

Radical coupling reactions of piceatannol and monolignols: A density functional theory study

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ARTICLE INFO

Keywords:

Stilbene
Piceatannol
Coniferyl alcohol
Sinapyl alcohol
p-coumaryl alcohol
Quinone methide
Density functional theory (DFT)

ABSTRACT

Recent experimental work has revealed that the hydroxystilbene piceatannol can function as a monomeric unit in the lignification of palm fruit endocarp tissues. Results indicated that piceatannol homo-couples and cross-couples with monolignols through radical reactions and is integrally incorporated into the lignin polymer. The current work reports on the thermodynamics of the proposed reactions using density functional theory calculations. The results indicated that, in general, the energetics of both homo-coupling and cross-coupling are not dissimilar from those of the monolignol coupling, demonstrating the compatibility of piceatannol with the lignification process. Moreover, the DFT methods appear to predict the correct courses of post-coupling rearomatization reactions.

1. Introduction

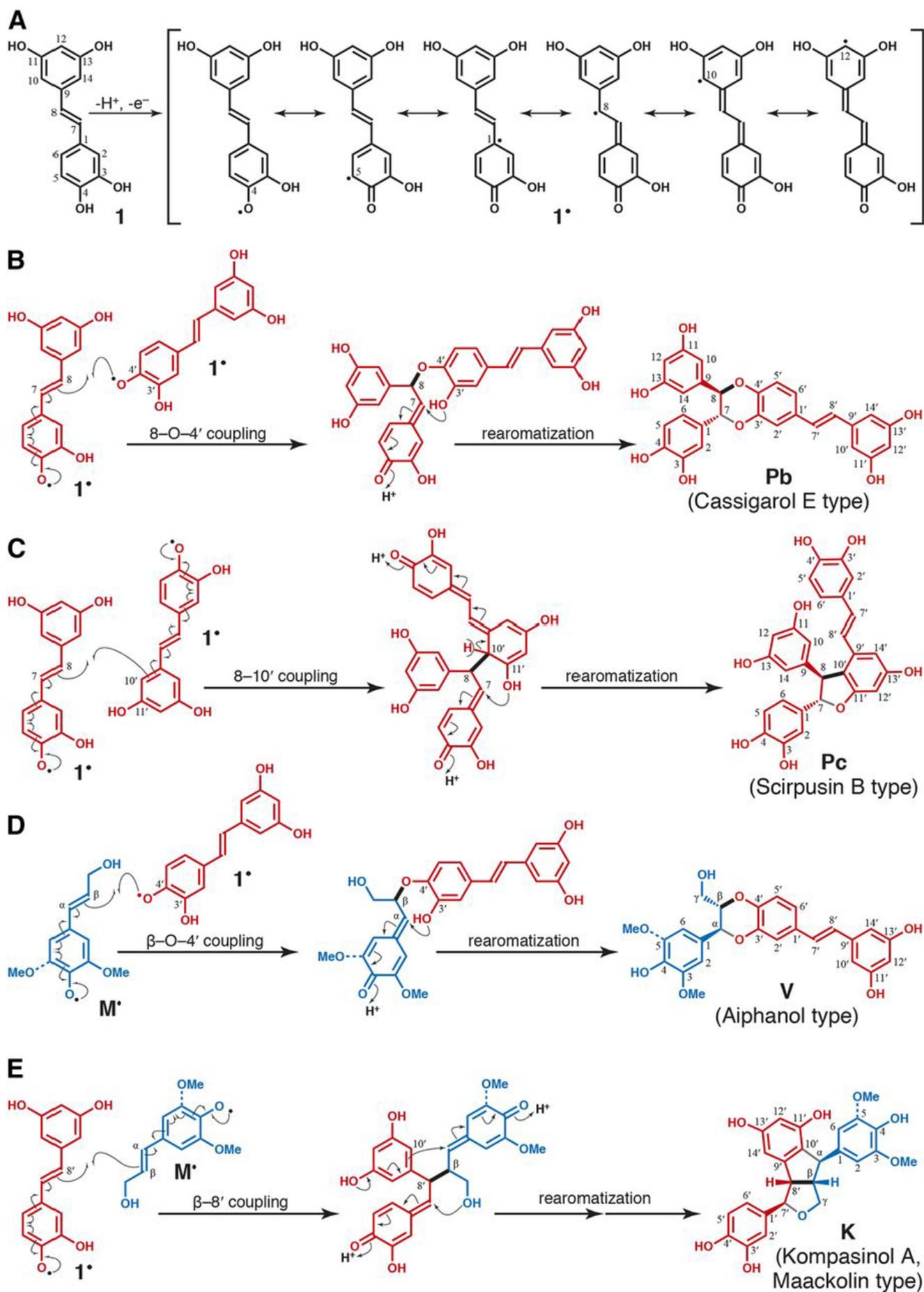
According to a strict definition, lignin is a polymer of the hydroxycinnamyl alcohol monolignols, sinapyl, coniferyl, and *p*-coumaryl alcohols (Vanholme et al., 2010). It has been found, however, that lignin can and does exhibit considerable plasticity with respect to its formation, incorporating numerous phenolic monomers even under normal growth and development of ‘natural’ plants, and more noticeably in plants with natural mutations or induced genetic modifications (Vanholme et al., 2012). A great number of monomers beyond the prototypical monolignols clearly act as monomers in various plants, such as ferulate esters (on arabinoxylan polysaccharides) in grasses (Ralph et al., 1995; Grabber et al., 2000; Bunzel et al., 2004), and the variously acylated monolignols (del Río et al., 2007, 2012, Martínez et al., 2008, Karlen et al., 2016, 2017, Lu et al., 2015a, b). More interestingly, recent reports have identified several phenolic compounds derived from outside the canonical monolignol biosynthetic pathway, such as the flavone triclin and the hydroxystilbene piceatannol, that behave as authentic lignin monomers participating in coupling and cross-coupling reactions with monolignols during lignification and being integrally incorporated into the lignin polymer (del Río et al., 2012; Lan et al., 2016a, 2016b; Rencoret et al., 2018). These

compounds are metabolic hybrids produced by combining the shikimate and acetate/malonate-derived polyketide pathways, rather than only the shikimate pathway that is responsible for monolignol formation.

The participation of piceatannol in lignification has been found to occur in the endocarp tissues of various palm fruits, such as macaúba (*Acrocomia aculeata*), carnauba (*Copernicia prunifera*), and coconut (*Cocos nucifera*) (del Río et al., 2017; Rencoret et al., 2018). Analyses of the lignins from these tissues revealed the presence of conventional guaiacyl and syringyl units along with piceatannol and small amounts of other hydroxystilbenes (resveratrol and isorhapontigenin). Subsequent results from NMR of isolated milled wood lignins and *in vitro* dehydrogenation polymer studies have revealed that piceatannol may both homo-couple and cross-couple with monolignols as shown in Fig. 1 (del Río et al., 2017), with further details of the scope and extent of both coupling modes still under investigation. Fig. 1A shows the dehydrogenation of piceatannol and the resonance structures of the resultant free radical. As with the monolignols, the hydroxycinnamates, hydroxycinnamaldehydes, and catechols, such as caffeoyl alcohol and 5-hydroxyconiferyl alcohol, the latter of which result from incomplete 3- and 5-OH methylation in the monolignol pathway, products invariably result from the 4-O-radical (that is conjugated through the sidechain).

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Fig. 1. A) Resonance structures from dehydrogenation of piceatannol. B) and C) homocoupling reactions of piceatannol. D) and E) Cross-coupling reactions of monolignols and piceatannol. del Río, J. C., Rencoret, J., Gutiérrez, A., Kim, H., Ralph, J., Hydroxystilbenes are monomers in palm fruit endocarp lignins. *Plant Physiol.* 2017, 174, 2072-2082. <https://doi.org/10.1104/pp.17.00362>. www.plantphysiol.org. Copyright American Society of Plant Biologists.

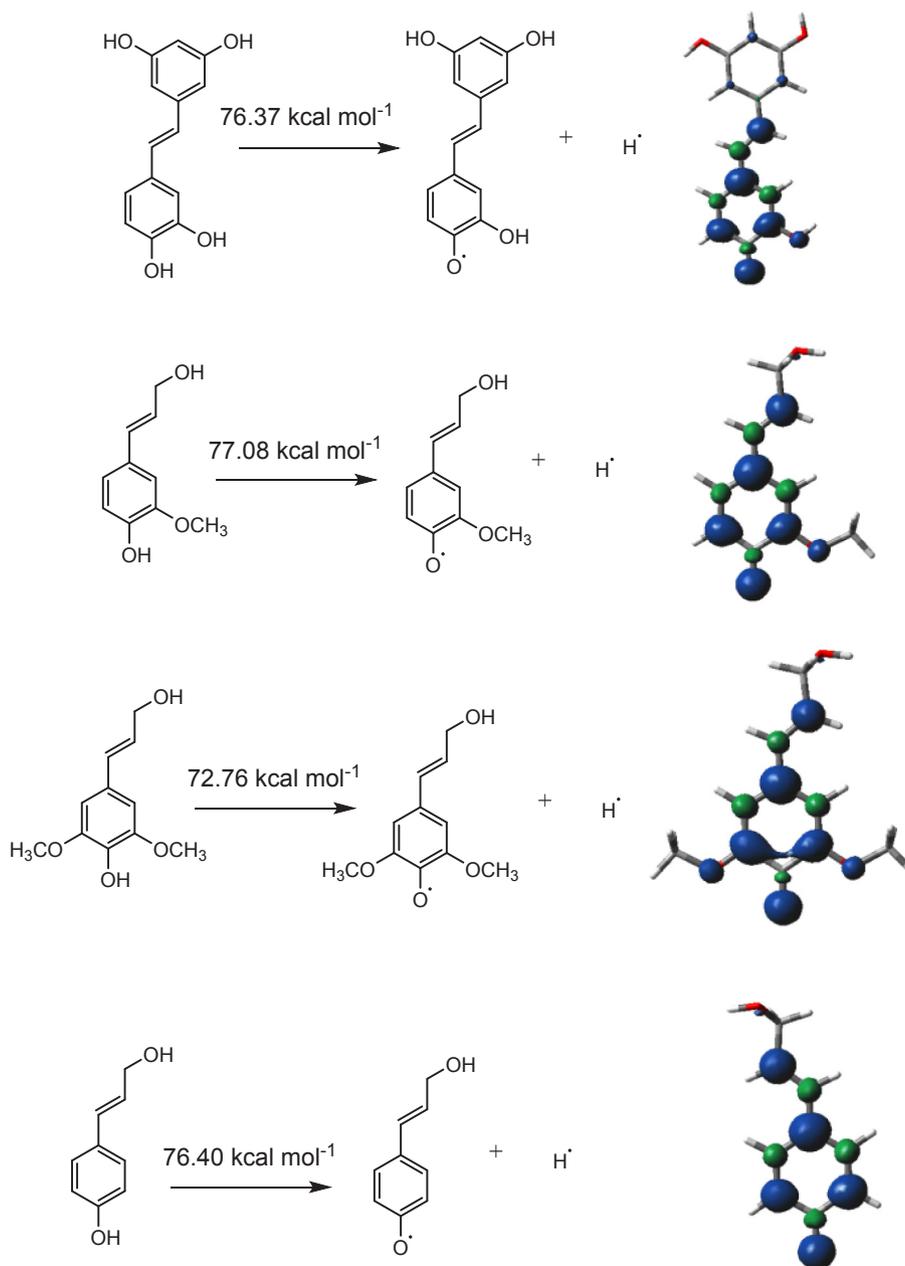


Fig. 2. Gibbs free energy of dehydrogenation reactions of piceatannol and the monolignols, and spin density plots for the free radicals.

Products of 3-*O*-coupling in caffeyl alcohol or 5-*O*-coupling in 5-hydroxyconiferyl alcohol are not evidenced. Fig. 1B and C illustrate two modes of piceatannol homo-coupling, namely 8-*O*-4' and 8-10' couplings, whereas 1D and 1E are the β -*O*-4' and β -8' cross-coupling reactions of piceatannol with a monolignol. In each of the coupling reactions at the favored β - or 8-positions on the sidechains, the first step results in the formation of a quinone methide. Quinone methides can be re-aromatized by proton-assisted nucleophilic attack by water or, as occurs in every case here, more favorably by internal trapping by appropriately situated (phenolic) hydroxyls, resulting in new 5- (in C and E) or 6-membered (in B and D) rings. The quinone methide in E is internally trapped by carbon attack of the electron rich aromatic ring (at its 10'-position), which can alternatively be regarded as electrophilic

aromatic substitution by the partially positively charged benzylic α -carbon of the quinone methide on the electron-rich aromatic ring.

Experimental results on milled wood lignin preparations from different palm fruit endocarps found that, among the different linkages involving piceatannol, the 8-*O*-4' and 8-10' homo-coupled structures predominate, with lower amounts of the cross-coupled β -*O*-4' structure, while the β -8' cross-coupled product was not found (del Río et al., 2017). Dehydrogenation polymers of piceatannol in the absence of monolignols resulted in the formation of 8-*O*-4' and 8-10' homo-coupled products in similar amounts. In the presence of monolignols, the β -*O*-4' cross-coupled products and 8-10' homo-coupled products were formed, the β -8' cross-coupled product was not detected and the 8-*O*-4' homo-coupled product appeared as a minor product, which can

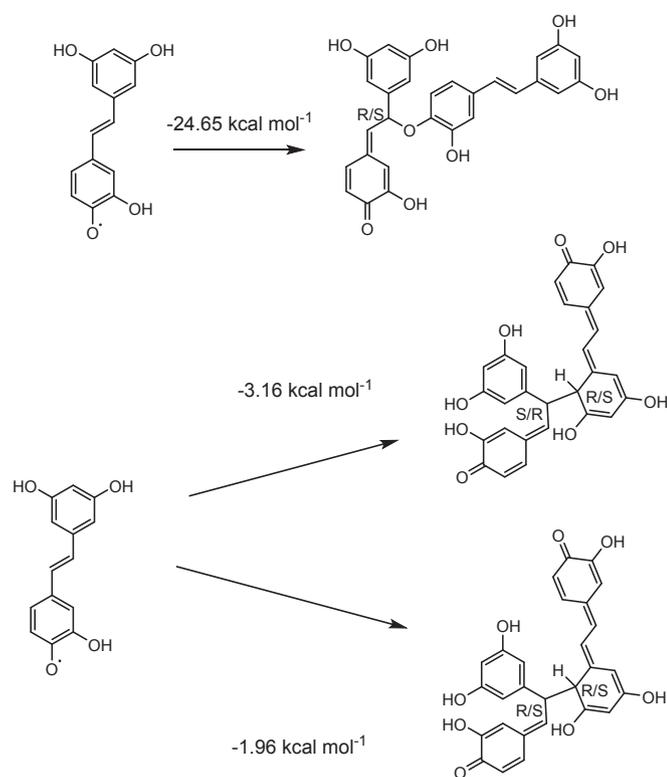


Fig. 3. Gibbs free energy of quinone methide formation by 8–O–4' and 8–10' homo-couplings.

be observed at close to the noise level in the 2D NMR spectra (del Río et al., 2017).

The chemical feasibility of such homo-coupled and cross-coupled reactions is supported by work that has isolated and identified stilbenolignan products from a number of plant sources (Cheng et al., 2014; Lam and Lee, 2010; Lee et al., 2001; Kulesh et al., 1995) but, as these products are usually optically active, dirigent proteins may be involved in guiding such coupling regiochemistries. In addition, synthetic preparations have been reported for these types of products (Pilkington and Barker, 2015; Chand and Banwell, 2007; Banwell et al., 2003, 2005; Kuboki et al., 2003). Although lignification and lignan formation are different processes, separated in time and space, the literature cited is indicative of the feasibility of such coupling reactions occurring *in planta*.

Given the experimental evidence that piceatannol can homo- and cross-couple during lignification, the objectives of the current work were to determine if there are thermodynamic preferences among the processes. Furthermore, the reactivity of the products toward the oxidation required for subsequent additions of monomers in the polymerization has been examined. This is accomplished by the application of density functional theory (DFT) calculations, to evaluate the energetics of quinone methide formation, the final rearomatization step, and the further dehydrogenation of the dehydrodimerization products. Such methods have been extensively applied to studies of lignin reactions and kinetics (Elder et al., 2017; Qi et al., 2017; Sangha et al., 2012, 2014; Beste and Buchanan, 2012; Kim et al., 2011; Parthasarathi et al., 2011). Also, because of its antioxidant properties, piceatannol, along with other stilbenes, has been extensively examined computationally (Mikulski and Molski, 2012; Nenadis and Stavra, 2017; Leopoldini et al., 2011; Lu et al., 2015a; b, 2017).

2. Results and discussion

Modeling of radical spin densities, and of radical coupling reactions to produce quinone methide intermediates, and then rearomatization to

the final products, was conducted to provide insight into coupling and cross-coupling propensities and the selectivity of these reactions.

2.1. Dehydrogenation of lignin monomers (piceatannol and monolignols)

The Gibbs free energy of the initial dehydrogenation reaction for each monomer is as shown in Fig. 2. These calculations were undertaken to ensure that the energy associated with hydrogen abstraction from the catechol moiety of piceatannol to produce the phenolic radical was consistent with that from the monolignols. If this were not the case, and if the essential initial oxidation reaction was found to be more energetic it might argue against the participation of piceatannol in the polymerization process. That said, it has been established that steric factors can determine the ‘radicalization’ of phenols, but that other mechanisms exist to obtain radicals. For example, horseradish peroxidase (HRP) readily effects 1-electron-oxidation of coniferyl alcohol but can do so only very slowly with sinapyl alcohol. Sinapyl alcohol can, however, be efficiently oxidized by radical transfer by, for example, *p*-coumarate (Ralph et al., 2004; Hatfield et al., 2008). It can be seen that the dehydrogenation energy of piceatannol is indeed not dissimilar to those of the monolignols, such that this initial reaction step is feasible. Fig. 2 also shows the unpaired spin densities for each of the free radicals. Those of the monolignols are, as would be expected from resonance structures, with the spin densities concentrated on the phenolic oxygen, and carbons 1, 3, 5 and β . Interestingly, for piceatannol, the spin density is largely restricted to the phenoxy oxygen, and carbons 1, 3, 5 and 8, with relatively little presence at positions 10, 12 and 14 for which resonance structures can be drawn (Fig. 1A). Although this is at variance with the experimental results that find the presence of 8–10' homo-coupling (Fig. 1C), the spin densities from this work are consistent with those in the previous literature (Nenadis and Stavra, 2017).

2.2. Quinone methide formation

2.2.1. Piceatannol homo-coupling: 8–O–4' and 8–10' couplings

The Gibbs free energy associated with the 8–O–4' and 8–10' homo-coupling reactions are shown in Fig. 3. As discussed in the Methods, such coupling reactions lead to the formation of chiral centers but are formed racemically. Since the energies of enantiomers must be equal and are generally within ‘chemical accuracy’ (Elder et al., 2016; John et al., 1989; Lewars, 2011) in our calculations, in this and the succeeding sections, the stereochemistry will be addressed by reporting the energies of one set of enantiomers.

The 8–O–4' coupling forming the *R/S* quinone methide product is an exergonic reaction, with a Gibbs free energy of $-24.65 \text{ kcal mol}^{-1}$. The 8–10' reaction is also exergonic with coupling producing the *SR/RS* stereoisomer more so than the *RR/SS* form. The striking observation, however, is that this reaction is markedly less exergonic than 8–O–4' coupling by over 20 kcal mol^{-1} . It will be recalled from Fig. 2 that the spin density at the 10' carbon is quite low which, in concert with the disruption of the aromatic system upon coupling, may account for the observed decrease in exergonicity with the formation of this linkage. However, the overall energies can be affected by the subsequent rearomatization step, with the rearomatization delivering a significant driving force.

2.2.2. Piceatannol and monolignol cross-coupling: β -O–4' and β -8' cross-couplings

The Gibbs free energy results from β -O–4' cross-coupling for each of the monolignols with piceatannol are shown in Fig. 4. These values are generally consistent with the analogous 8–O–4' homo-coupling in the range of *ca.* -23 – 26 kcal mol^{-1} , with the *p*-coumaryl alcohol product being measurably more exergonic than with the other monolignols. A possibly interesting relationship has been detected between the exergonicity of the quinone methides formed by β -O–4' cross-coupling and 8–O–4' homo-coupling of piceatannol, as shown in Fig. 5. Despite

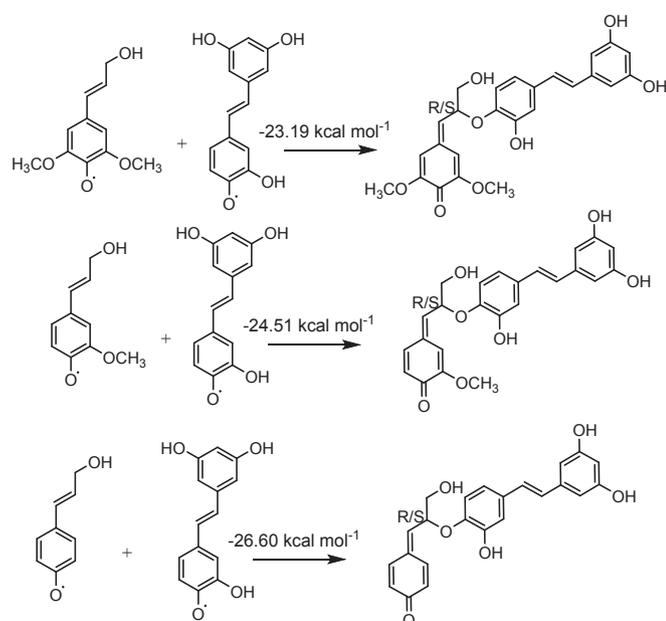


Fig. 4. Gibbs free energy of β - O -4' cross-coupling of piceatannol and mono-lignols.

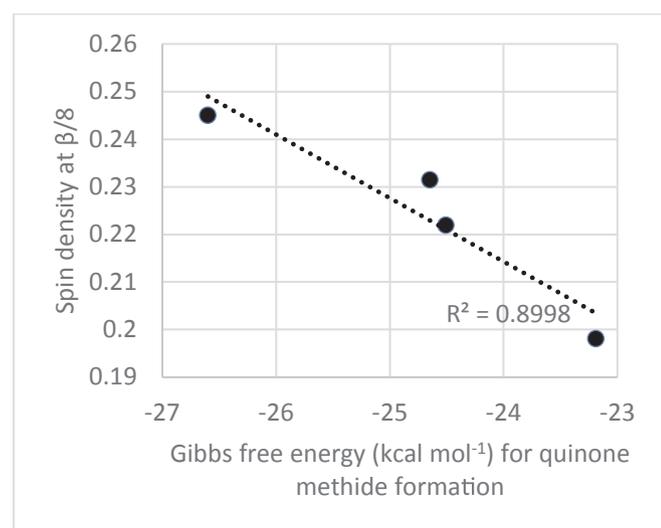


Fig. 5. Relationship of Gibbs free energy and spin density at the $\beta/8$ -carbon.

the small number of data points and minimal variation in the exergonicity, there is an excellent correlation (R -square = 0.90) with the spin density at the β -position in the monolignols and the corresponding C-8 in piceatannol. Such electronic results may be indicative of the underlying differences that lead to variations in thermodynamics.

Among the β -8' cross-coupled quinone methide products, the reaction between piceatannol and the *p*-coumaryl alcohol radical is the most exergonic, perhaps due to reduced steric hindrance (Fig. 6). The least exergonic is the formation of the sinapyl alcohol-piceatannol *SR/RS* quinone methide. These quinone methides are uniformly less stable than those from β - O -4' cross-coupling, by 1.35–4.26 kcal mol⁻¹ perhaps due to the lower spin density at the 8' position (0.23) in comparison to the O -4' (0.31). Stereochemistry has a minimal effect on the exergonicities of the coniferyl and *p*-coumaryl cross-coupled products, whereas the sinapyl alcohol products differ by more than 3 kcal mol⁻¹, with the *RR/SS* stereoisomer being the more stable. Given the presence of two methoxy groups, which are found to be eclipsed in the *SR/RS* stereoisomer, it might be proposed that the difference is due to steric factors.

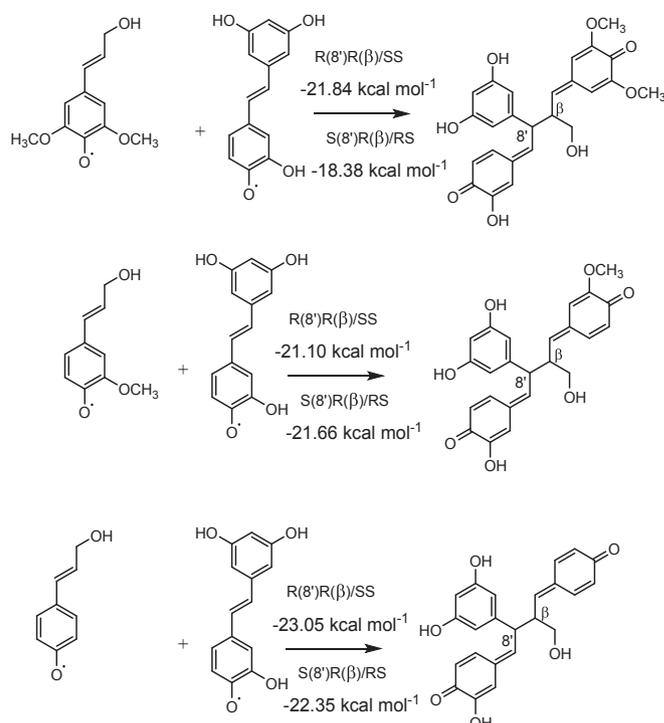


Fig. 6. Gibbs free energy of β -8' cross-coupling of piceatannol and cinnamyl alcohols.

Parenthetically, as a check on these results and for comparison purposes, calculations were also performed on quinone methides resulting from β - O -4' homo-coupling of the monolignols. Although such results have been reported in the previous literature (Qi et al., 2017; Sangha et al., 2014, 2012; Beste and Buchanan, 2012; Kim et al., 2011; Parthasarathi et al., 2011; Elder et al., 2016), they were repeated in the current study to ensure methodological consistency. The exergonicities were found to be -21.46, -26.23 and -29.37 kcal mol⁻¹ sinapyl, coniferyl and *p*-coumaryl alcohol radical couplings, respectively. The quinone methide formation reactions under examination in the current study do not differ markedly from these, and the order is the same for the homo-coupling of monolignol radicals and the β - O -4' cross-coupling reactions.

2.3. Rearomatization reactions

2.3.1. Piceatannol homo-coupling: 8- O -4' and 8-10' couplings

Fig. 7 shows the Gibbs free energy for the rearomatization reactions of the homo-coupled 8- O -4' and 8-10' quinone methides. Formation of the benzodioxane ring for the 8- O -4' structure by internal trapping of the quinone methide intermediate by the 3'-hydroxyl group, generates *SR/RS* and *SS/RR* stereoisomers for which the energy difference is minimal, but corresponds to the experimentally favored *trans* configuration of the benzodioxane ring. Similarly, the 8-10' structure generates, by the subsequent formation of the 11'- O -7 bond during rearomatization of the quinone methide intermediate, a phenylcoumaran structure with two chiral centers, for which the *RR/SS* products, also consistent with the *trans* configuration, are more stable by 1.90 kcal mol⁻¹. It can be seen that the rearomatization energies for these reactions are considerably larger than for the other quinone methides, but these reactants are made up of two quinone methides, each of which is rearomatized. It may be interesting to note that the rearomatization energies of 8-10' products, involving an additional aromatic center are roughly double that for the other reactions which involve single quinone methides.

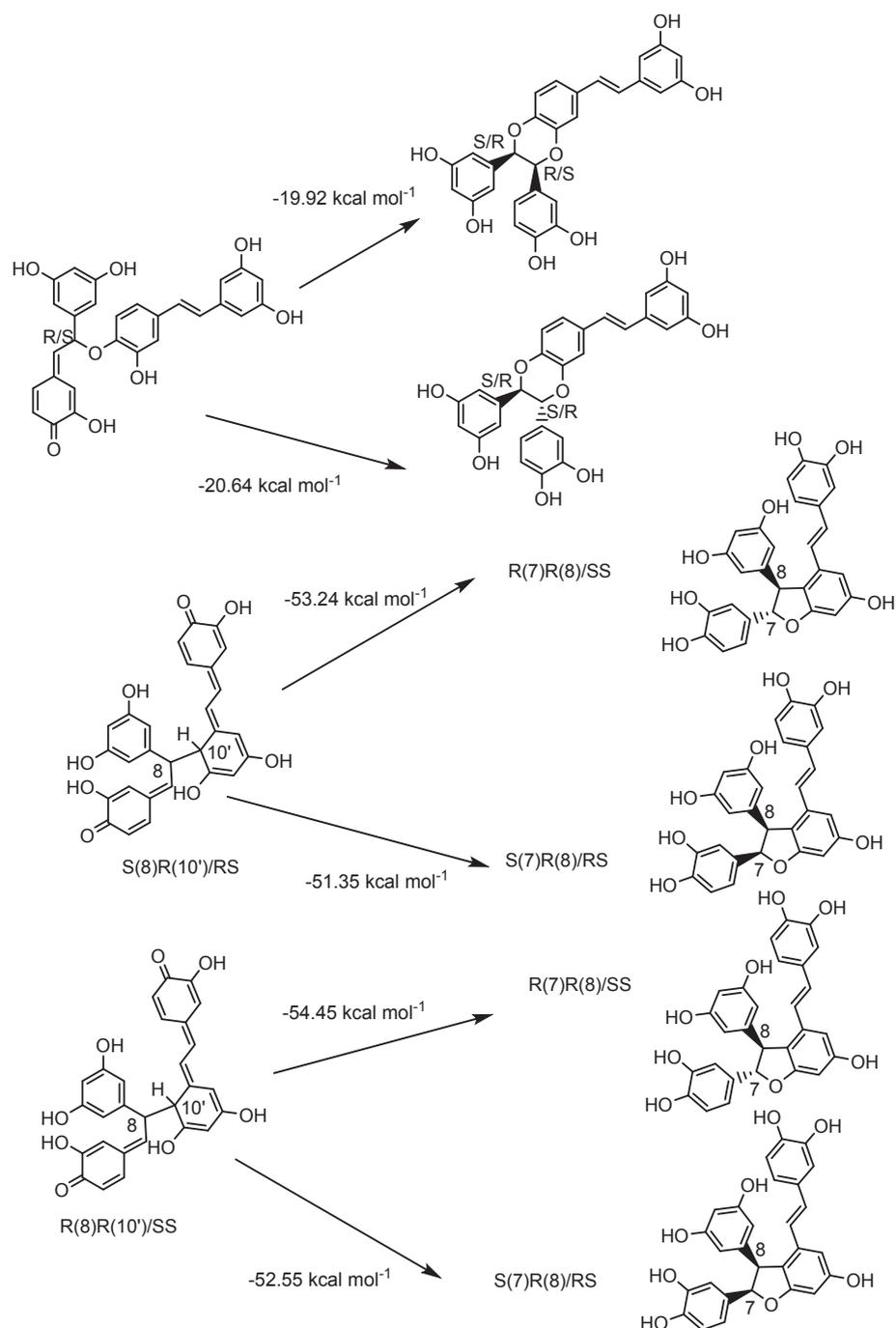


Fig. 7. Gibbs free energy of rearomatization for 8–O–4' and 8–10' homo-coupled quinone methides.

2.3.2. Piceatannol and monolignol cross-coupling: β -O-4' and β -8' cross-couplings

The Gibbs free energy of rearomatization reactions for the β -O-4' cross-coupled products, by internal trapping of the quinone methide intermediate by the 3'-hydroxyl group in the piceatannol unit forming a benzodioxane structure, are shown in Fig. 8. As in the previous section, two chiral centers are formed at the α - and β -carbons of the original monolignol. For the coniferyl alcohol cross-coupled product the *RR/SS* stereoisomeric product is 2.52 kcal mol⁻¹ more stable, which constitutes a measurable difference if a value of 2 kcal mol⁻¹ is assumed for chemical accuracy. It is also observed that these reactions are more exergonic than for the piceatannol homo-coupled product. The rearomatization reactions of the sinapyl alcohol cross-coupled product are less exergonic than for the coniferyl alcohol-piceatannol cross-coupling

product, but more exergonic than homo-coupled piceatannol, and the *RR/SS* stereoisomer is more stable by 1.49 kcal mol⁻¹. Cross-coupling of *p*-coumaryl alcohol continues the trends of increased exergonicity over homo-coupled piceatannol rearomatization, and the higher stability of the *RR/SS* stereoisomer. In all cases the formation of the *RR/SS*, or *trans*, stereoisomer is more exergonic.

The rearomatization of the β -8' cross-coupled quinone methides, by subsequent γ -O-7' and 10'- α bond formations producing the 3-oxabicyclo-[3.3.0]-octane ring structure, per Figs. 9–11, results in systems with 4 chiral centers and therefore 8 pairs of enantiomers. The rearomatization energies for these structures are analogous to those of the 8–10' homo-coupled piceatannols, in that there are two quinone methide groups present. The energies of the rearomatization reactions are generally similar for all of the monolignols but major differences were

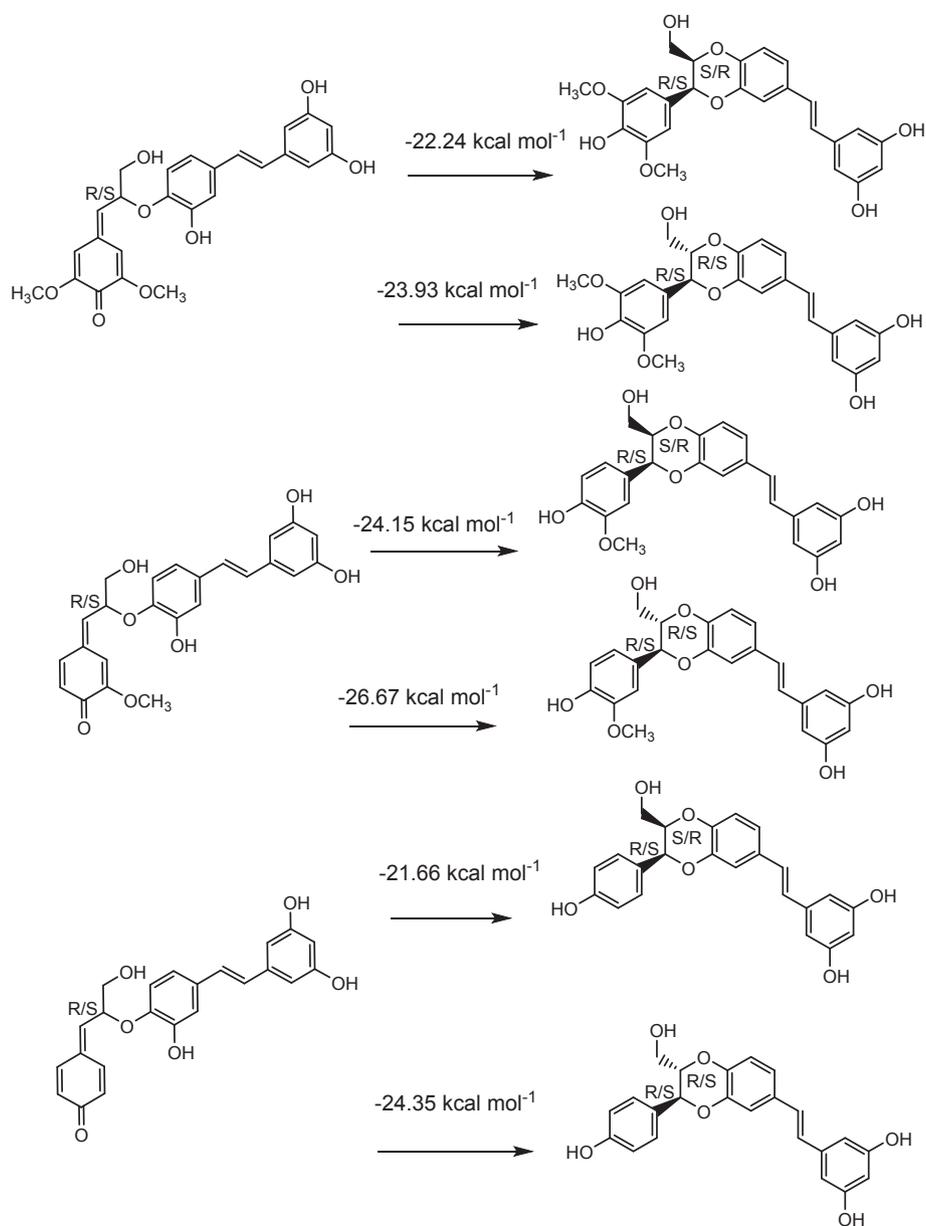


Fig. 8. Gibbs free energy of rearomatization for β -O-4' cross-coupled quinone methides.

found with stereochemistry of the products, due to the relative stability of the latter. Products with *RR/SS* stereochemistry at positions β and 8' are much more stable, which translates into more exergonic energies of rearomatization, and is consistent with the *cis* configuration reported for kompasinol A that has been reported in the experimental literature (Kobayashi et al., 1996).

2.4. Dehydrogenation reactions of homo- and cross-coupled products

Lastly, the dehydrogenation of homo- and cross-coupled dimers was examined, per the reactions in Fig. 12. This part of the study was undertaken to determine if the presence of piceatannol altered the energetics of the reaction, which could in turn affect the ability of piceatannol to participate in the polymerization or nucleation process. The Gibbs free energy of dehydrogenation for each reaction, cross-coupled monolignol (where appropriate) and stereochemistry is shown in Table 1. Reactions 1 and 2 of the homo-coupled dimers exhibit similar energies and minimal differences as a function of stereochemistry. Within the β -O-4' cross-coupled dimers in reaction 3 the energetics are

consistent for the coniferyl and sinapyl alcohol-linked structures but cross-linking with *p*-coumaryl alcohol results in a markedly higher energy of dehydrogenation. This observation generally holds true for the β -8' dimers (reaction 4) as well, although there are two stereoisomers among the *p*-coumaryl alcohol cross-linked structures with dehydrogenation energies that are more similar to those from the other monolignols. It is apparent from this that the introduction of a *p*-coumaryl group can have an impact on the thermodynamics of these reactions. Finally, for comparison purposes, the dehydrogenation energies of the β -O-4'-linked dimers (reaction 5) of the monolignols were determined and were found to be within the range of ~ 70 – 79 kcal mol⁻¹. Based on this, whether through homo-coupling or cross-coupling, piceatannol does not negatively impact the energies of dehydrogenation and the subsequent polymerization steps in lignin formation should be able to proceed.

In summary, experimental results from del Río and co-workers (del Río et al., 2017) reveal that the lignin from palm fruit endocarps contains the 8-10' and 8-O-4' products and smaller amounts of β -O-4' cross-coupled products, while the β -8' structure was not found.

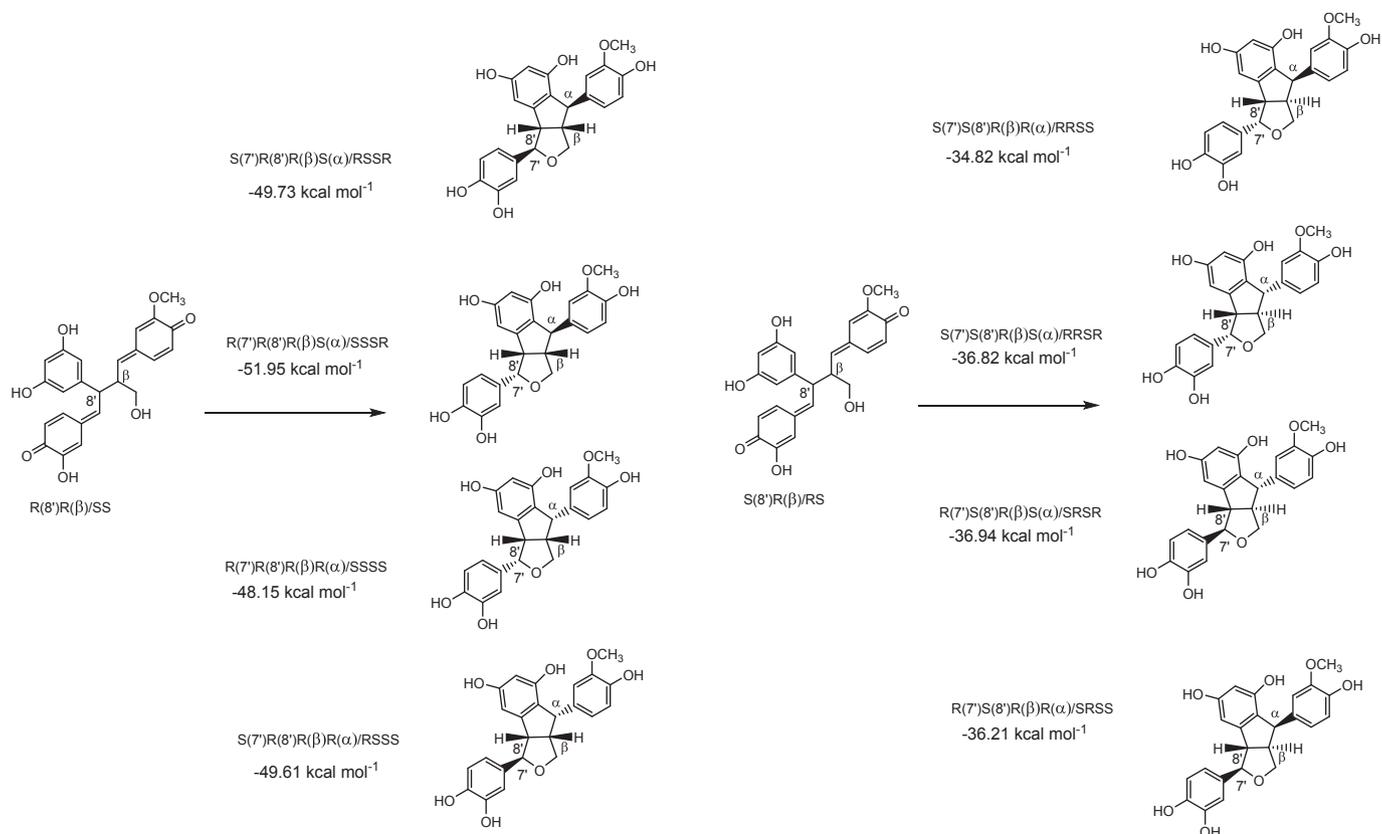


Fig. 9. Gibbs free energy of rearomatization for β -8' conferyl alcohol cross-coupled quinone methides.

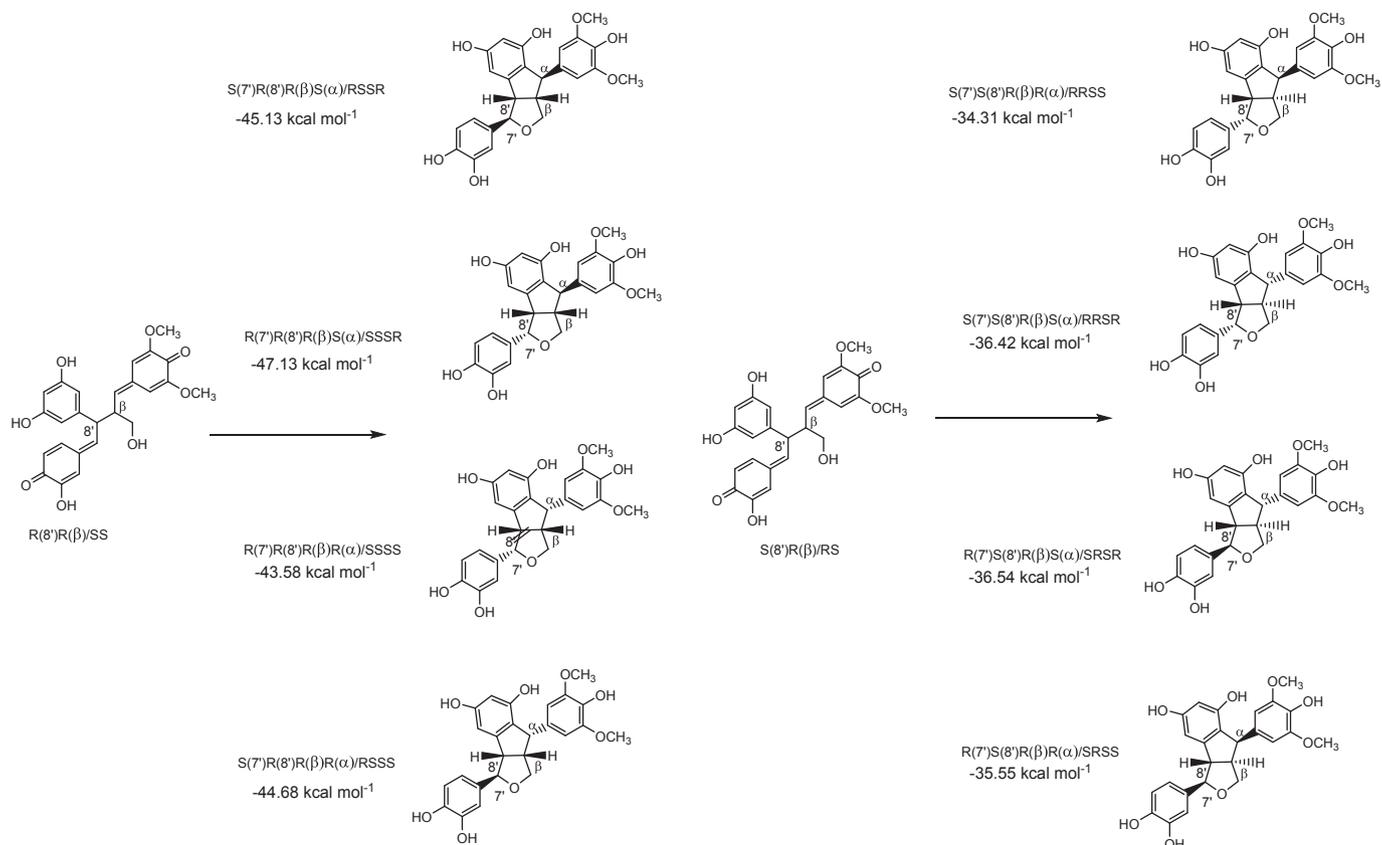


Fig. 10. Gibbs free energy of rearomatization for β -8' sinapyl alcohol cross-coupled quinone methides.

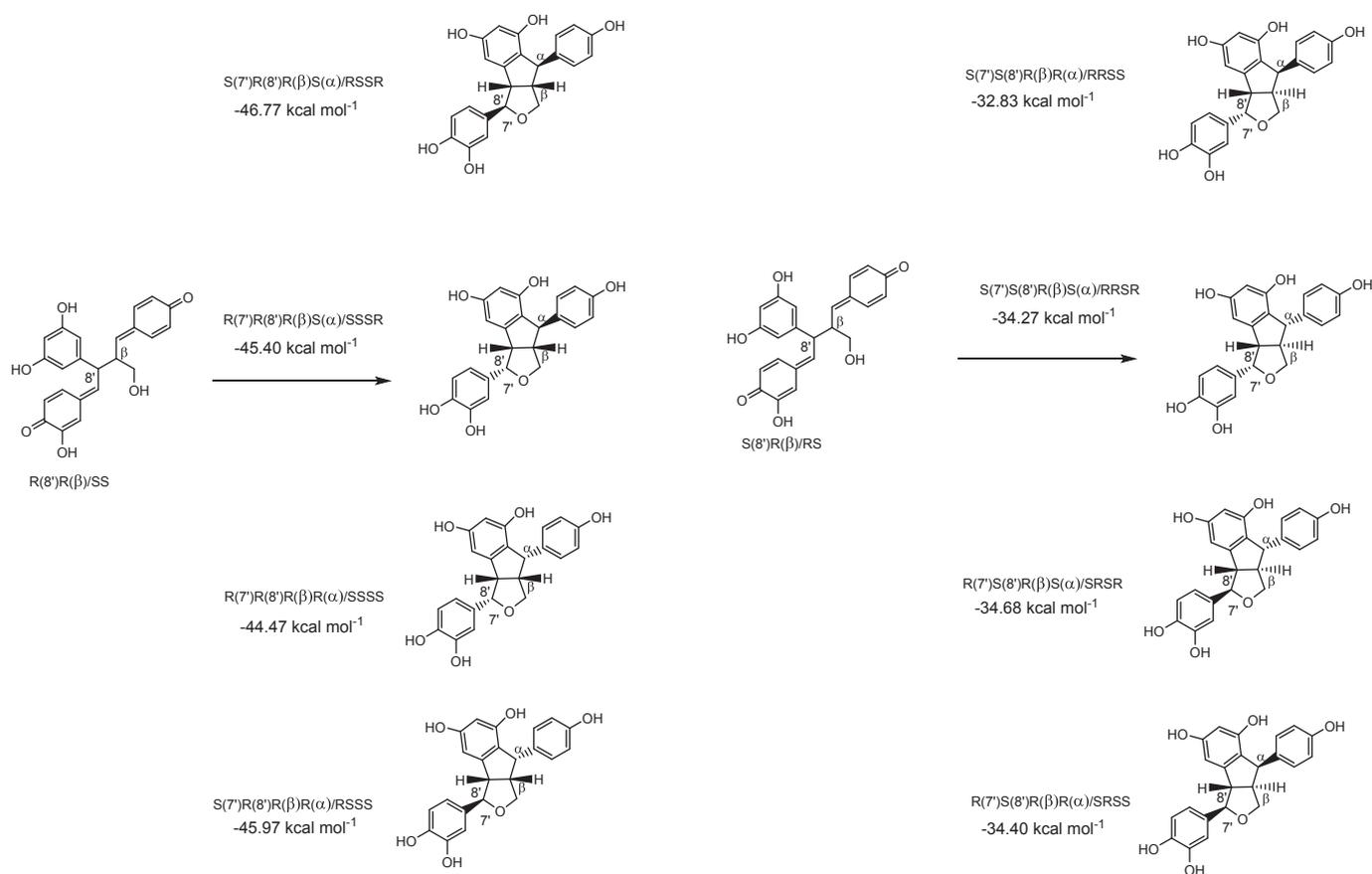


Fig. 11. Gibbs free energy of rearomatization for β -8' *p*-coumaryl alcohol cross-coupled quinone methides.

Dehydrogenation polymers formed with both piceatannol and monolignols as the substrates resulted in the formation of the 8-10' and β -O-4' products with the 8-O-4' as a minor product, whereas if piceatannol was the only substrate both 8-10' and 8-O-4' linkages were detected.

Based on these results the β -8' linked products are not formed under any of the conditions employed, although the analogous kompasinol A stilbenolignan has been prepared synthetically (Chand and Banwell, 2007) and isolated from plant sources (Kobayashi et al., 1996; Lam et al., 2008). Also, from the current work, the thermodynamics are favorable for the formation of these products. In contrast the 8-10' homo-coupled structure is found in all preparations. The free energies for initial quinone methide formation are only slightly exergonic but, when combined with the rearomatization step, the overall reaction is quite exergonic, perhaps accounting for its consistent formation. The consistent appearance of the 8-10' product is also at variance with the calculated spin densities, in which the unpaired electron density at the C-10 position is quite low, but is in agreement with the computational chemistry literature (Nenadis and Stavra, 2017). Finally, the observation that 8-O-4' structures are found in the native lignin as a major structure, but only as a minor product in dehydrogenation polymers derived from piceatannol and monolignols raises questions. It is obvious that the 8-O-4' bond can form and the thermodynamics are feasible. The differences in reaction that occur *in vitro* await further scrutiny.

Finally, an examination of the dehydrogenation energies for the homo-coupled and cross-coupled products revealed that in general the thermodynamics of the reaction are similar to those of the cinnamyl alcohol dilignols. This observation is important for the subsequent incorporation of such structures into the growing lignin polymer. The exceptions to this generalization are that several of the *p*-coumaryl alcohol cross-coupled products that are considerably more endothermic.

In conclusion, the thermodynamics associated with formation and

reactions of linkages that involve piceatannol are comparable with those of the monolignols and would therefore pose no obstacle to their participation in the lignification process.

3. Methods

The reactions evaluated in the current work are as shown in Fig. 1. With respect to Fig. 1D and E, all three of the monolignols (sinapyl, coniferyl and *p*-coumaryl alcohols) were cross-coupled with piceatannol. It can be seen that with both quinone methide formation and rearomatization, the formation of stereochemical centers occurs (Ralph et al., 2009). Although there are reports in the stilbenolignan literature regarding specific configurations, these may have been selected for biosynthesis due to their particular physiological properties, and may involve assisted coupling by dirigent proteins (Davin et al., 1997). Furthermore, there has never been any evidence found for optical activity in lignins (Ralph et al., 1999, 2008). Based on these points, no assumptions were made regarding stereochemistry such that an extensive examination of possible stereochemical configurations representing one set of enantiomers was performed. This limitation is justified based on the fact that, with the exception of rotation of plane polarized light, enantiomers are identical in all respects, including energetics. In previous work (Elder et al., 2016), inversions of chiral centers in lignin models were explicitly addressed and differences in energy were generally within the 1–2 kcal mol⁻¹ range described as “chemical accuracy” (John et al., 1989; Lewars, 2011). Although this means that the structures are not energetically distinguishable, they were not found to be identical as required of enantiomers. These discrepancies may be due to the geometric complexity of the lignin models in both the previous and current work and the multidimensional nature of the potential energy surface, which even with conformational searching, can result in inverted structures that vary slightly in both

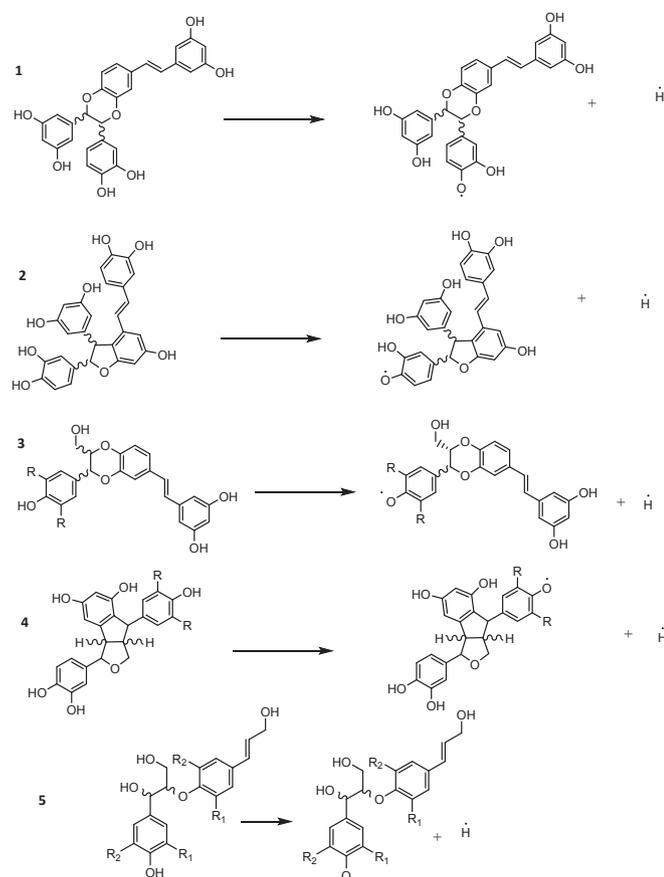


Fig. 12. Dehydrogenation reactions of 8–O–4' and 8–10' homo-coupled and β -O–4' and β -8' cross-coupled dehydrodimerization products. The dehydrogenation reactions of the β -O–4' dehydrodimerization product of monolignols is also shown. The numbering of the reactions corresponds to the numbering in Table 1.

geometry and energy. Constraining the current work to one set of enantiomers is based on these factors and findings.

With both the initial coupling and rearomatization, the products can have numerous rotational degrees of freedom. To address this, a conformational search was performed using a 1000-step Monte Carlo procedure, with MMFF minimization as implemented in Spartan'16 (Spartan, 2016). The unique conformations from this step were next optimized using the PM6 semi-empirical method in Spartan'16 (Spartan, 2016). The 10 resulting lowest-energy conformations were further refined with the M06-2X density functional method, the 6-31 + G(d) basis set, ultrafine integration grid, and GD3 empirical dispersion. Lastly, the lowest-energy conformation was submitted to M06-2X/6-311 + +G(d,p) optimization, again with the ultrafine integration grid, GD3 empirical dispersion, and the determination of harmonic vibrational frequencies to verify the identification of a stationary point and for thermal corrections to the electronic energy for the determination of Gibbs free energy. The density functional theory calculations were all performed with Gaussian 16, Revision A.03 using the default optimization criteria throughout (Gaussian16, 2016). The numbering scheme used throughout was as shown in Fig. 1.

Supporting information

Cartesian coordinates for low energy conformation of all compounds, optimized using M06-2X/6-311 + +G(d,p).

Table 1
Gibbs free energy of dehydrogenation for structures in Fig. 12.

Reaction	monolignol	stereochemistry	Energy (kcal mol ⁻¹)	
1	NA	RS/SR	80.12	
		RR/SS	79.44	
2	NA	RS/SR	79.41	
		RR/SS	79.63	
3	coniferyl	RS/SR	79.93	
		RR/SS	79.53	
		RS/SR	74.76	
		RR/SS	74.69	
4	coniferyl	RS/SR	99.96	
		RR/SS	99.63	
5	coniferyl	RRRR	78.52	
		RRRS	79.40	
		RRSR	80.38	
		RRSS	78.87	
		RSRR	79.09	
		RSRS	79.59	
		RSSR	79.45	
		RSSS	79.58	
		sinapyl	RRRR	74.56
			RRRS	73.92
			RRSR	75.23
			RRSS	74.30
p-coumaryl	RSRR	73.82		
	RSRS	74.60		
	RSSR	74.26		
	RSSS	74.28		
	RRRR	79.68		
	RRRS	99.76		
	RRSR	99.95		
	RRSS	79.40		
p-coumaryl	RSRR	99.79		
	RSRS	99.88		
	RSSR	99.81		
	RSSS	99.89		
	RS/SR	79.15		
	RR/SS	76.35		
	RS/SR	71.90		
	RR/SS	71.20		
p-coumaryl	RS/SR	79.99		
	RR/SS	78.65		

Declarations of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work used the Extreme Science and Engineering Discovery Environment (XSEDE), which is supported by National Science Foundation grant number ACI-1548562. Specifically, it used the Bridges system at the Pittsburgh Supercomputing Center (PSC) and Stampede2 at the Texas Advanced Computing Center (TACC), both via MCB-090159 to GTB. JR and HK were funded by the DOE Great Lakes Bioenergy Research Center (DOE Office of Science BER DE-FC02-07ER64494 and DE-SC0018409). JRe and JCdR were funded by the Spanish Projects CTQ2014-60764-JIN and AGL2017-83036-R (financed by Agencia Estatal de Investigación, AEI and Fondo Europeo de Desarrollo Regional, FEDER). GTB acknowledges support from the US Department of Energy Office of Energy Efficiency and Renewable Energy Bioenergy Technologies Office under Contract No. DE-AC36-08GO28308 to the National Renewable Energy Laboratory.

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