Lignin Monomers from Outside the Canonical Monolignol Biosynthetic Pathway

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Abstract

Lignin is a phenylpropanoid polymer derived from the oxidative radical coupling of three \( p \)-hydroxycinnamyl alcohols, the so-called monolignols: \( p \)-coumaryl, coniferyl, and sinapyl alcohols. Several other phenolic compounds, all deriving from the shikimate-derived monolignol biosynthetic pathway, have been found to behave as lignin monomers in many plants, including the monolignol ester conjugates (with acetate, \( p \)-coumarate, ferulate and \( p \)-hydroxybenzoate) or the incompletely methylated monomers caffeyl and 5-hydroxyconiferyl alcohols, among others. However, recent studies have demonstrated the occurrence of polyphenolic compounds derived from other biosynthetic pathways in the lignins of several plants. This is the case for the flavone tricin recently established as a monomer in the lignins from grasses and other monocots or the newly discovered hydroxystilbenes (resveratrol, isorhapontigenin and piceatannol) that are implicated as monomers in the lignification of palm fruit endocarps. The discovery of ‘non-conventional’ phenolic precursors arising from other biosynthetic pathways expands the traditional definition of lignin and reveals that any phenolic compound that are delivered to the cell wall may be oxidized and incorporated into the lignin polymer during lignification.

Keywords: monolignols; flavonoids; hydroxystilbenes; tricin; piceatannol.

Lignin monomers derived from the monolignol biosynthetic pathway

Lignin has long been considered to be a complex phenylpropanoid polymer derived essentially from the oxidative radical coupling of three \( p \)-hydroxycinnamyl alcohols differing in their degree of methoxylation, the so-called monolignols: \( p \)-coumaryl, coniferyl, and sinapyl alcohols. After their synthesis, the monolignols are delivered to the cell-wall where they are oxidized in a reaction mediated by peroxidases and/or laccases and then polymerized in a combinatorial fashion by free-radical coupling mechanisms, generating a variety of structures and linkages within the polymer [1,2]. Several other phenolic compounds, all deriving from the shikimate-derived monolignol biosynthetic pathway (Figure 1), have been found to behave as lignin monomers in many plants, participating in radical coupling reactions during lignification. This is the case for monolignol ester conjugates with acetates (1), \( p \)-hydroxybenzoates (2), \( p \)-coumarates (3) and ferulates (4), that are also used as lignin monomers in a variety of natural plants [3-7]. Several other monomers derived from truncated monolignol biosynthesis, such as the caffeyl and 5-hydroxyconiferyl alcohol, have been found as lignin monomers in the seed coats of several plants [8,9].

Figure 1. Examples of ‘non-conventional’ lignin monomers also derived from the monolignol biosynthetic pathway. Lignin monomers acylated at the \( \gamma \)-OH with acetates (1), \( p \)-hydroxybenzoates (2), \( p \)-coumarates (3) and ferulates (4); caffeyl alcohol (5); 5-hydroxyconiferyl alcohol (6).
Monomers from outside the canonical monolignol biosynthetic pathway

In all of the cases above, the lignin monomers are derived from the monolignol biosynthetic pathway. However, recent investigations have demonstrated that other phenolic compounds derived from outside the classical shikimate-derived monolignol biosynthetic pathway, namely, the flavonoid and hydroxystilbene pathways (Figure 2), can also participate in cross-coupling reactions with monolignols and/or lignin oligomers and become integrally incorporated into the lignin polymer in several plants [10-12].

![Flavonoids biosynthetic pathway](image)

**Figure 2.** Phenolic compounds from the flavonoid (tricin) and hydroxystilbene (piceatannol, isorhapontigenin, resveratrol) biosynthetic pathways incorporated into the lignins of several plants [10-12].

Phenolic compounds from the Flavonoids Biosynthetic Pathway

The flavone tricin was the first phenolic compound derived from outside the canonical monolignol biosynthetic pathway discovered in lignin preparations from wheat straw [10]. The tricin structure was determined by NMR and its characteristic correlation signals can be now easily assigned in the HSQC spectra of lignins (Figure 3). Tricin, unlike monolignols that originate from the shikimate biosynthetic pathway, is biosynthesized from a combination of the shikimate and acetate/malonate-derived polyketide pathways. Tricin’s only mode of incorporation into the lignin polymer is via 4′-O-β-coupling with a monolignol, and can therefore only appear at the initiating end of the lignin chain. Biomimetic radical coupling reactions confirmed the incorporation of this structure into the polymeric lignin and implicated tricin as a possible nucleation site for lignification [11,13]. Additional studies have expanded the range of plants with tricin incorporated into their lignins and indicated that tricin is in the lignin of all grasses [14-16] and that also occurs in the lignin of other monocots, such as in coconut coir [17] or in the vanilla plant [16]. Furthermore, a recent study showed that disruption of flavone synthase II gene in rice resulted in an altered cell wall lignin incorporating the flavone intermediate naringenin, instead of tricin, into the lignin polymer [18], illustrating again the full compatibility of flavones with lignification.

![NMR spectrum of lignin isolated from wheat straw](image)

**Figure 3.** Aromatic region of the 2D-NMR spectrum of the lignin isolated from wheat straw showing the main correlation signals of the flavone tricin (T). A trimeric model showing tricin incorporated into the lignin polymer through a 4′-O-β linkage is also shown.
Phenolic Compounds from the Hydroxystilbene Biosynthetic Pathway

Very recently, we also reported the occurrence of a second class of polyphenolic compounds, hydroxystilbenes (piceatannol, resveratrol, isorhapontigenin) that are present and act as authentic lignin monomers participating in coupling and cross-coupling reactions during lignification of palm fruit endocarps and that become integrally incorporated into the lignin structure [12]. Hydroxystilbenes are a class of non-flavonoid polyphenolics that, like the flavonoids, are metabolic hybrids resulting from a combination of the shikimate-derived phenylpropanoid and the acetate/malonate-derived polyketide pathways. Hydroxystilbenes, as with monolignols, can be oxidized to form radicals that are resonance-stabilized, as shown for piceatannol in Figure 4. Unlike tricin, which has only one possible mode of incorporation, piceatannol would be expected to couple and cross-couple with other piceatannols as well as with monolignols (and oligolignols) in a variety of ways, including the β-O-4′ coupling shown in Figure 4.

Figure 4. Piceatannol (P) radical and its different resonance forms (Top). The most stable phenolic radical is that from dehydrogenation of the 4-OH. Resonance forms show how coupling can occur at the 4–O, 5-, 8- and 10-positions, among others. Cross-coupling of monolignols M (sinapyl and coniferyl alcohol) with piceatannol P via β-O-4′-coupling to give the benzodioxane structure V (Bottom).

Signals from the hydroxystilbene piceatannol were observed in the 2D-HSQC-NMR spectra of lignins isolated from several palm fruit endocarps (Figure 5). The main aromatic correlation peaks corresponded to the different lignin (guaiacyl G and syringyl S) units as well as the pendant p-hydroxybenzoates (pB). The most striking feature was the presence of a previously unreported group of signals (Pb, Pc) appearing at δC/δH 100-107/5.8-6.8 from piceatannol-derived units. Likewise, the oxygenated-aliphatic region of the spectra also showed signals other than those commonly observed from conventional lignin structures (β-aryl ethers A, phenylcoumarans B, resinsols C, or cinnamyl alcohol end-groups I). These correlation peaks, labelled Pb, Pc and V are assigned here to structures involving piceatannol units. Definitive assignments of these signals were achieved by HSQC-TOCSY and HMBC experiments and by comparing with piceatannol polymerization products and in vitro biomimetic cross-coupling reactions [12]. Thus, Pb was identified as a benzodioxane structure formed via 8–O-4′-type radical coupling of a piceatannol unit at its 8-position with another piceatannol unit (at its O–4′ position) followed by internal trapping of the quinone methide intermediate by the 3′-hydroxyl group; Pc was identified as a phenylcoumaran structure formed by the radical coupling of a piceatannol unit (at its 8-position) with another piceatannol unit (at its 10′-position) followed by a subsequent 11′–O–7 bonding during rearomatization of the quinone methide intermediate. Structure V was
identified as arising from the cross-coupling of piceatannol and monolignols and are formed via radical coupling of a monolignol (at its β-position) and the catechol moiety of piceatannol (at its O-4’ position) followed by internal trapping of the quinone methide intermediate by the 3’-hydroxyl group in the piceatannol unit to form the benzodioxane structure V (Figure 4). Biomimetic cross-coupling reactions between piceatannol and p-hydroxycinnamyl alcohols successfully proved that benzodioxane structures V could be easily formed during the radical reaction [12]. The occurrence of these benzodioxane structures in the lignins from palm fruit endocarps demonstrates that the stilbene piceatannol acts as an authentic monomer participating in coupling and cross-coupling reactions during lignification.

Figure 5. Side-chain (δC/δH 48-98/2.6-6.5) and aromatic (δC/δH 96-155/5.6-8.0 ppm) regions of the 2D-HSQC-NMR spectrum of the lignin isolated from macaúba (Acrocomia aculeata) fruit endocarp showing the main signals from lignin and piceatannol units. The structures of the main piceatannol homodimeric coupling (Pb, Pc) and piceatannol-lignin cross-coupling structures (V) are also depicted.

Conclusions

Different phenolic compounds from outside the canonical monolignol biosynthetic pathway have been found to behave as lignin monomers in several plants. The lignins from grasses and other monocots incorporate the flavone tricin (and naringenin has been found in the lignin in a rice mutant) whereas the lignins from palm fruit endocarps incorporate the hydroxystilbene piceatannol (as well as resveratrol and isorhapontigenin at lower levels). These discoveries indicate that lignification is a flexible mechanism and that the plant is capable of using a variety of phenolic compounds from different biosynthetic pathways for the formation of the lignin polymers, further expanding the traditional definition of lignin. The discovery of ‘non-conventional’ phenolic precursors, different from the three canonical monolignols, illustrates the high metabolic plasticity of lignification and reveals that any phenolic compound that is delivered to the cell wall may be oxidized and incorporated into the lignin polymer during lignification via radical coupling reactions, subject exclusively to simple chemical compatibility. The incorporation of non-conventional monomers, not usually present in the lignins of other plants, as is the case for the tricin or the piceatannol described here, can open up new ways to design and engineer the lignin structure to produce polymers and plant-based biomaterials with altered properties.
References


Acknowledgements

This study has been funded by the Spanish projects AGL2014-53730-R and CTQ2014-60764-JIN (co-financed by FEDER funds) and the CSIC project 2014-40E-097. John Ralph and Hoon Kim were funded by the DOE Great Lakes Bioenergy Research Center (DOE BER Office of Science DE-FC02-07ER64494).