threo*	α*R* *0.00 (0.00)*	54.93 (58.63)
	αS,βR *erythro*	α*S*	58.92 (59.74)
	αR,βS *threo*	α*R*	56.05 (59.74)

Figure 5. Ring-opening reactions for (A) *α-O* cleavage of self-coupled 5-hydroxyconiferyl alcohol benzodioxane, (B) *α-O* cleavage of cross-coupled 5-hydroxyconiferyl alcohol and coniferyl alcohol benzodioxane, (C) *β-O* cleavage of self-coupled 5-hydroxyconiferyl alcohol benzodioxane, and (D) *β-O* cleavage of cross-coupled 5-hydroxyconiferyl alcohol and coniferyl alcohol benzodioxane. Values not in parentheses are from the conformational search, while values in parentheses are from ring opening followed by optimization. Relative enthalpy values are based on the lowest energy enantiomer for each product.

intermediate in exothermicity between the self-coupled reactions.

The rearomatization reactions are shown in Figure 3. All are exothermic, with the formation of the benzodioxane through internal trapping being less exothermic than the nucleophilic addition of water, resulting in the acyclic *β-O*-4. This energetic preference notwithstanding, there is ample experimental evidence for the presence of high levels of the benzodioxane,[7] suggesting that the reaction is under kinetic control, with the intramolecular reaction proceeding more rapidly than the bimolecular reaction required for acyclic *β-O*-4 formation. The difference in enthalpies of reaction between the benzodioxanes and acyclic *β-O*-4 dimers may be due to the greater flexibility of the latter, allowing for the assumption of lower energy conformations. For the benzodioxane products there are small (∼1 kcal/mol) differences in the enthalpies of reaction with stereochemistry, but in general the reactions forming the *erythro* products (*RS*/*SR*) are slightly more exothermic. Experimentally, it has been found that the *trans*(*threo*, *RR*/*SS*) form of the benzodioxane ring system is dominant, with low levels of the *cis*(*erythro*, *RS*/*SR*) detected.[5,10] If the reaction were under thermodynamic control, a marked preference for

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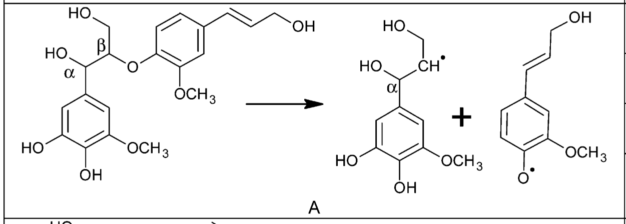
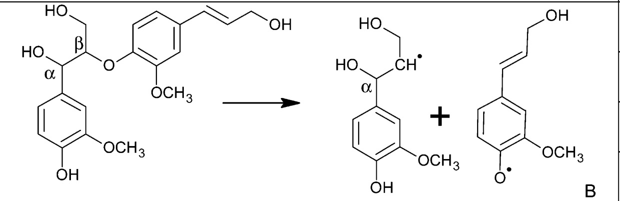
reaction	chirality		Enthalpy of reaction (298 K) kcal/mol
	reactant	product *relative enthalpy* kcal/mol	
	α*R*,β*R* *threo*	β*S* *0.00*	69.79
	α*S*,β*S* *threo*	β*R* *0.00*	70.84
	α*S*,β*R* *erythro*	β*S*	66.25
	α*R*,β*S* *erythro*	β*R*	67.81
	α*R*,β*R* *threo*	β*S* *0.40*	69.88
	α*S*,β*S* *threo*	β*R* *0.00*	71.30
	α*S*,β*R* *erythro*	β*S*	68.06
	α*R*,β*S* *erythro*	β*R*	66.63

Figure 6. Homolytic cleavage of *β-O* bond for (A) cross-coupled 5-hydroxyconiferyl alcohol and coniferyl alcohol and (B) self-coupled coniferyl alcohol. Relative enthalpy values are based on the lowest energy enantiomer for each product.

the *trans*(*threo*, *RR*/*SS*) would have been expected, rather than the small differences observed computationally. Based on these observations, it is apparent, therefore, that the rearomatization step is not under thermodynamic control. In contrast, among the acyclic *β-O*-4 dimers, the formation of the *threo* (*RR*/*SS*) products is more exothermic. This may be due to the markedly different geometries found between the stereoisomers, as shown in Figure 7, in which the *threo* dimers exhibit a severely folded structure with parallel aromatic rings, while the *erythro* dimers are more extended. In model studies, coupling and rearomatization of quinone methides from coniferyl alcohol result in a 50:50 ratio of stereoisomers, while a 75:25 *erythro*/*threo* ratio is found for sinapyl alcohol, with the rearomatization reaction proposed to be under kinetic control.[36,37] The relative enthalpies for the stereoisomers of each product are also shown, and as previously discussed for the quinone methides, the enthalpies of the enantiomeric dimers are not all identical. Among the benzodioxanes errors of 0.00−0.77 kcal/mol are found while the acyclic *β-O*-4 dimers differ by 1.02−1.81 kcal/mol, such that all values fall within the range of chemical accuracy. The higher values for the acyclic dimers could be due to their increased flexibility over the benzodioxanes.

Upon formation of the dimeric products, the next step in the polymerization is a second oxidation/dehydrogenation reaction. Monolignol radicals can be formed directly by the action of oxidative enzymes, and it is proposed that subsequent oxidations occur through a redox shuttle mechanism in which the activated monolignol abstracts a hydrogen from the end of the growing polymer to create a new radical center. This product next couples with a radical to form a quinone methide, followed by rearomatization in the "endwise" polymerization process.[33] In the current work, this is modeled as radical transfer between a coniferyl alcohol radical and the dimeric products. Interestingly, there is a recent literature report on the oxidation of model dimers in the presence of horseradish peroxidase and hydrogen peroxide, as shown by their consumption and formation of oligomers. This would indicate that dimers as well as monomers can be substrates for oxidative enzymes and participate in radical transfer leading to higher molecular weight products. This observation notwithstanding, coupling between dimers and monolignols is not precluded by the authors.[38] Based on this, the enthalpies of hydrogen abstraction from the benzodioxanes and acyclic *β-O*-4 dimers by the coniferyl alcohol radical are as shown in Figure 4. The reactions are mixed between exothermic and endothermic, but the differences between products and reactants are quite small. Abstraction from the self-coupled benzodioxanes (Figure 4A) is somewhat exothermic, while the cross-coupled products are endothermic (Figure 4B). The greater exothermicity of the hydrogen abstraction from the *threo* benzodioxane isomers may be partially due to the higher energies of the reactants, imparting a slight advantage. Oxidation of the coniferyl alcohol dimer (Figure 4C) exhibits very mixed results, but the reactions of the *threo* stereoisomers are exothermic, while *erytho* is endothermic. The corresponding cross-coupled reactions are all exothermic, with the *threo* reactants markedly more so at an average of −9.6 kcal/mol (Figure 4D). Figure 4 also shows the relative enthalpies of the stereoisomers of each product. The differences between enantiomers range from 0.00 to 1.43 kcal/mol, well within the range of chemical accuracy.

The bond dissociation energies of the ring-opening reactions are shown in Figure 5. The examination of these reactions is based on the proposed applications of the highly linear catechol alcohol lignins in carbon fibers, the processing of which occurs at elevated temperatures. Baker and Rials[39] have proposed that lignin structure, particularly the presence of syringyl groups in hardwood lignin, decreases the processing temperatures required for carbon-fiber production, through an increase in the free volume of the polymer. The linear nature of the catechol lignins under consideration here would result in an extended polymer, with perhaps even larger free volumes, and concomitant decrease in processing temperatures. Under such elevated temperatures homolytic cleavage reactions have been reported and investigated experimentally and computationally.[23,24,40] As would be expected, the triplet structures resulting from conformational searching have uniformly lower enthalpies of reaction than those from simple ring opening and optimization (these values are shown in parentheses), but the differences are quite small. Likewise, there are minimal energetic differences between the different stereochemical arrangements. Whether considering the benzodioxane homo- or heterodimers of 5-hydroxyconiferyl alcohol and coniferyl alcohol, the *α-O* cleavage reaction enthalpies lie consistently around an average of 51.7 kcal/mol (Figures 5A,B). In contrast,

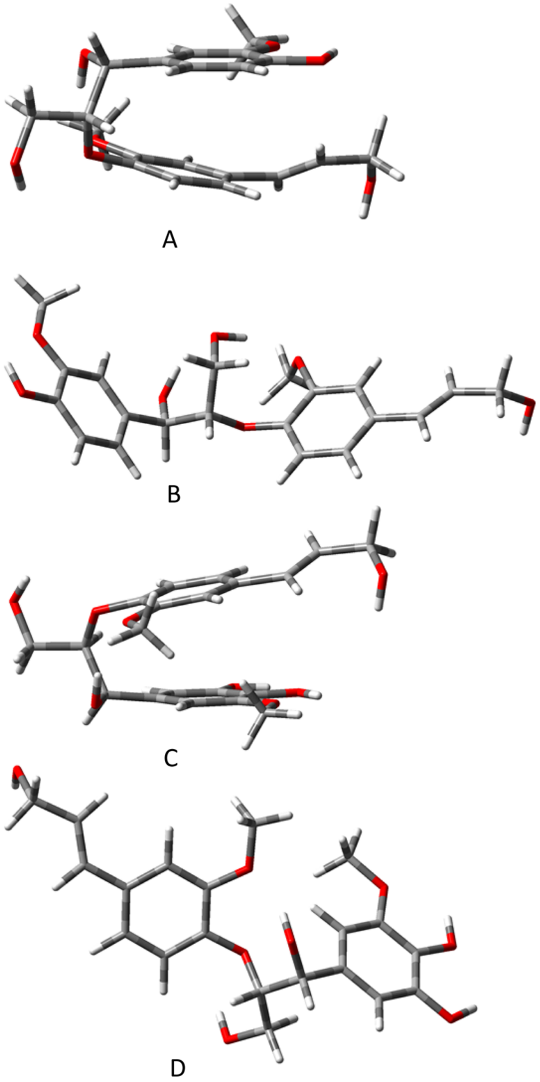


Figure 7. Structures of acyclic (A) *β-O*-4 self-coupled coniferyl alcohol (*threo-RR*), (B) *β-O*-4 self-coupled coniferyl alcohol (*erythro-RS*), (C) *β-O*-4 cross-coupled 5-hydroxyconiferyl alcohol-coniferyl alcohol (*threo-RR*), and (D) *β-O*-4 cross-coupled 5-hydroxyconiferyl alcohol-coniferyl alcohol (*erythro-RS*).

the cleavage of the *β-O* bond is more endothermic (Figures 5C,D), with even higher dissociation enthalpies for the cross-coupled dimers (Figure 5D). Furthermore, the differences in results obtained from either the conformational search or the ring-opened optimization approaches are greater for the *β-O* cleavage reactions, relative to the *α-O* case. This indicates that greater geometric changes are required for the *β-O* ring-opened systems to relax to a global minimum conformation. For comparison purposes, Younker et al.[23] report a bond dissociation enthalpy of ~50 kcal/mol for *α-O* cleavage in phenylcoumaran, while for a dibenzodioxocin model, the enthalpies of the *α-O* and *β-O* cleavage reactions were found to be ~46 and 57 kcal/mol, respectively.[28] The relative enthalpies of the enantiomeric products of these reactions are also as shown in Figure 5. As previously, the two values reported for each enantiomer represent the results from a conformational search and simple ring opening, followed by optimization (these values are in parentheses). All are within chemical accuracy except the *β-O* cleavage of the cross-coupled benzodioxane, resulting from the conformational search, in which the enantiomers differ by 2.87 kcal/mol.

Lastly, the cleavage of the acyclic *β-O*-4 dimers was examined (Figure 6). The enthalpies are similar, with the cross-coupled dimer being somewhat less endothermic than the self-coupled coniferyl alcohol dimer. It can also be seen than the reactions of the *threo* dimers are more endothermic, which would be due to the relative stability of the reactants. The relative enthalpies for the enantiomeric products are also shown in Figure 6, all of which are within chemical accuracy.

In summary, the current results show that the incorporation of 5-hydroxyconiferyl alcohol results in a considerable energetic preference for the coupling of radicals to form quinone methides, over self-coupling of the coniferyl alcohol radical. It was also found that the rearomatization reaction forming the acyclic *β-O*-4 dimers is more exothermic than the benzodioxane products. Given the extensive formation of the latter when 5-hydroxyconiferyl alcohol is present, this may indicate that the reaction is under kinetic rather than thermodynamic control.

The enthalpies of reaction for the hydrogen abstraction from the dimers were generally small, but among the benzodioxane reactants, the self-coupled 5-hydroxyconiferyl reaction was exothermic, while the cross-coupled reaction was endothermic. Within the acyclic *β-O*-4 dimers, the cross-coupled reactant was energetically preferred. It should be noted that both of these reactions involve dehydrogenation from the 5-hydroxyconiferyl alcohol phenolic group, which is consistent with the lower enthalpy of reaction for the dehydrogenation of the monolignol in comparison to coniferyl alcohol.

The ring-opening reactions of the benzodioxane linked dimers, as might occur at elevated temperatures, exhibit little difference, with the *β-O* bond cleavages being somewhat more endothermic. The bond dissociation enthalpies for the *β-O*-4 linked dimers are very similar, with higher values associated with the *threo* stereoisomers.

With respect to the last point, while previous literature[12,13] has taken stereochemistry into account in identifying low energy structures, the current paper presents an extensive report of the effect of stereochemistry on the thermodynamics of these lignin models. In general the differences observed were varied and within documented error ranges for computational methods especially for enantiomers. This observation may be of use in subsequent computational work in which stereochemistry is addressed, indicating that comparisons might justifiably be limited to diastereomers, reducing the number of calculations required for a given compound.

The exception to the generalization regarding energetic similarities was the acyclic *β-O*-4 structures which had different energetics attributable to their structure. The *threo* stereoisomers were severely folded with the aromatic rings parallel, separated by ~3.6 Å, which is similar to the inter-ring distance reported for sandwich benzene dimers,[41] perhaps accounting for their relative stability.

In conclusion, these results may be of utility in assessing the formation, reactions, and geometry of novel lignins and provide insights into targets for lignin modification through processing, synthesis, or genetic manipulation.

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