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Elastic molecular movements in lignin β-O-4 oligomers under tensile and compressive forces as approximated by *ab initio* calculations of selected bond rotations

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Abstract: *Based upon the most stable three-dimensional geometry for lignin β-O-4 dimeric structures, the C_α-C_β bond rotation is implicated in an elastic response to external tensile forces. Estimates of the C_α-C_β rotation barrier at the* ab initio *computational level indicate that this response is independent of monolignol type. In contrast, the barrier to aromatic ring rotation in response to external compressive forces increases from* p-*hydroxyphenyl to guaiacyl to syringyl β-O-4 repeating units. One of the functions of the methoxyl group is apparently to modulate the elasticity of the lignin polymer.*

Keywords: β-O-4 oligomer, ab initio, rotation barriers, lignin elasticity

Lignin is a naturally occurring polymer synthesized within the cell wall of woody plants. The three-dimensional structure of lignin is unknown, although lignin is second only to cellulose in natural abundance and thus occupies an important position in the global carbon cycle. Without *in vivo* three-dimensional structural information, the biological function of lignin at the molecular level remains uncertain. Lignin deposition within the cell wall involves the incorporation of one or more of the three different monolignols (*p*-coumaryl, coniferyl, or sinapyl alcohol) into the lignin polymer via a free-radical mechanism *(1–3)*. The amount of each monolignol incorporated varies with species, cell type, and specific location within the cell wall *(4–6)*. Thus, there is perhaps no single, three-dimensional structure for the lignin polymer. If there exists a difference in physical and mechanical properties in the lignins formed from the three monolignols, then this difference must necessarily involve the presence or absence of the methoxyl substituents on the aromatic ring.

Lignin is polymerized *in situ* within a preformed cell wall matrix *(4–10)*. The native, three-dimensional structure of the lignin polymer is constrained by this matrix and is unlikely to exist in its absence. Lignin is deposited throughout the middle lamella and in both the primary and secondary cell walls of woody tissue. Electron microscopy has revealed a lamellar nature for the space available for lignin in the secondary cell wall *(11)*. The framework that defines the space for lignin deposition varies in chemical composition and geometry. Both of these factors are interrelated: specific hemicellulose composition promotes specific geometrical patterns. Hemicelluloses are thought to coat the cellulose microfibrils *(12–14)*.

Once an available space for lignin deposition can be described and geometrically detailed at the molecular level with respect to microfibrils and hemicelluloses, the

question of initiation must be considered. Ferulic acid residues esterified to the polysaccharide matrix have been suggested as initiation sites for lignin polymerization in grasses *(7)*. It is not known if ferulic acid residues are a necessary condition to initiate lignin polymerization. Dimeric or trimeric quinone methide intermediates may react with polysaccharide hydroxyls, providing another type of anchor for continued monolignol deposition. The more flexible and exposed side-chain residues of the hemicelluloses can be targeted for reactions of this type. The number and location of initiation sites within a geometrically constraining matrix not only determine how lignin deposition begins, but also how lignification proceeds directionally. The proximity of phenoloxidases to the initiation site and the directional supply of monolignol free radicals will also affect the directional progression of lignification.

Lignin is a water-insoluble polymer. Even dimers and trimers are poorly soluble in aqueous environments. Yet the prelignified cell wall matrix is highly hydrated. Any oligomeric lignin in this aqueous environment will preferentially seek a nonaqueous or hydrophobic resting place. In the early stages of lignification of the lamellar space, this hydrophobic environment is most likely supplied by polysaccharide surfaces. In later stages, lignin itself becomes a more suitable respite. The process of lignification can, therefore, be considered a surface phenomenon: oligomeric lignin interacts noncovalently with the preexisting polysaccharide surfaces within the cell wall. Monolignol free radicals react with surface-exposed lignin.

Soon after lignin deposition has started, a lignin–water interface develops. The aromatic nature of lignin tends to exclude water molecules, creating a self-containing hydrophobic environment *(4)*. The three-dimensional geometry of this interface is constrained by the polysaccharide matrix and is continually reshaped with the addition of each new monolignol. It would perhaps not be unreasonable to view lignification proceeding as a wave of synthesis, the reactive edge consisting of previously formed lignin situated at the border between a hydrophobic and considerably more hydrophilic region. The packing of the aromatic rings in the newly formed lignin is influenced by this hydrophobic–hydrophilic interface and interactions with the presumably highly water-solvated polysaccharide matrix. Lignin deposition within a defined geometric space in the cell wall matrix will not drive out all the preexisting water molecules. The water that does remain is very likely tightly hydrogen-bonded to backbone residues in the polysaccharide matrix. In the contact region between lignin and the polysaccharide cell wall matrix, the strength of any hydrogen bonds that form will be intensified by the exclusion of water. This loss of water could alter the shape of cell wall polymers. The warping and cracking of wood during the lumber drying process is an extreme example of the physical effects of water removal. These observations also suggest that strong forces, due to polymeric conformational changes, are controlled to some extent by the presence or absence of water.

The fate of phenol oxidases involved in lignin polymerization may also be subject to the directional effects of lignification and the lignin–water interface. Preferring a hydrophilic or water phase, their mobility within the cell wall matrix could have a decided effect upon the direction of lignin deposition.

The recent discovery of the first dirigent protein has significantly changed our thinking with regard to monolignol coupling and lignin polymerization. This protein supplies the correct three-dimensional arrangement of amino acids to promote

stereospecific coupling of two guaiacyl free radicals to produce (+)pineoresinol *(15)*. It would perhaps be closer to the truth to consider every linkage in lignin to be specifically controlled by the previously described mechanisms and/or by proteins that have yet to be discovered.

Forces determine the structure of a molecule. Chemical forces, inherent in the nature of matter, are partly responsible for the preferred conformational state of any molecule. The three forces that create the covalent chemical bond between elements with a distinctive length and angle between bound atoms are: van der Waals' forces, hydrogen bonding, and electrostatic interactions.

Molecular mechanics and molecular quantum mechanics (*ab initio*) are two different computational approaches used to predict the results of these chemical forces in a quantitative manner. They differ both in theoretical basis and in the mathematical formulations used to describe the interactions of atoms within a molecule. Computationally, the net forces on a molecule at a stationary point (energy minimum) are zero. Its structure has no thermodynamic reason to change. Unless perturbed by an external force such as kinetic (thermal) energy from surrounding molecules, it will remain at rest. An equilibrium structure is incapable of generating the internal growth stresses known to be present in the living tracheid. Only a structure displaced from equilibrium can generate a force because of its tendency to return to that equilibrium state.

The physical properties of a molecule are intimately related to the structure of the molecule. The measured response of a polymeric material to externally applied forces serves to classify the properties of that material. Isolated lignins have been investigated in this regard *(16–18)*. However, studies of bulk lignins are difficult to relate to the lamellar structure of the secondary cell wall. Nevertheless, one point is clear: water is a plasticizer for lignin. The thermal softening temperature of lignin varies with the water content of the preparation *(19)*. This phenomenon not only occurs in bulk lignin but also in the intact woody cell wall *(20)*. Since water competes with hydroxyl groups for hydrogen bonding, we have focused considerable attention upon the intramolecular hydroxyl hydrogen bonding possibilities inherent in the β-O-4 linkage.

Mechanical stress is also part of the microenvironment that may well shape the three-dimensional structure of lignin as it is deposited. The natural state of a wood tracheid cell is to be under stress. Some of this stress is static due to gravitational loading. Some stress varies with time, such as that due to wind force and branch loading by snow or rain. These are external forces. Internal forces or growth stresses are also operative. Thus, the tracheid is in a constant state of stress from multiple external and internal forces. The three-dimensional structure of the β-O-4 linkage is also displaced from equilibrium as a result of these forces. Isolated thin sections of wood as well as isolated lignins are released from the forces found in the living tree, and will adjust their structure accordingly. The conclusions based upon Atalla's work *(21)* concerning lignin conformation should be considered in this context.

Our approach was to first gain some understanding of the molecular behavior of the isolated β-O-4 linkage through the use of molecular mechanics and *ab initio* computational methods. That initial investigation led to the identification of the most stable conformations of dimeric β-O-4 structures (and oligomers of repetitive monomers) in which the chemical forces that determine structure are balanced. For cell wall

polymers such as lignin, external forces must also be considered. Lignins are assumed to contribute to the strength and elasticity of the cell wall of woody plants. Therefore, lignins have to be dynamic polymers capable of conformational adjustment to both compressive and tensile forces. How the β-O-4 linkage may react to external forces is the subject of this work.

COMPUTATIONAL METHODS

The structural formulas for the repeating units in a β-O-4 oligomer are illustrated in **Fig. 1** as Schemes 1–3. For investigation of the full β-O-4 dimer, the structures in **Fig. 2** (Schemes 4–6) were utilized.

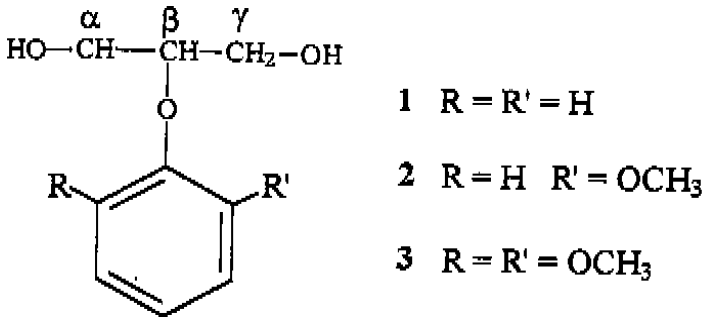

Figure 1. Structural formulas for the repeating units in β-O-4 oligomers, shown as Schemes 1–3.

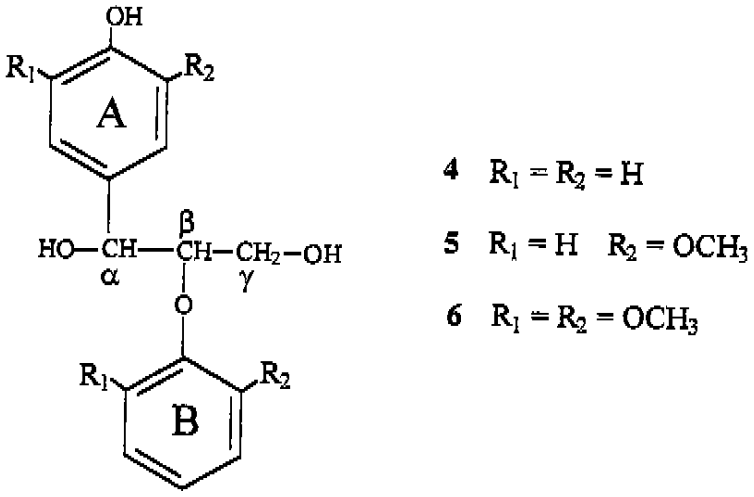

Figure 2. Structural formulas for the full β-O-4 dimers, shown as Schemes 4–6.

Determination of the global energy minimum for the *p*-hydroxyphenyl dimer was accomplished by converting the HF/6-31G*-optimized intramolecular hydrogen-bonded conformers of Scheme 1 *(22)* to the full dimer (Scheme 4), and then optimizing these larger structures at the same HF/6-31G* level. The full β-O-4 dimer is generated by addition of the appropriately substituted aromatic ring at the α-carbon such that either the *threo* or *erythro* diastereomer is produced.

The global energy minima for the guaiacyl *threo* and *erythro* β-O-4 dimers (Scheme 5) have been previously determined at the *ab initio* HF/6-31G* level *(23)*.

Location of the syringyl β-O-4 dimer (Scheme 6) global minimum on the HF/6-31G* potential energy surface involved far fewer possible structures than for either the *p*-coumaryl or the guaiacyl monomer types. With dual methoxyl groups, the potential for intramolecular hydrogen bonding patterns is simplified. Direct optimization of these possibilities at the HF/6-31G* level resulted in the lowest-energy conformers.

Gaussian 94, Revision D.3 *(24)*, employing the self-consistent field direct (SCF=Direct) option, was used for all *ab initio* quantum calculations. All structure optimizations used the program default method of redundant internal coordinates. Coordinate input files were used routinely, and constraints on the optimization were implemented by "freezing" specific torsions using the AddRedundant option. Gaussian computations were performed on a parallel processor (Power Challenge, Silicon Graphics Inc.) maintained by the University of Georgia Computing and Networking Services.

RESULTS

Global energy minima for *erythro* β-O-4 dimers

Schemes 4–6 in Fig. 2 indicate the structural formulas for the β-O-4 dimers discussed in this report. These are the simplest structures possible for the β-O-4 linkage that allow investigation of the rotational movements of the aromatic rings in proximity to the propanediol subgroup. The global energy minima for the *threo* and *erythro* diastereomers of these structures were established at the *ab initio* level (HF/6-31G*). The details of these calculations are presented here under Computational Methods. **Figure 3** depicts three-dimensional representations of the global energy minima of the *erythro* β-O-4 of *p*-coumaryl, guaiacyl, and syringyl dimers (3A–3C, respectively). Both a wire-frame and Corey–Pauling–Koltun (CPK) representation are shown for each structure from the same vantage point. Inspection of these conformations reveals a high degree of geometrical similarity. For all three monolignol types, both hydroxyl groups form intramolecular hydrogen bonds with the C_β ether oxygen. Although poorly directional, these nonbonded interactions are significant *(22)*. In the guaiacyl (Fig. 3B) and syringyl (Fig. 3C) types, the hydroxyl groups also form highly directional, intramolecular hydrogen bonds with the methoxyl oxygens: the α-hydroxyl in the case of guaiacyl, and both the α- and γ-hydroxyls in the case of the syringyl dimer. These interactions stabilize the B aromatic ring (Fig. 2) in an oblique orientation.

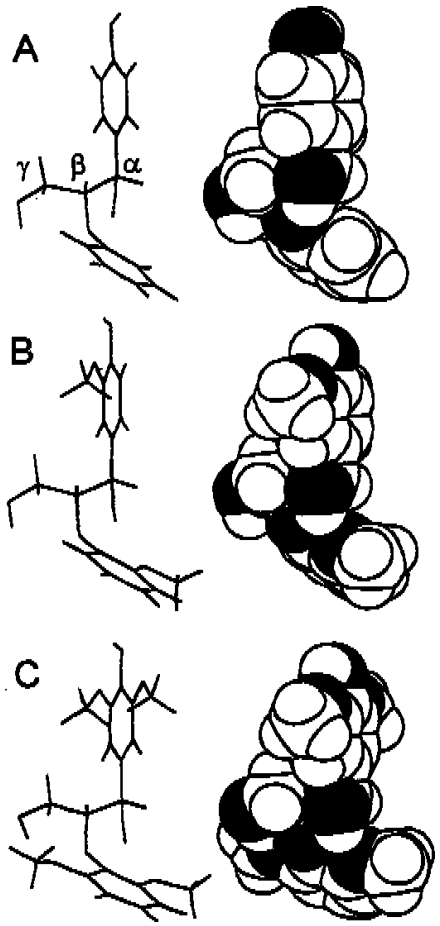

Figure 3. Wire-frame and CPK representations of the global energy minima for *erythro* β-O-4 dimers *(4–6)* estimated at the *ab initio* (HF/6-31G*) computational level. A: *p*-hydroxyphenyl-; B: guaiacyl-; and C: syringyl-type ring substitutions. Both the wire-frame and CPK representations are from the same vantage point. In the CPK representations, oxygens are black; carbons and hydrogens are void.

The *erythro* β-O-4 linkage under tension

Potential energy minima are not calculated at the *ab initio* level under the influence of externally applied forces. These conformational states usually involve single molecules, in essentially a gas-phase environment, with no interactions with solvent or other molecules. The multiple energy minima on a potential energy surface (including the global minimum) are equilibrium structures, and are relatively stable conformers. They contribute (in relation to their relative energy) to the measurable properties of the molecule. Between these energy minima are steric energy barriers. A molecule must acquire enough kinetic energy from its surroundings to surpass this barrier to reach a nearby stable conformation. Thermal energy can do this if the barrier is not too high and the temperature sufficient. Externally applied forces can result in the same transition between stable states.

When tensile force is applied to the ends of a molecule, the structure adjusts in response to this force. These adjustments are bond rotation or torsional movements, bond angle opening, and bond length stretching. Bond rotations or torsional changes occur first. These require the least force to modify. Bond angles typically require a stronger force to change, and covalent bonds are the strongest structural feature of molecules. A molecule will fail under extremes of tension when the weakest covalent bond in the structure breaks. To a first approximation, changes in the length of a molecular structure under tension can be attributed to changes in torsion angles.

Working with a handheld CPK model of the *erythro* guaiacyl β-O-4 global energy minimum, it was apparent that when tension was applied to the ends of the molecule, the structure would straighten out. Two major bonds in the β-O-4 linkage rotated in response to this stress: C_α-C_β and C_β-O (see Fig. 2 (Scheme 5) and Fig. 3B). Of these two, bond rotations around the C_α-C_β produced the greatest change in length of the structure. Maximal extension of the β-O-4 linkage requires changes in both torsion angles. Twisting of the aromatic rings does not produce any change in the overall length of the structure.

The C_α-C_β rotation barrier of the *erythro* guaiacyl dimer was investigated at the *ab initio* HF/6-31G* level. Starting with the geometry of the global minimum, the value of the C_{AR}-C_α-C_β-O dihedral angle was held constant at –130º, –120º, and –110º with full relaxation of all other torsions, bond angles, and bond lengths. This is presented graphically in **Fig. 4**. The maximum energy with respect to the global minimum was found at the –120º torsion value. At this value, the C_α and C_β hydrogens are eclipsed, and other unfavorable steric interactions exist in other parts of the structure. The first stable conformer past the rotation barrier is at –70º. **Table I** presents the relative energy values for these *erythro* conformers.

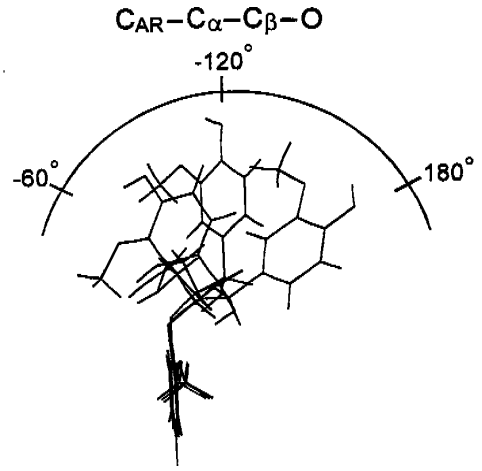

Figure 4. Wire-frame representation of the *erythro* β-O-4 guaiacyl-type linkage *(5)* as a function of C_{AR}-C_α-C_β-O torsion angle. From this perspective, a counterclockwise rotation increases the overall length of the β-O-4 linkage. Starting from the global energy minimum (–177º), the maximum extension is reached at approximately –120º. Relative energy estimates for these conformers are listed in Table I.

Table I. Relative energies of *erythro* β-O-4 conformers as a function of the C_{AR}-C_α-C_β-O torsion angle [*]

C_{AR}-C_α-C_β-O torsion angle, degrees	Relative energy, kcal/mole
–177.4	0
–130.0	8.29
–120.0	9.21
–110.0	8.55
–70.5	2.82

[*] See Fig. 4 for wire-frame representations

In the process of extension, the B aromatic ring of the dimer does undergo some relative change in conformation. For the *p*-coumaryl- and guaiacyl-type dimers, the C_β-O torsion changes such that nearly full extension of the dimer is achieved. This change occurs simultaneously with the rotation of C_α-C_β, and is also automatic as a result of the optimization procedure. For the syringyl dimer, this did not occur automatically, and the C_β-O bond torsion was also constrained to obtain the energy barrier at full extension of the molecule. In essence, all torsions involved in the extension of the β-O-4 have been modified such that maximal extension is realized. This does not include bond angle opening or bond length stretching, which can occur simultaneously but will not contribute significantly to the change in overall length. However, these changes will generally require significantly higher forces. Consequently, once torsional mobility has been exhausted, a β-O-4 linkage will stiffen considerably under applied tension.

The energy difference between the initial (contracted) and transition (extended) state conformations is a measure of the force required to effect the molecular movement from the contracted to the extended states of the β-O-4 linkage. The higher the barrier to convert these states, the greater is the amount of energy required to effect this conversion.

The trajectory from the global minimum to the extended transition state is a reversible path, and does not involve any other stable conformers or energy minima. This has been confirmed by *ab initio* optimization of the –130º conformer, allowing full relaxation of all molecular parameters. The result of this optimization is a direct change in molecular structure back to the original global minimum. Thus, the extension of the β-O-4 in the direction of chain length is an elastic process. Under tension in the direction of chain length, the linkage is extended primarily by rotation of the C_α-C_β bond. When the external tension is released, the structure returns to the initial equilibrium geometry. This elastic process is dramatized in **Fig. 5** with oligomeric constructs based upon *ab initio* HF/6-31G* dimer optimizations. All the major structural information required to construct a β-O-4 oligomer is contained in a β-O-4 dimer. Such an oligomer—constructed with *erythro* (R,S) guaiacyl repeating units derived from the established global energy minimum—is presented in Fig. 5A and can be described as a left-handed helix with approximately six repeating units per turn. There is no evidence that such an oligomer exists in lignins, and it is not our intention to suggest that it does. This, and the accompanying construct (Fig. 5B) are presented to illustrate the proposed elastic

conformational changes calculated for a single β-O-4 linkage as described previously (Fig. 4). The construct in Fig. 5B is derived from the calculated β-O-4 in which the C_{AR}-C_{α}-C_{β}-O torsion is held constant at –120º. An increase in length of approximately 25% and a decrease in the effective diameter of the structure are obvious. A helical motif is apparent in many oligomers constructed from *ab initio*-optimized, low-energy guaiacyl β-O-4 repeating units, both *threo* and *erythro* (data not shown). A helical structure has also been proposed by others *(25)* for an all-*erythro* oligomer. It should be noted, however, that a helical oligomer configuration is not a necessary condition for the previously mentioned elastic process. Any sequential arrangement of global minima (*threo* and/or *erythro*) β-O-4 repeating units will exhibit a similar elastic behavior.

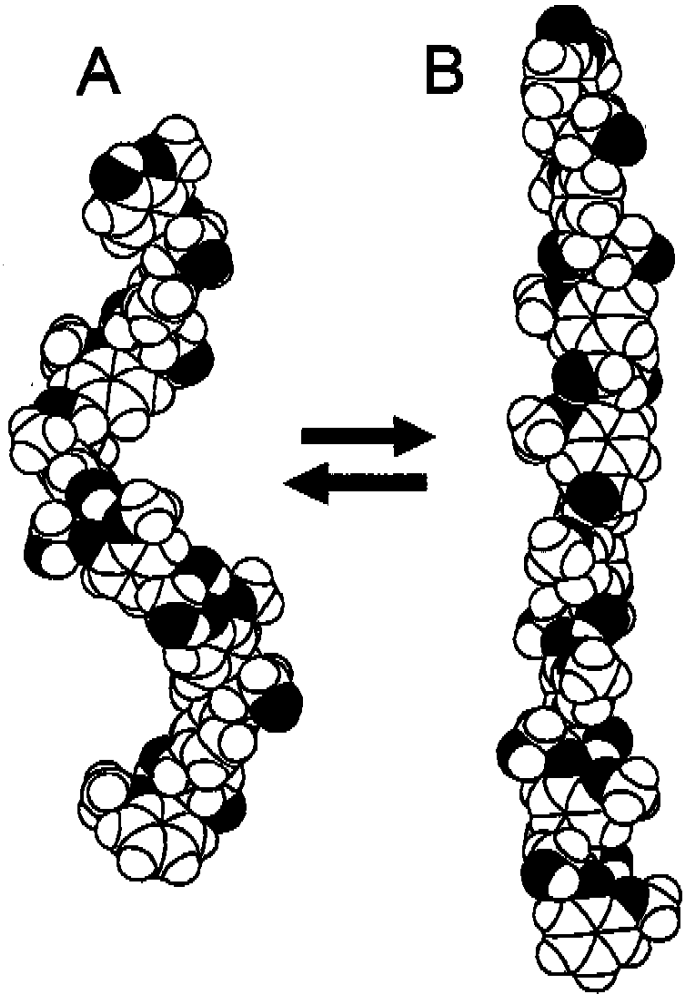

Figure 5. Oligomeric constructs of *erythro* guaiacyl β-O-4 repeating units *(2)* dramatizing the calculated elastic changes in the β-O-4 linkage under external tensile forces. The arrows indicate that the conformational change induced by tensile force applied to the ends of the structure results in an unstable conformation that will elastically return to the original structure when the external load is released.

During this proposed elastic extension of the *erythro* guaiacyl β-O-4, the intramolecular hydrogen bonds present in the initial equilibrium structure are not broken. In fact, several are improved slightly in either donor/acceptor distances and/or directional orientation. The α-hydroxyl/methoxyl hydrogen bond in particular remains intact even in the fully extended *erythro* structure. This is in contrast to the *threo* diastereomer of the guaiacyl β-O-4 global energy minimum dimer in which the γ-hydroxyl/methoxyl hydrogen bond is weakened considerably at the rotation barrier. Consequently, the fully extended *threo* β-O-4 linkage lies approximately 3.5 kcal higher in energy than the *erythro* form, and will require more tensile force to be extended. However, the lack of a reasonably strong hydroxyl/methoxyl hydrogen bond will simultaneously lessen the force necessary to effect ring rotation.

There is a problem with a γ-hydroxyl/methoxyl oxygen intramolecular hydrogen bond in the *threo* guaiacyl global minimum. It has been concluded that the most likely hydroxyl group to form intermolecular hydrogen bonds is the γ-hydroxyl *(26)*. As a primary alcohol, the γ-hydroxyl has more rotational flexibility and extension than the α-hydroxyl. It might be assumed that the most stable *threo* conformer where an α-hydroxyl/methoxyl hydrogen bond stabilizes the structure might be more populated. In this instance, the full extension of the linkage allows the maintenance of the hydrogen bond, and the rotation barrier is quite similar to the *erythro* conformer. It is obvious that hydroxyl orientation can lead to different physical properties of a β-O-4 oligomer. Water can compete with intramolecular hydrogen bonds and, in doing so, changes the hydroxyl orientation. This is in keeping with the experimentally measured effect of water upon the elastic modulus of isolated lignin *(18)*.

A comparison of the *erythro* β-O-4 C_{AR}-C_{α}-C_{β}-O rotational barrier energy for each of the three monolignols is presented in **Table II**. There is essentially no difference in these energy barriers. All three monolignols connected via *erythro* β-O-4 linkages are predicted to show similar elastic characteristics under tension. Since no hydrogen bonds to the methoxyl groups are broken during the extension process, it is not surprising that all three monolignols will show similar rotational barriers.

Table II. Rotation barrier of the *erythro* β-O-4 C_{AR}-C_{α}-C_{β}-O torsion angle as a function of monolignol type

Monolignol type	Rotation barrier, * kcal/mole
p-Coumaryl	9.01
Guaiacyl	9.21
Syringyl	8.86

* Energy difference between the global energy minimum and the fully extended dimeric structure

The *erythro* β-O-4 linkage under compression

Given that an α-hydroxyl/methoxyl hydrogen bond exists not only in the low-energy equilibrium state but is also maintained in extension of the *erythro* β-O-4, the question arises as to what type of external forces will cause disruption of this stabilizing force. It is obvious that rotation of the B aromatic ring C_{AR}-O bond (Fig. 2 (Scheme 4), Fig. 3) will effect just such a disruption. The type of force that can effect ring rotation is compression in a direction perpendicular to chain length in an oligomer. This compressive force is thought to be transmitted to an isolated oligomer through contact from adjacent molecules of either lignin or hemicellulose. The precise mechanism of this contact is not known, but its effect is to force the β-O-4 rings to assume the least space possible. The maximum packing of aromatic rings in a system of β-O-4 oligomers requires a vertical ring orientation (in our structural perspective). An external compressive force directed perpendicular to chain length causes ring rotation from the oblique to a vertical orientation while simultaneously forcing chain extension. This correlates with the macroscopic behavior of a material under compression where the material expands in directions at right angles to the direction of the compressive force.

Ring rotation in a β-O-4 oligomer is not free from steric constraints. The C_β hydrogen is a steric block to free rotation at the methoxyl end of the ring, and the C_γ hydrogens are involved at the opposite end of the ring. These steric constraints have been investigated separately at the HF/6-31G* level, and the energy barriers to ring rotation are reported and discussed below.

Figure 6 presents a comparison of ring rotation in the *erythro* β-O-4 repeating unit for each of the three monolignols (Fig. 1, Schemes 1–3). In this instance, unfavorable steric interactions between the aromatic ring and the C_β hydrogen are present. All initial geometries are the global energy minimum for each of the respective monolignol types. These same geometries are found in the most stable *threo* dimer conformer as well. Figure 6 shows both wire-frame and CPK representations from the same vantage point. The arrows represent conformational changes that, in the forward direction, may or may not result in a stable product conformer. If no return arrow is shown, then the product is both a stationary point and a stable energy minimum on the HF/6-31G* potential energy surface. A return arrow indicates that the product conformation is unstable and will revert back to the initial conformer when rotational constraints are relaxed. Thus, the combination of a forward and a reverse arrow indicates an elastic response when the change in conformation is induced by externally applied forces.

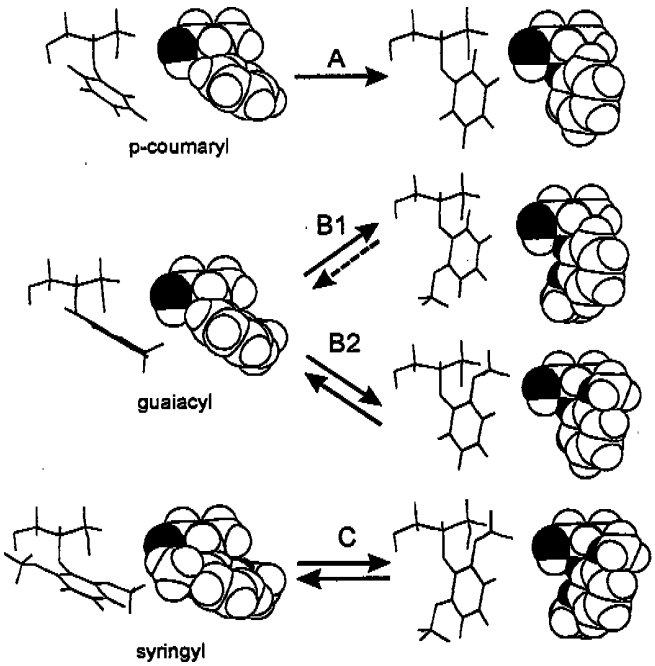

Figure 6. Conformational changes for *p*-hydroxyphenyl, guaiacyl, and syringyl β-O-4 repeating units *(1–3)* due to aromatic ring rotations around the C_{AR}-O bond. Both a wire-frame and CPK representation are shown for each conformer. In the CPK representation, oxygens are black; carbons and hydrogens are void. In A, the resulting *p*-hydroxyphenyl vertical ring conformer is stable. For the guaiacyl-type structure, two vertical ring conformers are possible: in B1, the ring hydrogen is topmost and in close proximity to the C_β hydrogen; in B2, the methoxyl group is in steric conflict with the C_β hydrogen. The reversible arrow designation indicates an elastic response to an external compressive force. The oblique to vertical transition is the result of an external compressive force applied perpendicular to the direction of chain elongation. When the compressive force is released, the structure returns to the original oblique conformation. In B1, the resulting vertical ring conformer is stable if the ring methoxyl is rotated out of the plane of the ring. In B2, the resulting ring conformer is unstable and will elastically return to the oblique conformer when the external force is released. In C, a single, unstable vertical ring conformer is produced by ring rotation in the syringyl repeating unit. Associated relative energies for these elastic transitions are listed in Table III.

For the *p*-hydroxyphenyl β-O-4 repeating unit, the conformational transition indicated in Fig. 6A results in a stable vertical ring orientation. This vertical conformer is not only a stationary point on the HF/6-31G* potential energy surface, but is a local energy minimum as well. The primary torsional rotation involves the C_{AR}-O bond, and the barrier to this rotation has been reduced through the effect of hydrogen bonding to the ether oxygen by both hydroxyl groups *(22)*. The relative energy difference between these two *p*-hydroxyphenyl conformers is listed in **Table III**.

Table III. Relative energy change of β-O-4 repeating units due to aromatic ring rotation

Monolignol type	Conformational change *	Relative energy change, kcal/mole
p-Coumaryl	A	0.87
Guaiacyl	B1	4.09
	B2	6.6
Syringyl	C	8.6

* Conformational change as diagramed in Fig. 6

As previously reported *(22)*, a stable vertical ring orientation is not uncommon for the *p*-hydroxyphenyl repeating unit, either with or without intramolecular hydrogen bonds. The situation with guaiacyl and syringyl ring orientations is quite different. Ring rotation with a guaiacyl repeating unit can occur in one of two different ways: a ring hydrogen can be rotated toward the top of the vertical orientation as in B1; or alternatively, the methoxyl group can be directed toward the propane chain as in B2. There are both stability and energy differences in the resultant conformers.

While a stable vertical ring orientation (with the ring hydrogen close to the propane chain) is possible for the guaiacyl repeating unit, there is a necessary prerequisite: the ring methoxyl must be orientated out of the place of the aromatic ring for the vertical orientation to exist as an energy minimum on the HF/6-31G* potential energy surface. Full optimization of the in-plane methoxyl orientation results in the conformer returning to the original, ring oblique form. This is represented by the dashed arrow in B1 (Fig. 6).

In the transition represented in B2, the resulting vertical ring orientation with the methoxyl group positioned near the propane chain is not stable. This conformer is the result of holding the C_{AR}-O torsion constant at 180º and allowing the rest of the structure to relax. Releasing the constraints on this torsion results in a return to the ring oblique orientation. There is approximately a 2.5-kcal difference in the guaiacyl β-O-4, depending upon which group—a hydrogen or methoxyl—is rotated to within steric conflicts with the C_β hydrogen. The direction of ring rotation determines whether a hydrogen or methoxyl barrier is encountered, and this will be specified by how the aromatic rings pack in a system of multiple β-O-4 oligomers.

Molecular movements as the result of compression forces will in all probability tend to effect ring orientation in the β-O-4 repeating unit. Full rotation to the vertical orientation may not always occur, but some degree of rotation will be effected. The full vertical orientation is, however, a convenient configuration for comparison of the three monolignol types. The differences in the energy barriers listed in Table III for the guaiacyl and syringyl monolignol types are the result of the breaking of intramolecular hydrogen bonds. When the ring is rotated, the methoxyl group must break free of any intramolecular hydrogen bonds present in the oblique orientation. In the case of the syringyl β-O-4 repeating unit, two intramolecular hydrogen bonds are disrupted by ring rotation.

In the *erythro* guaiacyl repeating unit, once the ring rotation barrier has been passed, the β-O-4 is now in a different conformation, with the methoxyl group located in the proximity of the γ-hydroxyl group. This could well be a factor in hysteresis when wood is subjected to compression forces in excess of its elastic limit. The unsubstituted or symmetrically substituted *p*-coumaryl and syringyl repeating units would not be expected to show this behavior.

In the syringyl β-O-4 repeating unit, ring rotation to a vertical orientation must necessarily result in the positioning of a methoxyl group near the C_β hydrogen. This transition is shown in Fig. 6C. As in the case of guaiacyl, the vertical ring orientation is not stable and will revert back to the oblique orientation in the absence of torsional constraints.

As mentioned previously, there are two regions of steric constraint for ring rotation: the C_β hydrogen and the C_γ hydrogens. Computational investigation of this second instance of steric constraints utilized the full β-O-4 dimer of Scheme 5 (*threo* and *erythro*) and involved rotation of the A aromatic ring, constraining the C_{AR}-C_α bond while allowing full relaxation of the remaining geometry. Only the guaiacyl-type dimer was investigated, since the methoxyl groups are not directly involved in this instance. As such, this rotation barrier will essentially be independent of monolignol type. The A aromatic ring was rotated in both a clockwise (CW) and counterclockwise (CCW) manner, since some differences in the respective barriers were anticipated due to the direction of approach to the C_γ hydrogens. Using the global minima as initial structures, the values for the C_{AR}-C_α rotation barriers were as follows:

- CCW: *threo* 7.85 kcal, *erythro* 7.72 kcal
- CW: *threo* 6.40 kcal, *erythro* 7.66 kcal.

DISCUSSION AND CONCLUSIONS

Based upon *ab initio* (HF/6-31G*) calculated rotational barriers, the β-O-4 under tension appears to be independent of monolignol type. Some differences, however, may be expected between the *threo* and *erythro* forms because of variations in the intramolecular hydrogen bonding patterns. In addition, once torsional movements become competitive with bond angle opening, the C_β-O-C_{AR} angle will begin to open up. The tensile force required to increase this angle may well vary with monolignol type. The intramolecular hydrogen bonding to the C_β-ether oxygen is different for each monolignol, and this will effect variation in this angle. These possibilities have not yet been investigated.

Most of the rotation barriers investigated in this report appear to be essentially independent of monolignol type, but they are substantial in magnitude. As such, they provide a molecular basis toward an understanding of the function of lignins within the cell wall. For the three monolignol types, the major difference in rotation barriers occurs during ring rotation under compression, specifically rotation of the C_{AR}-O bond (Fig. 6 and Table II). For an additive effect to occur under compression in a system of many β-O-4 linkages, the aromatic rings must be positioned such that an external force is transmitted almost instantaneously across a series of aromatic rings. The entire system of aromatic rings will then become engaged at the instant of loading and begin to rotate, more or less in unison. An ordered alignment of chains may be a necessary condition to

form this system. Water situated between polymeric chains can be considered part of the force transmission arrangement. If the barriers to ring rotation are additive in an arrangement of chains of β-O-4 oligomers, significant differences in the elastic properties of these three polymer types will exist. In addition, the magnitude of the elastic forces involved will be dependent upon the quantity of lignin present.

The methoxyl group appears to play multiple molecular roles. With *p*-hydroxyphenyl as the reference β-O-4 structure, the addition of methoxyl groups increases the barrier to ring rotation: the guaiacyl ring rotation is restricted more than *p*-hydroxyphenyl, and syringyl more than guaiacyl. If one compares oligomers composed exclusively of β-O-4 linkages, one would predict the syringyl type to be the least elastic polymer and the strongest in resisting external loading. Since the *p*-hydroxyphenyl β-O-4 is stable in either an oblique or vertical configuration, β-O-4 oligomers of this monomer will tend to exhibit plastic rather than elastic deformation. However, covalent bonds (secondary linkages) to the β-O-4 aromatic rings will also restrict ring rotation. This is more likely in *p*-hydroxyphenyl structures with no methoxyl groups to block the bonding sites. It is not possible at all in syringyl structures, and the opportunity is decreased but still possible in the guaiacyl type monolignol. It is not known how these factors are balanced *in vivo*, but one of the primary roles of the methoxyl group is apparently to modulate the elasticity of the lignin polymer.

The living tree withstands limited external forces by design. Occasional winds displace branches from their equilibrium positions. Tensile, compressive, and shear forces are generated in different sections of the branch. When the external force subsides, the branch returns to its original position after a brief period of damped oscillations. The previously described elastic mechanism for the β-O-4 linkage undoubtedly plays a role at the molecular level in the elastic response of the living tree.

Hardwoods and softwoods utilize different engineering design strategies to maintain trunk and branch alignment and to distribute external loads *(27)*. In softwoods, compression wood forms on the underside of limbs and on the low side of displaced trunks. The compression wood exerts a positive expansive force in line with the cell axis to maintain branch alignment and to bring displaced trunks into vertical alignment. In hardwoods, tension wood forms on the top of branches. The tension wood maintains branch alignment by resisting the tensile force generated by the extended limb. These different mechanical solutions to the gravity problem are reflected by differences at the cellular and at the molecular level. Both of these engineering strategies utilize, with some variation, the same basic components: cellulose, hemicelluloses, lignins, and water to construct cell walls with different mechanical properties. The *in vivo* function of lignin in either of these systems is currently unknown at the molecular level.

Hardwood lignins (total wood) are known to contain guaiacyl and syringyl monomers in approximately a 50:50 ratio, with small amounts of phenylcoumarin structures. Due to the cyclic ether in phenylcoumarin, this structure cannot undergo the elastic extension under tension as proposed for the β-O-4 linkage. The insertion of a phenylcoumarin structure into a β-O-4 oligomer stiffens the chain under tension. A sinapyl alcohol monolignol cannot form a phenylcoumarin linkage. Coniferyl alcohol, however, can. Therefore, a mixed polymer allows the insertion of a phenylcoumarin linkage into an otherwise all-syringyl oligomer to fine-tune the elastic properties of the material. A mixture of guaiacyl- and syringyl-type β-O-4 repeating units would make

sense from a design point of view if stiffness under tension is advantageous to the hardwood fiber or vessel. One can speculate that hardwood lignins are heteropolymers in at least some regions of the woody cell wall.

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EXECUTIVE SUMMARY

We have used computational methods (molecular mechanics and *ab initio*) to establish the global energy minima for the *threo* and *erythro* β-O-4 model dimers formed from *p*-coumaryl, coniferyl, and sinapyl alcohol. All *threo* global energy minima have similar geometric profiles, as do all *erythro* global minima conformers.

The main distinguishing feature in the guaiacyl and syringyl conformers is the presence of a stabilizing intramolecular hydrogen bond from one or both hydroxyl groups to either one or both ring methoxyl oxygens. This stabilization cannot occur in the *p*-hydroxyphenyl β-O-4, since this structure lacks methoxyl groups.

An oligomeric construct based upon the global energy minimum and containing only *erythro* (R,S) guaiacyl β-O-4 linkages produces a right-handed helical structure. The all (S,R) guaiacyl β-O-4 structure is a left-handed helical structure.

To extend this helical structure, as if under tension, rotation around the C_α-C_β bond must occur. This process has been simulated with *ab initio* calculations on the β-O-4 dimer, and the rotation barrier has been estimated for both *threo* and *erythro* conformers of all three monolignol types. For all three types, the rotation barriers are essentially the same. In the *erythro* conformers, the intramolecular hydrogen bonds to the methoxyl oxygens are not broken during this process. In addition, there are no intervening local energy minima on the HF/6-31G* potential energy surface up to the point where the energy barrier is encountered. Thus, the extension of the β-O-4 in this manner can be described as an elastic process. This is important for an understanding of the elastic properties of wood and other plants that contain lignin. None of the other linkage types proposed for lignin have the capacity to exhibit elasticity of this magnitude.

If the oligomeric construct is subjected to compressive forces, perpendicular to the direction of chain elongation, ring rotation will occur. Rotation barriers involving ring rotation have been determined at the HF/6-31G* level for all three monolignol β-O-4 linkages. Significant differences in these barriers have been documented. They increase in the order *p*-hydroxyphenyl < guaiacyl < syringyl. The increase is due to the breaking of intramolecular hydrogen bonds in the guaiacyl and syringyl linkages. Ring rotation up to the rotation barrier is again an elastic process for the syringyl β-O-4 linkage. For the *p*-hydroxyphenyl linkage, it is best described as a plastic deformation. The guaiacyl structure can exhibit either of these properties, depending upon the direction of ring rotation.

Hardwood lignins are known to contain guaiacyl and syringyl monomers in about a 50:50 ratio, with small amounts of phenylcoumarin structures. However, it has not been known whether hardwood lignins are homo- or heteropolymers. From a design point of view, it is likely that hardwood lignins are at least partially composed of mixed guaiacyl/syringyl polymers. The insertion of a phenylcoumarin structure into a β-O-4 oligomer stiffens the chain. A sinapyl alcohol monolignol cannot form a phenylcoumarin linkage. Coniferyl alcohol, however, can. Therefore, a mixed polymer allows the

insertion of a phenylcoumarin linkage into an otherwise all-syringyl oligomer to fine-tune the elastic properties of the material. This may serve well in the architecture of hardwoods, where an increased resistance to tension in reaction wood maintains branch orientation.

Any molecular mechanism that restricts ring rotation in a β-O-4 oligomer will increase the strength of the molecule under external load. This can be accomplished in several ways. With a *p*-hydroxyphenyl as the reference β-O-4 oligomer, the addition of methoxyl groups restricts ring rotation: guaiacyl more so than *p*-hydroxyphenyl, and syringyl more so than guaiacyl. All else being equal, one would predict syringyl type lignin to be the least elastic. However, all else is not equal. Covalent linkages to the β-O-4 ring will also restrict ring rotation. This is more likely in *p*-hydroxyphenyl structures, where there are no methoxyl groups to block covalent bond formation to the aromatic ring. It is not possible at all in syringyl structures, while the opportunity is decreased but still possible in guaiacyl-type lignin. Thus one of the primary roles of the methoxyl group is to modulate the elasticity or flexibility of the resulting lignin polymer.