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Perspective

Coupling and Reactions of Lignols and New Lignin Monomers: A Density Functional Theory Study

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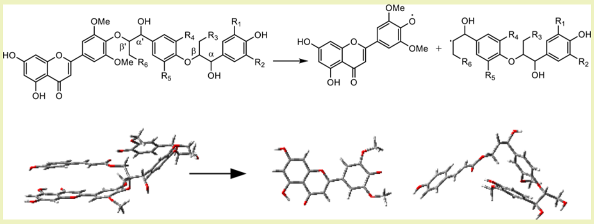

ABSTRACT: This perspective summarizes and compares computational results for the thermodynamics of bond dissociation, coupling, and rearomatization for a number of noncanonical lignin monomer–lignol combinations that have been found to occur experimentally. The noncanonical lignin monomers discussed are tricin, caffeyl alcohol, 5-hydroxyconiferyl alcohol, and piceatannol. Among dimeric combinations, the results for bond dissociation are generally similar, but in cases for which trimers have been reported (tricin-lignol adducts), this value can be quite variable, with stereochemical and structural preferences. Among the adducts examined thus far, the energies associated with quinone methide formation and rearomatization are not dissimilar and would not impede subsequent polymerization. These fundamental studies may help to elucidate how lignin monomers are incorporated into the lignin polymer, provide leads for targeted genetic modification, and be of use in deconstruction for the production of commodity chemicals.

KEYWORDS: *Tricin, Piceatannol, Caffeyl alcohol, 5-Hydroxyconiferyl alcohol, Lignin*

INTRODUCTION

According to the strictest definition, lignin is a phenylpropanoid polymer formed by the oxidative radical coupling of three cinnamyl alcohols, *p*-coumaryl, coniferyl, and sinapyl alcohols, the so-called monolignols produced in the general phenylpropanoid biosynthetic pathway. Increasingly, however, it is being found that other phenolic compounds, including phenolics from other biosynthetic pathways,[1] can also participate in radical coupling reactions during lignification and be fully integrated into the lignin polymer. These noncanonical compounds may be found naturally in some plant lines or may be the result of natural mutations[2] or induced genetic modifications.[3] Examples of the former include caffeyl alcohol, found in the lignin of the seed coats of vanilla orchid (*Vanilla planifolia*) and in some members of the Cactaceae, Euphorbiaceae, and Cleomaceae families,[4] hydroxystilbenes (especially piceatannol) identified in lignins from palm fruit endocarps,[5] hydroxystilbene glucosides in the lignin from spruce bark,[6] diferuloyl putrescine in the lignin from maize kernels,[7] and tricin in the lignins from grasses and other commelinid monocots.[8−11] Indeed, it has been recently reported that 35 different monomers have been detected in natural lignins.[12] The experimental work on newly discovered lignin monomers derived from beyond the canonical monolignol biosynthetic pathway has been recently reviewed.[13]

On the basis of the above discoveries of "new" lignin monomers, the current work describes and summarizes the application of contemporary computational methods to studies on the formation of noncanonical lignin monomer−lignol adducts and their reactivity. These studies have been undertaken to examine the thermodynamics of the coupling of the monomers with lignols, as well as bond dissociation reactions of the products. Results from the former are indicative of the ability of these noncanonical lignin monomers to participate in the lignification reaction, whereas the latter are related to the reactivity of modified lignins that may improve processability of biomass. Lastly, where feasible, comparisons of bond dissociation energies and ease of rearomatization between reactions with the different noncanonical lignin monomers are discussed. The specific lignin monomers to be discussed are the catechyl alcohols (caffeyl and 5-hydroxyconiferyl alcohols), piceatannol, and tricin. The reactions to be considered are the thermodynamics of bond dissociation for the tricin and catechyl alcohol adducts and the coupling and rearomatization for piceatannol and catechyl alcohol adducts.

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■ METHODS

Per the title, density functional theory calculations have been used in all of the studies that are discussed. There is ample precedent for the use of this method in work on lignin models. Extensive studies on bond dissociation of dimers have been reported.[14,15] Work on the ubiquitous β-ether units, with their characteristic and prevalent β−O−4′ linkages, has already been reported.[16,17] Ring-opening reactions for the various cyclic units have also been the subject of computational studies.[18−23] Except as noted, the values reported are from Gaussian09 or 16,[24] M06-2X/6-311++G(d,p) optimizations with thermal corrections for enthalpies and free energies from frequency calculations, which also verified the identification of a minimum by the absence of imaginary frequencies. The default convergence criteria and ultrafine integration grid were used for the optimizations. Given the flexibility of the structures in question, an initial 1000 step Monte Carlo conformational search was performed with Merck force-field minimization. The unique low energy conformations were subsequently optimized using the PM6 semiempirical method. The 10 low energy conformations were next refined with M06-2X/6-31+G(d), after which the lowest energy conformation was submitted to M06-2X/6-311++G(d,p) optimization as described. All species are neutral, and therefore, the calculations were performed in the gas phase.

Tricin−Lignol Conjugates. Tricin, 5,7-hydroxy-2-(4-hydroxy-3,5-dimethoxy-phenyl)-4*H*-chromen-4-one (Figure 1), is a flavonoid that can occur in plants as an extractive, as various tricin glycosides, as flavonolignan glycosides, or in polymeric lignin. Tricin has numerous potential biological applications, having antioxidant, antiviral, and anti-inflammatory activities.[25] The distribution of tricin derivatives across the plant kingdom has been reviewed.[25] Tricin associated with lignin has been found in wheat straw[26] and several other monocotyledons[10] at levels of ~0.026%−0.715%, based on the whole cell wall and up to 3.3% on a lignin basis.[9,10] *In vitro* studies have found that tricin couples with all three cinnamyl alcohols through the formation of a 4′−O−β bond,[8] which was also observed in lignin preparations by the application of nuclear magnetic resonance spectroscopy.[8,26] Furthermore, although other tricin−lignin linkages could theoretically occur, no evidence for coupling at the 3-position, for example, can be discerned,[8] although there is a lignan that appears to show such coupling;[27,28] only the 4′−O−β has been detected both *in planta* and *in vitro*, such that tricin can occur only at the starting end of the polymer, suggesting a role as a nucleation site for lignification,[8] much like that ascribed to ferulate on arabinoxylans in grasses.[29] This may be accounted for by the enhanced stability of the radical from the (phenolic) 4′−OH, which was found to be 9.09 and 20.14 kcal mol^{-1} more stable than those from the 7- and 5-OH, respectively, and as expected from a consideration of traditional resonance structures, but also as evident from Figure 1 exhibits the greatest delocalization of the unpaired electron.

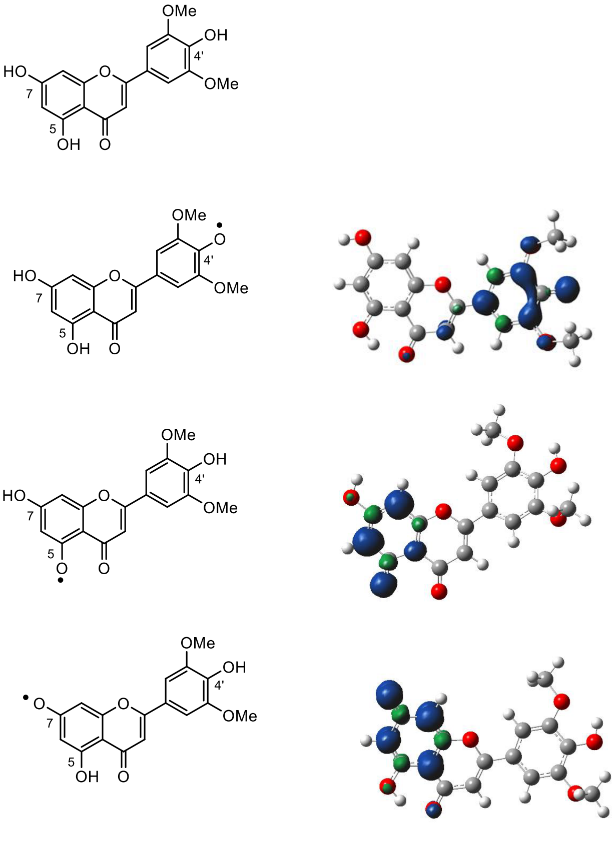
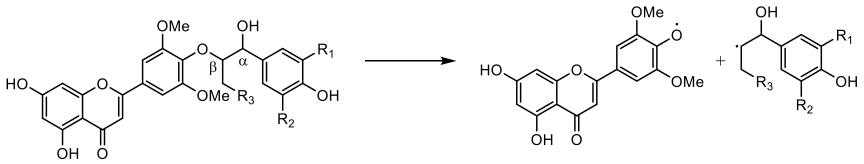


Figure 1. Tricin and phenoxy radicals from tricin and spin density plots (blue indicates regions of positive spin density; green indicates regions of negative spin density).

In an experimental study on tricin−oligolignol metabolites present in maize, the dimeric and trimeric structures shown in Figures 2 and 3 that included coupling to monolignols and their acetate and *p*-coumarate conjugates were reported.[9] The observation of combinatorial coupling, not just with regard to the stereochemistry and optical centers but also the presence and nature of the native γ−OH substitution,[9] suggested that we look at this feature to determine if acylation, a process that can be enhanced in transgenic plants or can be selected by breeding, could facilitate processing in addition to providing a potentially valuable source of "clip-offs"—small acids readily releasable from the polymer.[30] It has been found that the amount of tricin present in lignin is greater than the amount of extractable tricin,[10] such that the former may represent a valuable source of tricin, assuming that an efficient and industrially viable cleavage process can be developed. As such, the bond dissociation energies of the homolytic cleavage reactions shown in Figures 2 and 3 were determined to ascertain if the reactivities of these tricin−oligolignol conjugates are affected by the number of lignin units in the oligolignols, the methoxyl content, their state of (natural) derivatization on the γ−OH groups, or their stereochemistry. The dimers have two chiral carbons resulting in four optical isomers or two diastereomers. The configurations for this work are R(α)S(β) and S(α)S(β). The trimers have four chiral carbons, 16 optical isomers, and eight diastereomers. The configurations considered in the current work are S(α)S(β)S(α')S(β'), S(α)S(β)S(α')R(β'), S(α)S(β)R(α')S(β'), S(α)S(β)R(α')R(β'), S(α)R(β)S(α')S(β'), S(α)R(β)S(α')R(β'), S(α)R(β)R(α')S(β'), and S(α)R(β)R(α')R(β').

The energy of reaction for each dimer and stereoisomer is shown in Table 1A. The average overall value for the homolytic cleavage is 55.71 ± 2.53 kcal mol^{-1}, with a range of 51.15−59.45 kcal mol^{-1}. The colors in the table are centered on the average, with higher values in yellow and lower values in blue. Among these structures, a distinct difference with stereochemistry can be observed with 5 of the 6 values below the average having the RS configuration. Furthermore, acylated derivatives account for four of the structures below the average of which three are



	R1	R2	R3
3H	H	H	OH
3G	OMe	H	OH
3S	OMe	OMe	OH
3G′	OMe	H	OAc
3S′	OMe	OMe	OAc
3G″	OMe	H	O*p*CA
3S″	OMe	OMe	O*p*CA

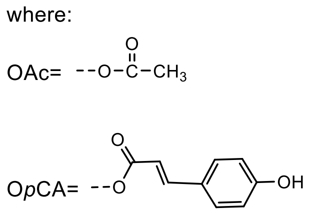
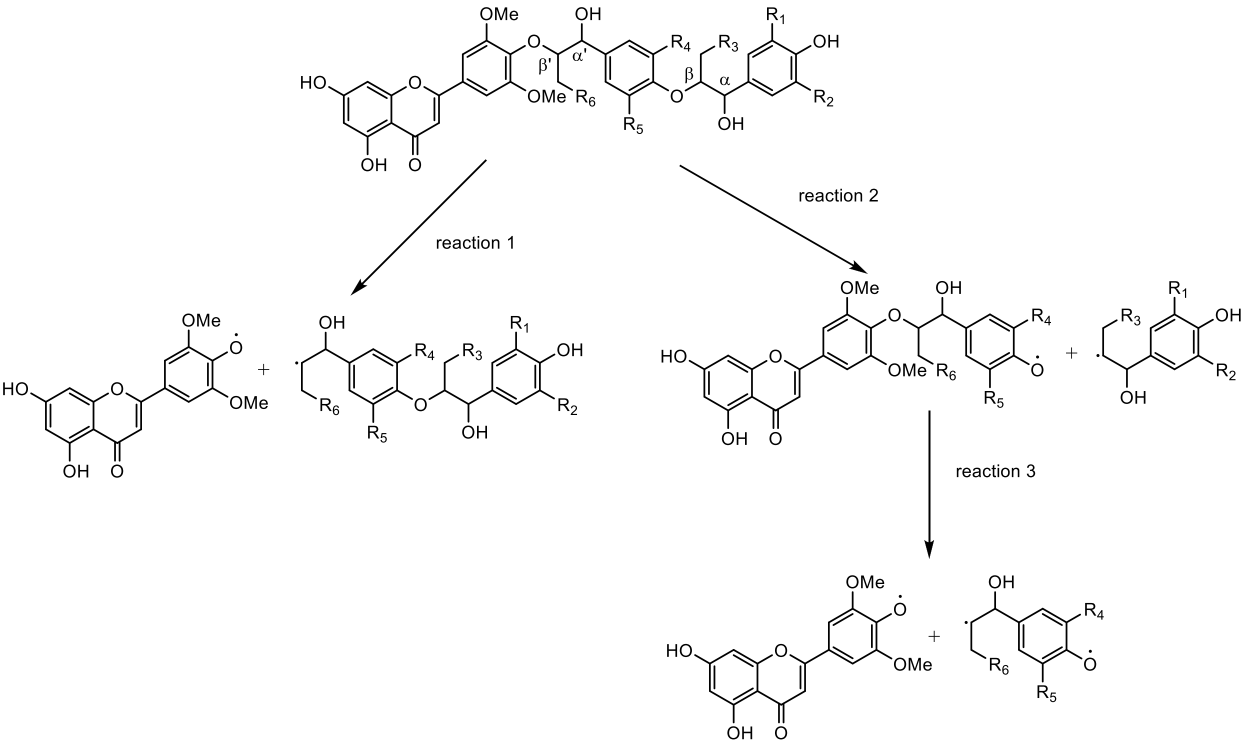


Figure 2. Dimeric tricin−lignol conjugates and products from homolytic cleavage. Ac, acetates; *p*CA, *p*-coumarates.



	R1	R2	R3	R4	R5	R6
4SG	OMe	H	OH	OMe	OMe	OH
4GG	OMe	H	OH	OMe	H	OH
4GG′	OMe	H	OAc	OMe	H	OH
4G′G	OMe	H	OH	OMe	H	OAc
4GG″	OMe	H	O*p*CA	OMe	H	OH
4G″G	OMe	H	OH	OMe	H	O*p*CA
4GS″	OMe	OMe	O*p*CA	OMe	OMe	OH

Figure 3. Trimeric tricin−lignol conjugates and products from homolytic cleavage. Ac, acetates; *p*CA, *p*-coumarates.

acetates. There do not appear to be any obvious trends between the G, S, and H lignols.

Table 1B shows the energy of reaction for reaction 1 (Figure 3) for each trimer and stereoisomer. The overall average energy of reaction is 61.83 ± 4.33 kcal mol^{-1}, with a range of 52.32−71.59 kcal mol^{-1}. Although the average value would indicate that this reaction requires more energy than the analogous reaction of the dimers, there are several instances in which the energies are similar. To facilitate comparison with the reactions of the dimers, the colors in Table 1B are centered on 59.45 kcal mol^{-1}, which is the highest energy of reaction for the dimers.

Within the 15 structures (out of a total of 56) that have energies of reaction below 59.45 kcal mol^{-1}, 11 exhibit *erythro* (RS/SR) stereochemistry for the distal lignol, positions C-*α* and

Table 1. Gibbs Energies of Reaction[a]

A	3H	3G	3S	3G′	3S′	3G″	3S″
RS	55.72	54.30	54.30	52.05	51.15	59.26	54.31
SS	56.48	57.50	57.43	52.72	57.75	59.45	57.52

reaction 1

B	4SG	4GG	4GG′	4G′G	4GG″	4G″G	4GS"
SSSS	67.95	70.54	71.59	63.34	65.83	67.49	59.98
SSSR	64.89	59.89	60.00	55.76	63.24	53.89	65.35
SSRS	65.60	54.48	61.95	58.51	61.01	58.59	60.19
SSRR	67.90	65.51	67.76	64.19	56.99	58.86	69.55
SRSS	62.82	60.69	59.91	60.14	65.33	65.20	65.07
SRSR	56.83	60.90	61.17	58.45	63.73	55.19	62.12
SRRS	58.84	61.81	60.05	58.44	59.67	52.32	63.66
SRRR	62.67	61.33	67.63	62.79	59.42	52.54	63.15

reaction 2

C	4SG	4GG	4GG′	4G′G	4GG″	4G″G	4GS"
SSSS	62.87	63.52	63.67	62.94	63.79	63.08	62.85
SSSR	62.37	62.57	60.42	64.90	58.23	51.72	66.30
SSRS	63.75	63.31	60.27	61.70	57.38	53.58	64.08
SSRR	62.95	62.90	63.03	65.31	63.13	58.60	62.70
SRSS	56.84	56.56	59.82	56.32	61.97	65.14	62.33
SRSR	55.73	64.41	65.56	61.26	61.94	54.10	65.97
SRRS	59.78	61.70	62.57	62.96	60.11	59.82	64.29
SRRR	58.02	57.36	59.91	62.13	62.25	58.76	65.40

reaction 3

D	4SG	4GG	4GG′	4G′G	4GG″	4G″G	4GS"
SSSS	58.72	57.75	57.59	55.67	56.31	53.98	59.74
SSSR	56.69	53.91	54.77	47.21	56.46	59.28	50.89
SSRS	55.29	50.48	54.83	49.48	50.25	57.92	56.33
SSRR	59.11	57.85	58.21	54.44	53.43	58.37	57.86
SRSS	57.74	59.74	56.66	58.63	59.59	53.64	56.67
SRSR	56.36	50.46	50.68	48.79	53.87	60.10	54.91
SRRS	55.55	51.10	51.14	48.84	53.05	50.59	53.87
SRRR	57.62	56.96	56.77	54.82	54.49	54.23	57.79

[a](A) Tricin−ignol dimers. (B) Reaction 1 for tricin−lignol trimers. (C) Reaction 2 for tricin−lignol trimers. (D) Reaction 3 for tricin−lignol trimers. All values are in kcal mol^{-1}. The colors use a spectral profile from yellow (high) to blue (low).

C-β. Eight of the members of this group are *p*-coumaroylated, of which six are derivatized through position R_6. There are four acetylated derivatives, two at each position. The remaining three representatives are nonderivatized. Among the trimers with the higher heats of reaction, all have *threo* (SS/RR) stereochemistry at the C-α and C-β positions. The practical ramification is therefore suggested to enhance monomer acylation (which is well known in, especially, grasses in which most acylation by *p*-coumarate is on S units)[31,32] and to enhance the syringyl levels, which also results in higher *erythro:threo* ratios.[33−36]

The energies for the sequential reactions 2 and 3 (Figure 3) are shown in Table 1C and D, the colors of which are again centered on the average for each reaction. The average energy of reaction for reaction 2 is 61.34 ± 3.25 kcal mol^{-1}, with a range of 51.72−66.30 kcal mol^{-1}. Among the 21 structures that have energies of reaction lower than the average, nine are *p*-coumaroylated, six are acetylated, and 12 have RS/SR (*erythro*) stereochemistry at positions C-α and C-β. The average energy of reaction for reaction 3, which is analogous to reaction 1 and the cleavage of the dimers, is relatively low at 55.13 ± 3.25 kcal mol^{-1} and a range of 47.21−60.10 kcal mol^{-1}. Within the 56 trimeric metabolites, 26 have energies of reaction below 55.13 kcal mol^{-1}, of which 12 are *p*-coumaroylated, 10 are acetylated, and 19 have RS/SR stereochemistry at positions C-α and C-β. Although there are instances that the energies of both reaction 2 and 3 are lower than that of the dimers, as the release of tricin is predicated on reactions 2 and 3 occurring sequentially, the total as can be seen would render this route thermodynamically unfavorable.

From these results, it can be seen that the dimers are generally more reactive toward cleavage, and with less variable energies, than the trimers. Among the trimers, acylation of the γ−OH enhances reactivity as does the *erythro* configuration for the chiral carbons of the distal lignol. If the release of tricin is an objective, structures that are representative of species with lower energies of reaction might constitute targets for genetic modification, as noted above.

Piceatannol−Lignol Conjugates. Piceatannol is a hydroxystilbene produced through a combination of the shikimate and acetate malonate pathways and was found in the lignin of endocarp tissues of the palm fruits of *Acrocomia acluleta*, *Copernicia prunifera*, and *Cocos nucifera*.[5,37] Analytical results based on milled wood lignins and crude polymers (radical oxidative dimers and polymers) have found that piceatannol can undergo homocoupling and cross-coupling with monolignols,[5] as shown in Figure 4. Given this experimental evidence, computational methods have been used to calculate the energetics of the coupling and rearomatization reactions to determine how or if cross-coupling between piceatannol and the monolignols (sinapyl, coniferyl, and *p*-coumaryl alcohol) might impact lignin formation.[38]

Piceatannol can be oxidized to form a resonance-stabilized radical, as shown in Figure 4A. The free energy of the dehydrogenation reaction is consistent with those of the monolignols, indicating the feasibility of this reaction. The unpaired spin density (Figure 5) is limited to the phenoxy oxygen and carbons 1, 3, 5, and 8, with small populations at carbons 10, 12, and 14 (numbering is as shown in Figure 4). We had expected that higher density might be on the latter carbons as resonance structures can be drawn to show delocalization to these positions, and experimentally, 8−10′ coupling products have been detected. This notwithstanding, these spin densities are in agreement with the literature[39] and with the evidence that significant 8-coupling is occurring; in fact, given that the 7,8-double bond cannot be detected in various product units in piceatannol−lignin spectra,[5,37] the substantial density on the 8-carbon is reassuring.

The Gibbs free energy for the 8−O−4′ piceatannol homocoupling reaction (Figure 4B) forming the quinone methide was found to be −24.65 kcal mol^{-1}, with the analogous β−O−4′ cross-coupling reactions (Figure 4D) ranging from −23.19 to −26.60 kcal mol^{-1}. These are also in good agreement with β−O−4′ homocoupling of the monolignols, which were

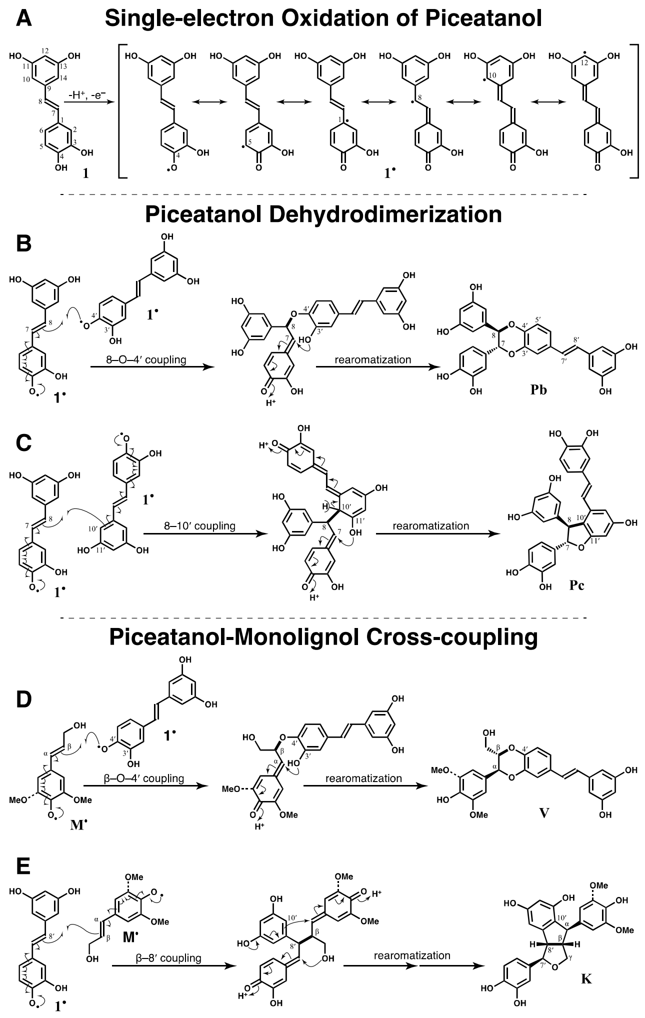


Figure 4. (A) Piceatannol and resonance structures from dehydrogenation. (B) 8−O−4′ homocoupling and rearomatization of piceatannol. (C) 8−10′ homocoupling and rearomatization of piceatannol. (D) *β*−O−4′ cross-coupling of monolignols and piceatannol. (E) *β*−8′ cross-coupling of monolignols and piceatannol (ref 5). The straight bold bonds are those formed during the radical coupling step. For consistency, the compound designations are as in ref 5.

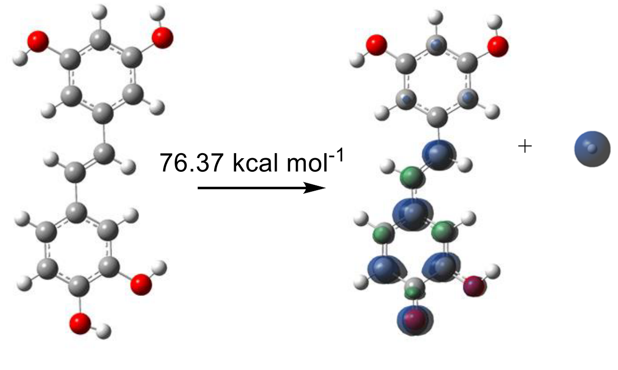


Figure 5. Gibbs free energy of dehydrogenation of piceatannol and spin density plot for the resultant products (blue indicates areas of positive spin density; green indicates areas of negative spin density).

found to be −21.46, −26.23, and −29.37 kcal mol^{-1} for sinapyl, coniferyl, and *p*-coumaryl alcohol, respectively. The *β*−8′ cross-coupled quinone methides (Figure 4E) exhibit energies of reaction ranging from −18.38 to −23.05 kcal mol^{-1}. These energies of reaction, which are not inconsistent with other coupling reactions, are interesting since *β*−8′ cross-coupled products have not been detected in either isolated lignins or dehydrogenation polymers. From these computational results, it would appear that the coupling reactions are thermodynamically feasible. In contrast to the general similarity in energy of reaction for these couplings, the 8−10′ homocoupling reaction (Figure 4C) is markedly less exergonic, with energies of reaction of −3.16 and −1.96 kcal mol^{-1}, which vary with the stereochemistry of the quinone methide. This is due to the disruption of the aromatic system and perhaps the low spin density found at the 10′ carbon. Nevertheless, as noted, experimental evidence has been provided for this coupling mode.[5,6]

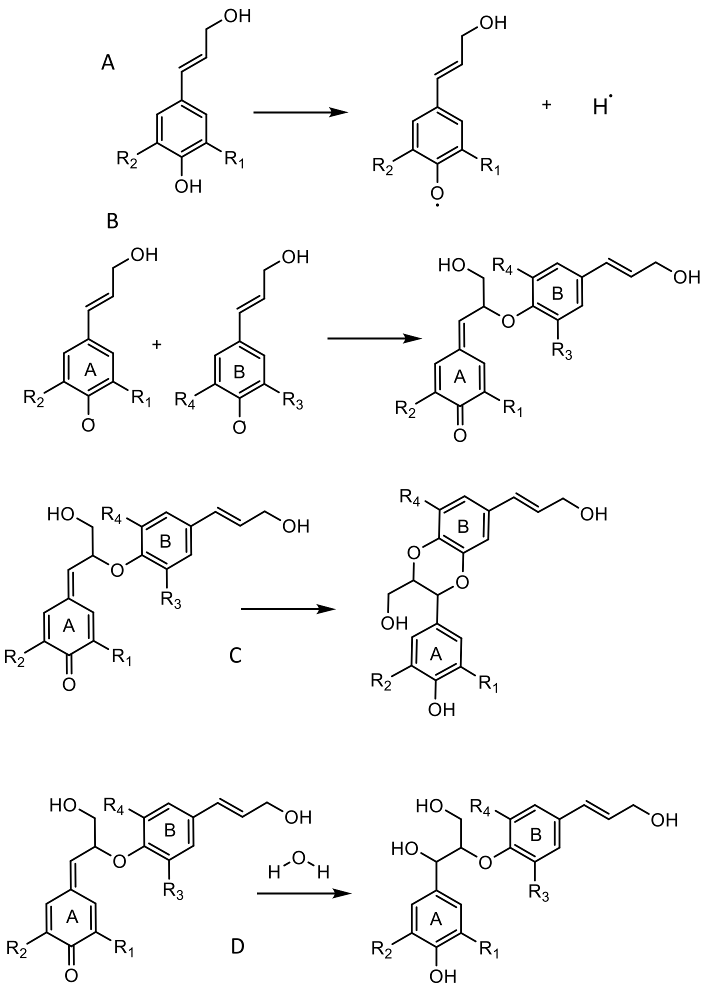
The rearomatization reactions of the 8−O−4′ homocoupled (Figure 4B) and *β*−O−4′ cross-coupled quinone methides (Figure 4D) are reasonably similar in the range from −19.92 to −26.67 kcal mol^{-1}, although the homocoupled piceatannols are among the less exergonic of these reactions. There is a small effect of stereochemistry between the 8−O−4′ homocoupled products, with the more stable form corresponding to the *trans* configuration of the benzodioxane ring and the isomer primarily (>90%) produced. Interestingly, the energy of rearomatization for quinone methides from *β*−O−4′ homocoupled monolignols is consistently less exergonic in the range from −12.53 to −20.04 kcal mol^{-1}. As such, the reactions that involve piceatannol would be thermodynamically favored; i.e., this is a case in which cross-coupling may be favored over homocoupling of monolignols when enough piceatannol is available. Studies into the details of these homo- and cross-coupling propensities are ongoing and will be published elsewhere.

The reactions involving the 8−10′ homocoupled quinone methides (Figure 4C) are quite exergonic ranging from −51.35 to −54.45 kcal mol^{-1}, due to the rearomatization of two quinone methide groups and the formation of a phenylcoumaran ring structure by internal trapping. As before, the *trans* stereoisomer is more stable; phenylcoumaran rings in nature are overwhelmingly *trans*.[40] Similarly, the rearomatization of the *β*−8′ cross-coupled quinone methides (Figure 4 E) are particularly exergonic, again due to reactions involving two quinone methides, each with two ring closures via internal trapping. Among these, there is a sharp difference with stereochemistry in which the *cis* form is about 10 kcal mol^{-1} more stable, a configuration which is consistent with the analogous lignan kompasinol A.[41]

Finally, the dehydrogenation reactions of the homocoupled and cross-coupled dimers were examined to determine if the presence of piceatannol would change the energetics of this step that is crucial for the continuation of the polymerization of lignin. In general, the energies for this reaction were in the range of 70−80 kcal mol^{-1}, which is not dissimilar to those for *β*−O−4′ linked dimers of sinapyl, coniferyl, and *p*-coumaryl alcohol. The exceptions to this generalization are the dimers in which piceatannol is cross-coupled with *p*-coumaryl alcohol, for which the dehydrogenation energies are as high as 99 kcal mol^{-1}.

In summary, the energetics of the formation and reactions of piceatannol with monolignols are similar to those of the monolignols alone and should therefore not provide an obstacle to their participation in lignification.

Catechyl Alcohols and C-Lignin. The catechyl alcohols, caffeyl and 5-hydroxy-coniferyl alcohols, occur naturally in the lignins of seed tissues of some plants and can also result from genetic manipulation. Lignins based solely on caffeyl alcohol



R1	R2	ΔHr
H	OH	86.7
OMe	OH	81.8

R1	R2	R3	R4	ΔHr
OH	H	OH	H	-46.7
OMe	OMe	OH	H	-35.0
H	OMe	OH	H	-38.2
H	H	OH	H	-41.2
OH	H	OMe	OMe	-41.9
OH	H	H	OMe	-47.4
OH	H	H	H	-47.0
OH	OMe	OH	OMe	-46.6
OH	OMe	H	OMe	-43.5
H	OMe	OH	OMe	-43.5

R1	R2	R3	R4	ΔHr
OH	OMe	OH	OMe	-19.5
H	OMe	OH	OMe	-20.2
OH	H	OH	H	-20.5
OMe	OMe	OH	H	-26.9
H	OMe	OH	H	-28.7
H	H	OH	H	-25.8

R1	R2	R3	R4	ΔHr
OH	OMe	H	OMe	-26.2
OH	H	OH	H	-24.6
OH	H	OMe	OMe	-24.3
OH	H	H	OMe	-19.8
OH	H	H	H	-21.5
OMe	OMe	OH	H	-30.7
H	OMe	OH	H	-33.2
H	H	OH	H	-30.2

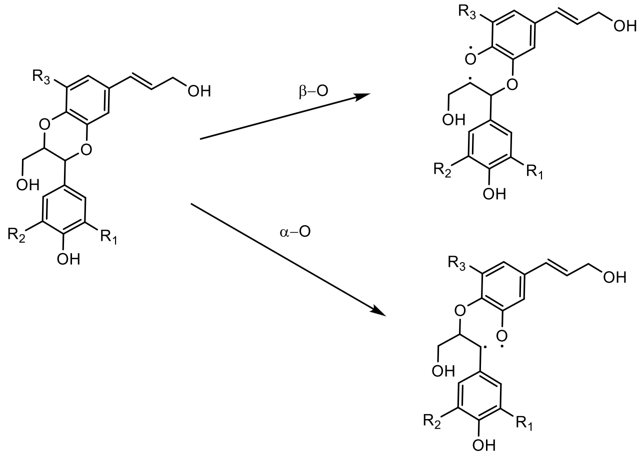
Figure 6. Reactions of caffeyl and 5-hydroxyconiferyl alcohol. (A) Enthalpies of dehydrogenation.(B) Enthalpies of reaction for quinone methide formation. (C) Enthalpies of rearomatization by benzodioxane ring formation. (D) Enthalpies of rearomatization by nucleophilic addition of water. All values are in kcal mol^{-1}.

(so-called C-lignin) have been isolated from the seed coats of the vanilla orchid and various cacti and have been induced in softwoods by downregulating the caffeoyl-CoA 3-*O*-methyltransferase enzyme.[4,42,43] 5-Hydroxy-coniferyl alcohol has also been found to be incorporated into the lignin of cacti[3] and can occur in angiosperms by downregulation of the caffeic acid *O*-methyltransferase enzyme, preventing the methylation of 5-hydroxyconiferyl alcohol to sinapyl alcohol, as reviewed many times now.[12,44−54]

Enthalpies of reaction for the dehydrogenation, radical coupling, and rearomatization reactions for homocoupled catechyl alcohols and cross-coupling with monolignols are as shown in Figure 6.[20,22] These are average values for all stereoisomers, for which there was minimal variability. Parenthetically, the calculations on caffeyl alcohol were performed using the Def2-TZVPP basis set, which like 6-311++G(d,p) is a triple ζ basis set with polarization functions. This similarity of methods notwithstanding, comparisons between the studies must be made with caution.

The enthalpies of reaction associated with dehydrogenation (Figure 6A) are somewhat variable but not inconsistent with coniferyl alcohol for which the value is 85.4 kcal mol^{-1}. The homocoupling of the caffeyl and 5-hydroxyconiferyl alcohol radicals to form quinone methides have virtually identical enthalpies of reaction of −46.7 and −46.6 kcal mol^{-1}, respectively. Cross-coupling of the 5-hydroxyconiferyl alcohol and coniferyl alcohol radicals is somewhat less exothermic at −45.5 kcal mol^{-1}. Cross-coupling enthalpies of caffeyl alcohol with monolignol radicals vary depending on the mode of coupling. Reactions for which the A-ring is derived from the monolignol are markedly less exothermic ranging from −35.0 to −41.2 kcal mol^{-1}, whereas if the A-ring arises from caffeyl alcohol, the enthalpy ranges from −41.9 to −47.4 kcal mol^{-1} (Figure 6B). The internal rearomatization (Figure 6C) reactions that occur by formation of a benzodioxane ring are very similar for the homocoupled products at −20.5 and −19.5 kcal mol^{-1} for caffeyl and 5-hydroxyconiferyl alcohol, respectively, and cross-coupling between 5-hydroxyconiferyl alcohol and coniferyl alcohol at −20.2 kcal mol^{-1}. The cross-coupling reactions of caffeyl alcohol with the monolignols are generally more exothermic ranging from -25.8 to -28.7 kcal mol^{-1}. Following the same pattern, rearomatization by the nucleophilic addition of water to the homocoupled quinone methides (Figure 6D) results in similar enthalpies of reaction at -24.6 and -26.2 kcal mol^{-1} for caffeyl and 5-hydroxyconiferyl alcohol, respectively. Cross-coupling of caffeyl alcohol and the monolignols varies sharply with the mode of coupling. Those for which the A-ring comes from the monolignol are substantially more exothermic, with enthalpies of rearomatization ranging from -30.2 to -33.2 kcal mol^{-1}, which have been noted to favor that model experimentally.[55]

The enthalpies of bond dissociation for the benzodioxane dimers are shown in Figure 7. Among the α–O cleavage reactions there are minimal differences between the homocoupled and cross-coupled structures, with a measurable difference between the caffeyl and 5-hydroxyconiferyl alcohol-based models. Furthermore, for the dimers composed of caffeyl alcohol, there are distinct differences with stereochemistry, which does not occur in the 5-hydroxyconiferyl alcohol structures. The enthalpies of reaction for β–O cleavage of the caffeyl alcohol-derived compounds are quite consistent at ∼65 kcal mol^{-1}, regardless of the monolignol or stereochemistry. As before, the dimers based on 5-hydroxyconiferyl alcohol exhibit energies of reaction that are lower but also do not differ with stereochemistry. The data suggest that homolytic cleavage of the α–O bond is easier than for the β–O bond, as might be predicted; certainly heterolytic cleavage (in base or acid) will always open the α–O bond in preference.[56]



	R$_1$	R$_2$	R$_3$	*threo*	*erythro*
α-O	OH	H	H	58.35	54.73
	OMe	H	H	58.83	54.46
	H	H	H	58.83	54.43
	OMe	OMe	H	58.33	54.43
	OH	OMe	OMe	53.34	52.49
	OMe	H	OMe	51.97	51.11
β-O	OH	H	H	65.41	65.31
	OMe	H	H	65.82	65.05
	H	H	H	66.03	65.21
	OMe	OMe	H	65.33	65.16
	OH	OMe	OMe	55.04	54.75
	OMe	H	OMe	55.49	58.36

Figure 7. Enthalpies of benzodioxane ring-opening reactions. All values are in kcal mol^{-1}.

Comparison of Computational and Experimental Results. The work reported and summarized in the current perspective involves the use of computational methods as a complement to experimental observation. This complementarity is somewhat limited by the observables that can be derived from calculations, the reported experimental data, and the ability to make direct comparisons between these sometimes disparate methods.

The most fruitful comparisons have involved stereochemistry, as detected by nuclear magnetic resonance spectroscopy, and optimized geometries from computational calculation. The *trans* isomers of 8–O–4′ and 8–10′ products of piceatannol homocoupling are found to be more stable by 0.72 and 1.89 kcal mol, respectively. It has been reported experimentally that the *trans* isomer predominates,[40] with levels in excess of 90%, which based on a Boltzmann calculation amounts to an energy difference of 1.25 kcal mol^{-1} that is in good agreement with the calculated energy differences. The *trans* isomers of the β–O–4′ products are also exclusively more stable by 1.70–2.68 kcal mol^{-1}. Products resulting from β–8′ cross-coupling of monolignols and piceatannol in its *cis* configuration are more stable by ∼10 kcal mol^{-1}, and the stereochemistry is consistent with the analogous kompasinol A lignan as reported in the literature.[41]

Experimentally, the 8–O–4′ and 8–10′ homocoupled piceatannols have been found to be the major products in milled wood lignins from palm fruit endocarps, with lower levels of β–O–4′ cross-coupled products (cf. Figure 4).[5] Crude polymers of piceatannol, in the absence of monolignols, resulted in the formation of 8–O–4′ and 8–10′ homocoupled products. Crude polymers produced with both piceatannol and monolignols gave β–O–4′ cross-coupled and 8–10′ homocoupled products, with small amounts of the 8–O–4′ homocoupled product. Interestingly, we could not detect β–8′ cross-coupled structures in either milled wood lignin preparations or crude polymers.[5] The formation of the 8–O–4′, 8–10′, and β–O–4′ products involving piceatannol are consistent with resonance structures drawn in Figure 4A. Figure 5, however, showing the calculated unpaired spin density for the piceatannol radical, is at some degree of variance with these results. As might be expected, there is large unpaired spin density at the phenoxy oxygen and C-8 positions, whereas the population at the C-10 position is relatively low, although consistent with previous computational literature.[39] Conversely, the large spin density at C-8 in piceatannol would seemingly represent a favorable reaction indicator for the formation of the β–8′ cross-coupled product that has not been confirmed in lignin polymers. In addition, the thermodynamics associated with this product are not unfavorable for its formation.

As before, the benzodioxanes formed by homocoupling of the catechyl alcohols, caffeyl and 5-hydroxyconiferyl alcohols, are found to be mainly in the *trans* configuration, with smaller but observable levels of the *cis* isomers reported.[42,57−60] The computational results for caffeyl alcohol are consistent with this observation, albeit with very small energetic differences between the stereoisomers. In contrast, the *cis* isomers of the benzodioxanes formed from 5-hydroxyconiferyl alcohol are found to be somewhat more stable, with relative enthalpies of reaction less than 2 kcal mol^{-1}. Depending on the values assigned to "chemical accuracy" (typically 1–2 kcal mol^{-1}), discrimination between the stability of the *cis* and *trans* isomers would be tenuous.

As discussed in the section concerned with tricin, experimental results have found that coupling occurs only through the O–4′ position, which is in accord with spin density

(Figure 1) and relative stability of radicals. Furthermore, liquid chromatography—mass spectrometric analyses of the 3G dimer and 4SG trimer found that the tricin—lignol conjugates were composed of a mixture of stereoisomers.[8] The 3G dimer is made up of 80% *erythro* (RS) and 20% *threo* (SS), but computationally the *threo* isomer was found to be more stable by 2.44 kcal mol^{-1}. The trimer was reported to be composed of 13% SSSS/SSRR, 13% SSSR/SSRS, 37% SRSS/SRRR, and 37% SRSR/SRRS, in which the configuration corresponds to α—β—α'—β' carbons. A line plot (Figure 8) of the relative energy and inverse of the population reveals reasonable similarity in the trends between these measurements.

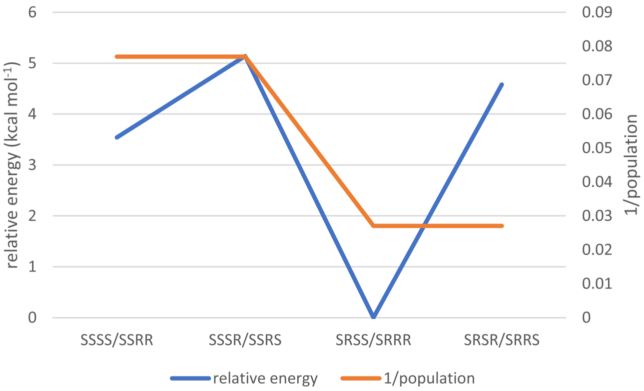


Figure 8. Plot of relative energies and the inverse of the observed populations of the 4SG trimer

In summary, the divergences found between experimental observation and computational calculation in these studies could be due to several factors. The computational results reported are enthalpies and energies, but the reactions in question may be under kinetic rather than thermodynamic control. The former has been explored for monolignol coupling indicating relatively low activation energy barriers for such reactions.[61−63] In addition, instrumental methods represent bulk measurements of an ensemble of conformations, whereas the computational results are based on a single structure for the final step.

Inter-lignin—Monomer Comparisons. From these results, comparisons can be made between the varying noncanonical lignin monomer—lignol combinations to determine how such combinations might impact the coupling reactions in lignification or reactivity of the structures. For consistency purposes, these comparisons are be based on available free energies.

It can be seen from Table 1 that the homolytic cleavage reaction for the tricin—lignol adducts depends quite markedly on the stereochemistry, and among the trimers, the reaction can be quite exergonic, reaching over 70 kcal mol^{-1}. The Gibbs free energy of the benzodioxane ring-opening reactions of homocoupled 5-hydroxy coniferyl alcohol and cross-coupled with coniferyl alcohol are in Table 2. These are generally consistent with the bond dissociation energies of the tricin—lignol dimers, and with the exception of the α—O cleavage of the cross-coupled dimer, the differences with stereochemistry are within chemical accuracy.

Although there are instances in which the bond dissociation energies are comparable for products that are cross-coupled with tricin or catechyl alcohols, the former is much more variable, and many of the products exhibit much higher energies of reaction.

Table 2. Gibbs Free Energies of Benzodioxane Ring-Opening Reactions[a]

	R_1	R_2	R_3	*threo*	*erythro*
α-O	OH	OMe	OMe	51.58	49.81
	OMe	H	OMe	51.06	46.86
β-O	OH	OMe	OMe	55.25	53.89
	OMe	H	OMe	56.72	55.32

[a] All values are in kcal mol^{-1}.

Furthermore, the products based on 5-hydroxyconiferyl alcohol have minimal variation with stereochemistry, in comparison to the wide variability among the tricin cross-coupled products. As such, it can be concluded that there are thermodynamic differences both between the different noncanonical lignin—monomer conjugates and within those based on tricin.

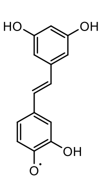
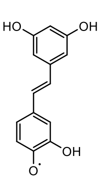
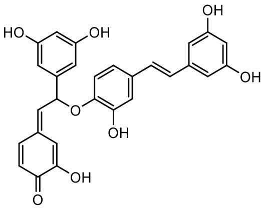
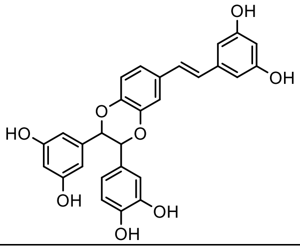
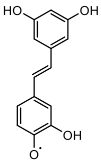
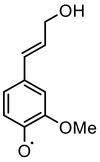
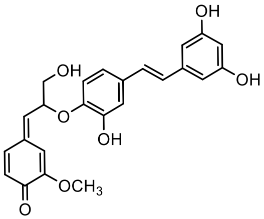
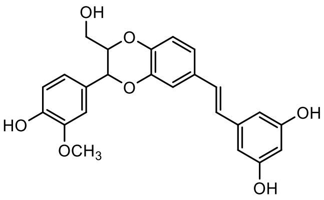
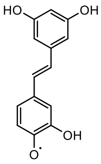
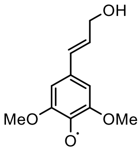
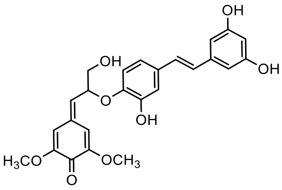
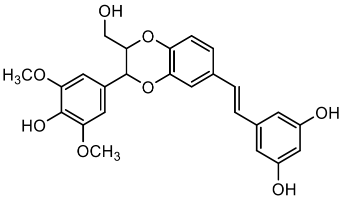
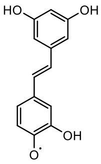
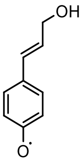
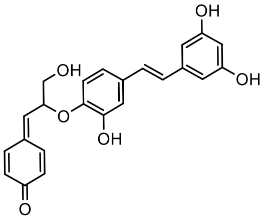
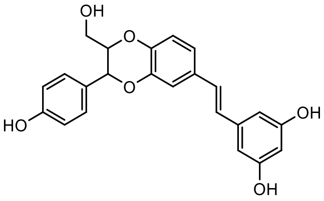
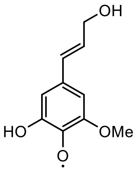
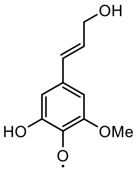
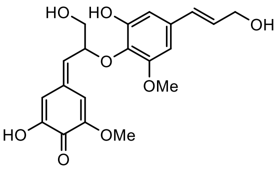
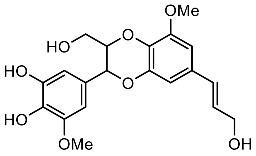
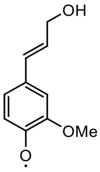
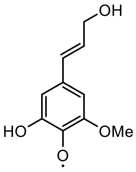
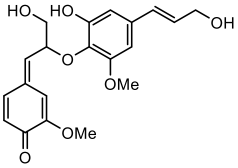
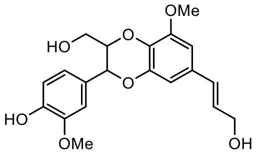
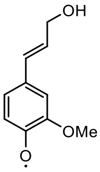
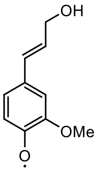
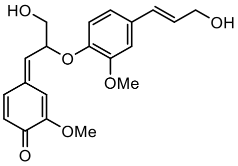
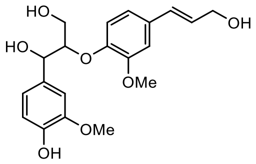
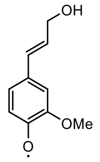
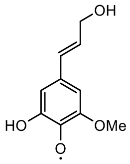
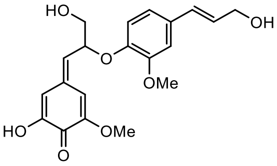
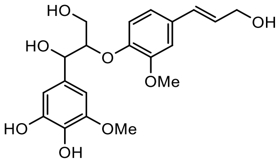
The thermodynamics of coupling and rearomatization reactions have been examined for piceatannol and catechyl alcohol-based adducts. Although piceatannol can, as discussed above, couple through several types of linkages, the linkages identified for the catechyl alcohols appear to be limited to β—O—4′ bonds; the subsequent discussion is therefore restricted to these bond types. The free energies of reaction for coupling to form the quinone methides and subsequent rearomatization reactions of piceatannol and 5-hydroxyconiferyl alcohol are shown in Table 3. In general, the energies associated with quinone methide formation are not markedly different between the two noncanonical lignin monomers. It may be of interest, however, that homocoupling of 5-hydroxyconiferyl alcohol is the most exergonic of these reactions suggesting that, even in a mixed monomer environment, homocoupling products will be favored. The high level of benzodioxanes, estimably higher than the level of 5-hydroxyconiferyl alcohol, suggests that this is borne out during lignification. The rearomatization reactions of the piceatannol derivatives are slightly more exergonic but not markedly so. As might be expected, the formation of acyclic structures is generally less exergonic. From these results, the nature of the lignin monomer does not affect the thermodynamics of coupling or rearomatization, such that their incorporation into the polymer should be feasible.

CONCLUSIONS

On the basis of these results, it has been found that the noncanonical lignin monomers that are coupled to monolignols have generally similar thermodynamics of bond dissociation. As the lignol group increases in size, however, this value becomes much more variable and in many cases reaches the point of thermodynamic infeasibility. As such, genetic manipulation methods that can limit the length of the lignol group may be desirable for deconstruction of lignin or the release of valuable lignin monomers. In addition, the energies associated with coupling and rearomatization reactions of noncanonical lignin monomers to lignols have been found to be similar for those of the monolignols themselves and should, therefore, not present an impediment to the polymerization reaction. As mentioned in the Introduction, there are many more noncanonical lignin—monomer combinations yet to be examined in this regard, which may be of use as a complement to ongoing experimental work. The predictions from calculations made herein are gratifyingly consistent with experimental observations in cases in which those have been reported.

The discovery and analyses, both experimental and computational, of lignin that contains novel lignin monomers provides

Table 3. Free Energies of Quinone Methide Formation and Rearomatization for Dimers Derived from Piceatannol and or 5-Hydroxyconiferyl Alcohol

	reactants		quinone methide	ΔG_r	rearomatization product	ΔG_r
piceatannols				-24.65		*erythro* -19.92 *threo* -20.64
				-24.51		*erythro* -22.24 *threo* -23.93
				-23.19		*erythro* -24.15 *threo* -26.67
				-26.60		*erythro* -21.66 *threo* -24.35
5-hydroxy coniferyl alcohol				-30.96		*erythro* -18.56 *threo* -15.29
				-26.22		*erythro* -20.10 *threo* -18.70
				-27.80		*erythro* -14.77 *threo* -20.64
				-29.81		*erythro* -13.00 *threo* -13.75

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information regarding the plasticity of the polymerization process and possible enhancements in processing of lignin and biomass.

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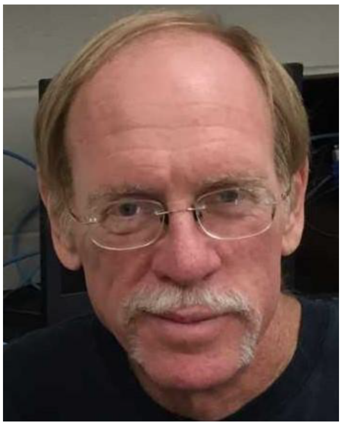
Author Contributions

T.E. performed the calculations and wrote the manuscript. J.C.d.R., J.Ra., H.K., and J.Re. identified and experimentally verified the presence and structure of the lignin−monomer−lignols combinations. G.T.B. and M.F.C. provided computer access and time allocations. All authors participated in editing the manuscript.

Notes

The authors declare no competing financial interest.

Biographies



Thomas Elder is a Research Scientist with the United States Department of Agriculture, U.S. Forest Service. He holds a Ph.D. from Texas A&M University. He is an Emeritus Professor in the School of Forestry and Wildlife Sciences and Affiliate Professor in Biosystems Engineering at Auburn University. He is also an Adjunct Professor at the Center for Renewable Carbon at the University of Tennessee−Knoxville. He has been a visiting scientist at the University of Wisconsin, the University of Copenhagen, and the University of Natural Resources and Life Sciences, Vienna (BOKU). His research is concerned with the chemical characterization and utilization of wood. He is active in the application of computational chemistry to the reactions and structure of lignin.

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John Ralph is a Professor of Biochemistry at the University of Wisconsin−Madison and since 2015 a Distinguished Professor of the Tokyo University of Agriculture and Technology. He obtained his B.Sc.-Hons in Chemistry at Canterbury University, New Zealand, in 1976, and his Ph.D. in Chemistry/Forestry at the University of Wisconsin−Madison in 1982. Ralph's group is recognized for its work on lignin biosynthesis, including delineation of the pathways of monolignol biosynthesis, lignin chemistry, and lignin reactions. The chemical/structural effects of perturbing lignin biosynthesis have been a focus, and extensions are aimed at redesigning lignins to be more valuable or more readily degraded. The group develops analytical methods and synthetic methods for biosynthetic products, precursors, intermediates, molecular markers, and cell-wall model compounds. Ralph was elected as a Fellow of the American Association for the Advancement of Science (AAAS) in 2005 and has been named by the Institute for Scientific Information as one of the 10 most cited authors in the plant and animal sciences every year since 2007.

Jorge Rencoret is a Senior Researcher at the Institute of Natural Resources and Agrobiology of Seville (IRNAS-CSIC, Spain) and expert in structural elucidation of lignins by 2D NMR, DFRC, thioacidolysis, Py-GC/MS, and GPC. As a Ph.D. student, to learn the most advanced analytical techniques on plant cell-wall characterization, he completed stays at the Biological Research Center (CIB-CSIC, Spain) and the Royal Institute of Technology (KTH, Sweden). After finishing his Ph.D. (2008), he joined Dr. John Ralph's lab at the University of Wisconsin−Madison (USA) and worked on the characterization of lignin in genetically modified plants (2009−2011). His research work has been recognized by the Royal Academy of Sciences of Seville (distinguished young researcher, 2013), the University of Seville (USBruker prizes, 2013 and 2015), and the Spanish National Research Council (recognition awards, 2013 and 2016). At present, his research is aimed at the characterization and valorization of lignins for their improved industrial use and also the development of biotechnological tools for plant cell-wall deconstruction.

Hoon Kim is a Distinguished Scientist at the DOE Great Lakes Bioenergy Research Center (GLBRC) and the University of Wisconsin−Madison, USA. He joined Dr. John Ralph's lab as a Ph.D. student in 1995 and received his Ph.D. in wood (forest product)/organic chemistry at UW−Madison in 2001. He has worked nearly 25 years with the Ralph group as a postdoc, Associate Scientist, and Senior Scientist, most recently being awarded UW's Distinguished Scientist title. He has established himself as a scientist in lignin and plant cell wall chemistry with both substantial and practical experience in NMR techniques. Lignin chemistry is his main focus, and his recent study is the analysis of whole plant cell-wall components, including polysaccharides and metabolites. His most notable contribution to this research area has been the development of a gel-state 2D NMR method for whole plant cell walls that has significantly enhanced biomass research at large. His expertise also includes oxidative radical reactions of monolignols, synthesis of lignin model compounds, and structural analysis of transgenic and mutant lignins.

Gregg T. Beckham is a Senior Research Fellow and Group Leader at NREL. He received his Ph.D. in Chemical Engineering at MIT in 2007. He currently leads and works with an interdisciplinary team of biologists, chemists, and engineers at the National Renewable Energy Laboratory on conversion of biomass to chemicals and materials including in metabolic engineering, fermentation, separations, chemical catalysis, biopolymers, and lignin valorization. He also works in the burgeoning area of plastics recycling, biodegradation, and upcycling. He was the founding cochair of the Lignin Gordon Research Conference in 2018. For lignin research in particular, he is keenly interested in many aspects of this interesting biopolymer, including development of improved analytical chemistry methods, molecular modeling of lignin, catalytic depolymerization to useful monomers, and biological conversion of aromatic compounds, all towards realistic lignin valorization processes.

Michael F. Crowley is a principal scientist in the National Renewable Energy Laboratory's (NREL's) Biosciences Center, having joined the laboratory in 2007. He leads the theory, modeling, and simulation efforts for biofuels research. Crowley is a developer of both CHARMM and Amber molecular dynamics software for biological macromolecular simulation. Crowley's group has designed and implemented new methods and algorithms specifically for biomass and biofuel-related questions. An author on more than 130 peer-reviewed scientific articles, Crowley has given more than 200 presentations, more than 50 of those

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