7

Lignin Monomers Derived from the Flavonoid and Hydroxystilbene Biosynthetic Pathways

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7.1 Lignin Monomers Derived from the Monolignol Biosynthetic Pathway

7.1.1 'Canonical' Monolignols

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 (Figure 7.1a). Extensive research has been devoted during the past decades to elucidating the monolignol biosynthetic pathway and the mechanisms of gene regulation, which are now basically clarified (Boerjan et al. 2003; Dixon and Reddy, 2003; Vanholme et al. 2008, 2010, 2019). The biosynthesis of the monolignols starts with the deamination of phenylalanine (and/or tyrosine), which is derived from the shikimate biosynthetic pathway, and involves successive hydroxylation reactions of the aromatic ring, followed by phenolic *O*-methylation and reduction of the side-chain carboxylic acid group to an alcohol (Dixon et al. 2001; Boerjan et al. 2003; Ralph et al. 2004; Liu, 2012; Vanholme et al. 2008, 2010, 2019; Barros et al. 2016). These reactions are catalyzed by different enzymes that are well documented, and include phenylalanine ammonia-lyase (PAL), tyrosine ammonia-lyase (TAL), cinnamate 4-hydroxylase (C4H), 4-coumarate:coenzyme A ligase (4CL), ferulate 5-hydroxylase (F5H), *p*-coumarate 3-hydroxylase (C3H), *p*-hydroxycinnamoyl-CoA:quinate/shikimate hydroxycinnamoyltransferase (HCT), caffeoyl shikimate esterase (CSE), caffeoyl-CoA *O*-methyltransferase (CCoAOMT), caffeoyl-CoA reductase (CCR), caffeic acid *O*-methyltransferase (COMT), and cinnamyl alcohol dehydrogenase (CAD).

Recent Advances in Polyphenol Research, Volume 7, First Edition. Edited by Jess Dreher Reed, Victor Armando Pereira de Freitas, and Stéphane Quideau.

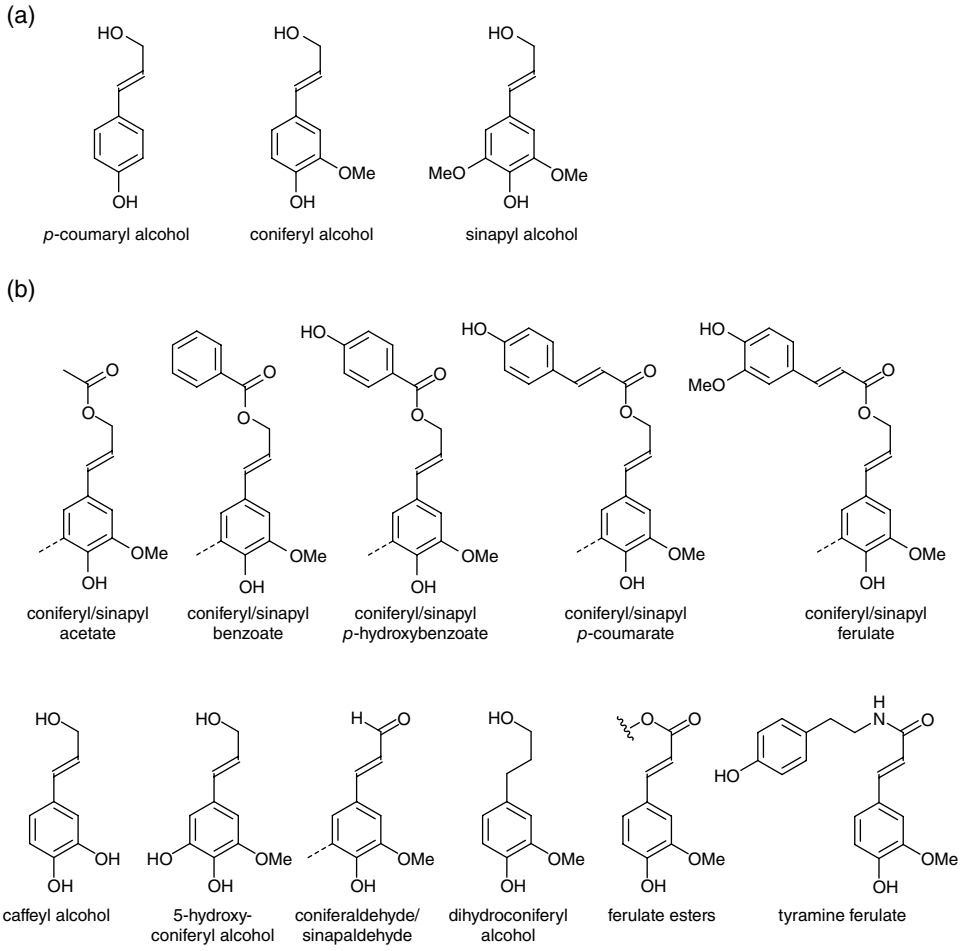

Figure 7.1 (a) Structures of the three canonical monolignols, *p*-coumaryl, coniferyl, and sinapyl alcohols. (b) 'Non-conventional' lignin monomers also derived from the monolignol biosynthetic pathway. Lignin monomers acylated at the γ-OH with acetate, benzoate, *p*-hydroxybenzoate, *p*-coumarate, and ferulate; caffeyl alcohol; 5-hydroxyconiferyl alcohol; hydroxycinnamaldehydes; dihydroconiferyl alcohol; ferulate esters; tyramine ferulate.

After their synthesis, the monolignols are delivered to the cell wall (by largely unknown mechanisms) 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 (Boerjan et al. 2003; Ralph et al. 2004, 2019). The most frequent interunit linkage is the β−*O*−4 alkyl-aryl ether; other common linkages and structures in the lignin polymer include β−5 phenylcoumarans, β−β resinols, 5−5 dibenzodioxocins, 5−*O*−4 biphenyl ethers, and β−1 spirodienones. The relative abundance of each of these linkages largely depends on the supply of the individual monolignols to the lignifying zone, which greatly varies among taxa and cell type as well as environmental conditions or growth stage (Terashima et al. 1993; Campbell and Sederoff, 1996; Vermerris and Boon, 2001; Donaldson, 2001; Rencoret et al. 2011; Lourenço et al. 2016).

7.1.2 Other 'Nonconventional' Lignin Monomers

During the last few years, a number of papers have demonstrated that several other phenolic compounds, all deriving from the shikimate-derived monolignol biosynthetic pathway (Figure 7.1b), also behave as lignin monomers in many plants, participating in radical coupling reactions during lignification. This is the case for monolignol ester conjugates with acetates, *p*-hydroxybenzoates, *p*-coumarates, or the newly found ferulate and benzoate analogs, that are also used as lignin monomers in a variety of natural plants (del Río et al. 2007, 2008; Martínez et al. 2008; Ralph, 2010; Lu et al. 2015; Karlen et al. 2016, 2017). Monolignol acetates are ubiquitous in angiosperms and have been found in high amounts (sometimes reaching up to 80% acetylation degree) in the lignins of kenaf, sisal, and abaca, as well as in many hardwoods (Ralph, 1996; Lu and Ralph, 2002; del Río et al. 2004, 2007, 2008); *p*-hydroxybenzoates are widely found in palms, poplars, and willows (Smith, 1955; Landucci et al. 1992; Rencoret et al. 2013, 2018; Lu et al. 2004, 2015) and have also been recently found in high amounts in the lignin of the seagrass *Posidonia oceanica* (Kaal et al. 2018; Rencoret et al. 2020); *p*-coumarates are typical of grasses and are also found in significant levels in other monocots, such as curaua and abaca (Ralph et al. 1994; Lu and Ralph, 1999; Martínez et al. 2008; del Río et al. 2008; Ralph, 2010); monolignol ferulates have recently been described in a large number of plant species (Karlen et al. 2016); likewise, benzoates have also been recently reported in the lignins of some palms, such as date and macaúba palms (Karlen et al. 2017; Rencoret et al. 2018) and C3H and C4H downregulated transgenic *Populus trichocarpa* (Kim et al. 2020). Lignins derived exclusively from caffeyl alcohol were discovered in seed coats of vanilla orchid and in some members of the Cactaceae, Euphorbiaceae, and Cleomaceae families and are almost homopolymers linked through β−*O*−4-coupling and producing chains of benzodioxane structures (Chen et al. 2012, 2013; Tobimatsu et al. 2013), and unusual lignins composed of 5-hydroxyguaiacyl units derived from 5-hydroxyconiferyl alcohol were found in the seeds of three species of Escobaria (Chen et al. 2013). Hydroxycinnamaldehydes, the immediate precursors of monolignols, have been detected in various mutant and transgenic plants (Kim et al. 2003; Lapierre et al. 2004), but are also evidenced at low levels in essentially all lignins; in 'normal' wild-type plants, it remains unclear whether the aldehyde is delivered to the wall as such, i.e., as an intermediate of incomplete monolignol biosynthesis, or if it results from the oxidation of the monolignols in the oxidizing environment required for lignification. Dihydroconiferyl alcohol has also been found in 'all' gymnosperm lignins, and is an abundant component in a CAD-deficient pine mutant (Ralph, Kim et al. 1999; Lapierre et al. 2000). Polysaccharide hydroxycinnamate esters (particularly ferulates) are also incorporated into lignins, principally in grasses, where they appear to act as nucleation sites for lignin polymerization (Ralph et al. 1995; Hatfield et al. 1999). Finally, the amide tyramine ferulate has been found incorporated into the lignins of tobacco plants and presumably other Solanaceae (Ralph et al. 1998), and the related diferuloyl putrescine has recently been identified in the lignin of maize kernels (del Río et al. 2018).

The incorporation of these 'non-conventional' lignin monomers into the lignin polymer generates characteristic and diagnostic structures, such as the tetrahydrofuran structures produced from the β−β coupling of γ-acylated monolignols (Lu and Ralph, 2002, 2005; del Río et al. 2007, 2008; Ralph, 2010; Lu et al. 2004, 2015) or the benzodioxane structures produced from the incorporation of caffeyl and 5-hydroxyconiferyl alcohols (Chen et al. 2012, 2013; Tobimatsu et al. 2013), that provided compelling evidence for their

participation in coupling and cross-coupling reactions during lignification. Therefore, these phenolic compounds should be considered to be authentic lignin monomers, in addition to the three canonical monolignols. Indeed, from examination of a large number of lignins, it appears unlikely that any lignin polymer is derived solely from the three monolignols!

In all of the cases above, however, the 'non-conventional' lignin monomers are still derived from the established monolignol biosynthetic pathway – they are either intermediates in the pathway or arise from derivatization reactions of these intermediates or, in the case of the variously acylated monolignols, of the monolignols themselves. Recent investigations have demonstrated that other phenolic compounds derived from outside the classical shikimate-derived monolignol biosynthetic pathway – namely, the flavonoid and hydroxystilbene biosynthetic pathways (i.e., the flavone tricin, the hydroxystilbenes piceatannol, resveratrol, and isorhapontigenin, and the hydroxystilbene glucosides piceid, isorhapontin and astringin; Figure 7.2) – can also participate in radical coupling reactions with monolignols and/or lignin oligomers and become integrally incorporated into the lignin polymer in several plants (del Río et al. 2012, 2017, 2020; Lan et al. 2015; Lan, Morreel et al. 2016; Lan, Rencoret et al. 2016; Lan et al. 2019; Rencoret et al. 2018, 2019; Neiva et al. 2020). These phenolic compounds should therefore be also considered as authentic lignin monomers participating in lignification.

7.2 Flavonoid and Hydroxystilbene Biosynthetic Pathways

Flavonoids and hydroxystilbenes are metabolic hybrids as they derive from a combination of the shikimate-derived phenylpropanoid and the acetate/malonate-derived polyketide pathways (Figure 7.3). Flavonoids and hydroxystilbenes are synthesized from a coenzyme A (CoA) activated hydroxycinnamic acid unit and three malonyl-CoA extender units. The first step is the deamination of L-phenylalanine or tyrosine catalyzed by phenylalanine ammonia-lyase (PAL) or tyrosine ammonia-lyase (TAL) to *trans*-cinnamic or *p*-coumaric acids, respectively. The cinnamic acid is hydroxylated by cinnamate-4-hydroxylase (C4H) to produce *p*-coumaric acid, which is then activated by *p*-coumarate:CoA ligase (4CL) to

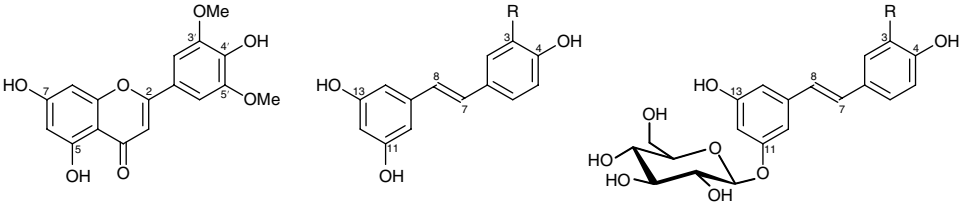

Figure 7.2 Phenolic compounds derived from the flavonoid (tricin) and hydroxystilbene (resveratrol, isorhapontigenin, and piceatannol, and their respective glucosides piceid, isorhapontin, and astringin) biosynthetic pathways that have been found to act as true lignin monomers in several plants. *Source:* del Río et al. 2012, 2017, 2020; Lan et al. 2015; Rencoret et al. 2019.

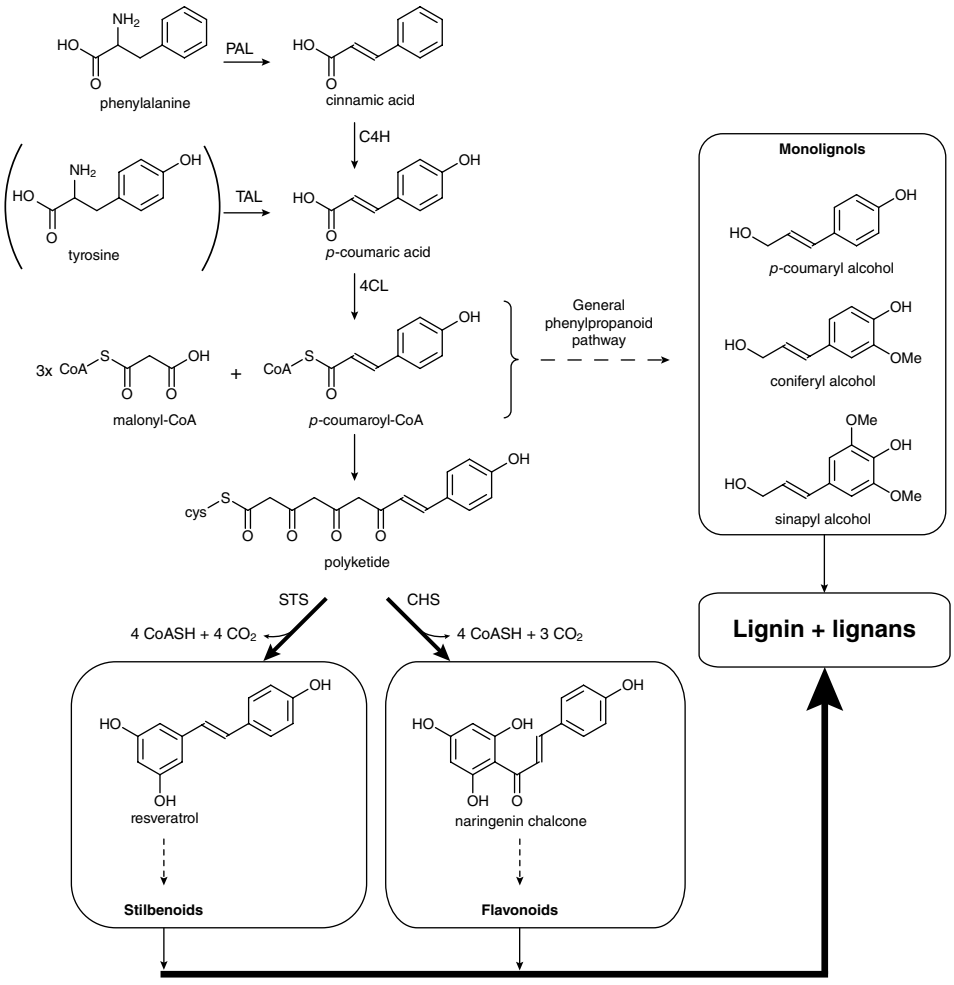

Figure 7.3 Simplified scheme of the general polyphenolics biosynthetic pathways leading to the biosynthesis of monolignols, flavonoids, and stilbenes. PAL, phenylalanine ammonia-lyase; TAL, tyrosine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate:CoA ligase; STS, stilbene synthase; CHS, chalcone synthase.

produce *p*-coumaroyl-CoA, which is also the entry point for the production of monolignols intended for lignin (and lignans). A polyketide synthase then sequentially adds three acetate extender units, derived from malonyl-CoA, to an activated *p*-coumaroyl-CoA unit. Depending on the polyketide synthase activity, chalcone synthase (CHS; EC 2.3.1.74) or stilbene synthase (STS; EC 2.3.1.95), cyclization of the generated tetraketide intermediate results in the production of either a chalcone or a stilbene (Watts et al. 2006). Whereas CHS cyclizes the polyketide intermediate by an intramolecular Claisen condensation, cyclization by STS involves an aldol condensation accompanied by an additional decarboxylation leading to the loss of one carbon as CO_2 (Austin and Noel, 2003). Flavonoids include different classes of compounds, such as flavones, flavanones, flavonols, isoflavones,

anthocyanins, chalcones, aurones, xanthones, and flavanols (Andersen and Markham, 2006; Quideau et al. 2011). Simple hydroxystilbenes, however, have a more restricted number of compounds that consist of two phenolic moieties joined by an ethylene bridge and, similarly to monolignols, are particularly prone to participating in oxidative coupling reactions to produce dimers and higher oligomers (Lin and Yao, 2006; Quideau et al. 2011; Rivière et al. 2012; Keylor et al. 2015).

7.3 Radical Coupling of Flavonoids and Hydroxystilbenes with Monolignols – Flavonolignans and Stilbenolignans

Flavonoids and hydroxystilbenes are known to participate in oxidative radical cross-coupling reactions with monolignols to produce 'nonconventional' lignans (termed flavonolignans and stilbenolignans) that have two phenylpropanoid units linked together through a diversity of linkages (Begum et al. 2010; Chambers et al. 2015). Although lignification and lignan formation are distinct processes separated in time and space (Ralph, Peng et al. 1999; Umezawa, 2003), the wide natural occurrence in plants of these types of hybrid compounds arising from the cross-coupling with monolignols is an indication that at least some members of the flavonoids and hydroxystilbenes might also be compatible with lignification.

7.3.1 Flavonolignans

Flavonolignans have a broad structural diversity and include a wide variety of compounds that present different linkages and structure types (Figure 7.4), including simple 4′−*O*−β′′ ethers (as in aegecin and salcolins A and B), benzodioxane structures (as in silybins A and B, dehydrosilybin, silandrins A and B, or hydnocarpin D), phenylcoumaran structures (as in silychristins A and B, dehydrosilychristin, silyhermin, and neosilyhermins A and B); cyclohexane bridges (as in neohydnocarpin), and tricyclic ketones (as in silydianin) (Begum et al. 2010). Different classes of flavonoids belonging to the families of flavanonols, flavonols, flavanones, and flavones have been found coupling with monolignols in several flavonolignans (Begum et al. 2010; Chang et al. 2010; Chambers et al. 2015). However, and despite the high number of flavonoids identified in plants, only a limited number of them (taxifolin, quercetin, eriodictyol, dihydrotricin, apigenin, luteolin, selgin, and tricin) have been reported to form flavonolignans (Figure 7.4a). The flavanonol taxifolin is the most important one, and was found in silybin, silychristin, isosilybin, isosilychristin, silydianin, and other flavonolignans isolated from milk thistle (Lee and Liu, 2003; Csupor et al. 2016; AbouZid et al. 2016, 2017; Martinelli et al. 2017). The flavonol quercetin was found in dehydrosilybin and dehydrosilychristin (Begum et al. 2010). The flavanone eriodictyol was found in neosilyhermin, silandrins A and B, silyhermin, and neosilyhermin, also isolated from milk thistle (Csupor et al. 2016), whereas the flavanone dihydrotricin was present in the calquiquelignans A and B from rattan palm (Chang et al. 2010). The flavone apigenin was found in lignoside and isolignoside (Begum et al. 2010); another flavone, luteolin, was present in hydnocarpin, hydnocarpin D, hydnowightin, isohydnocarpin, neohydnocarpin and sinaicitin (Begum et al. 2010), whereas selgin was found in palstatin, 5′-hydroxyhydnocarpin and 5′-hydroxyhydnocarpin D (Chambers et al. 2015), and tricin was found in aegecin and salcolins A and B as well as in their acetylated and *p*-coumaroylated γ-carbon

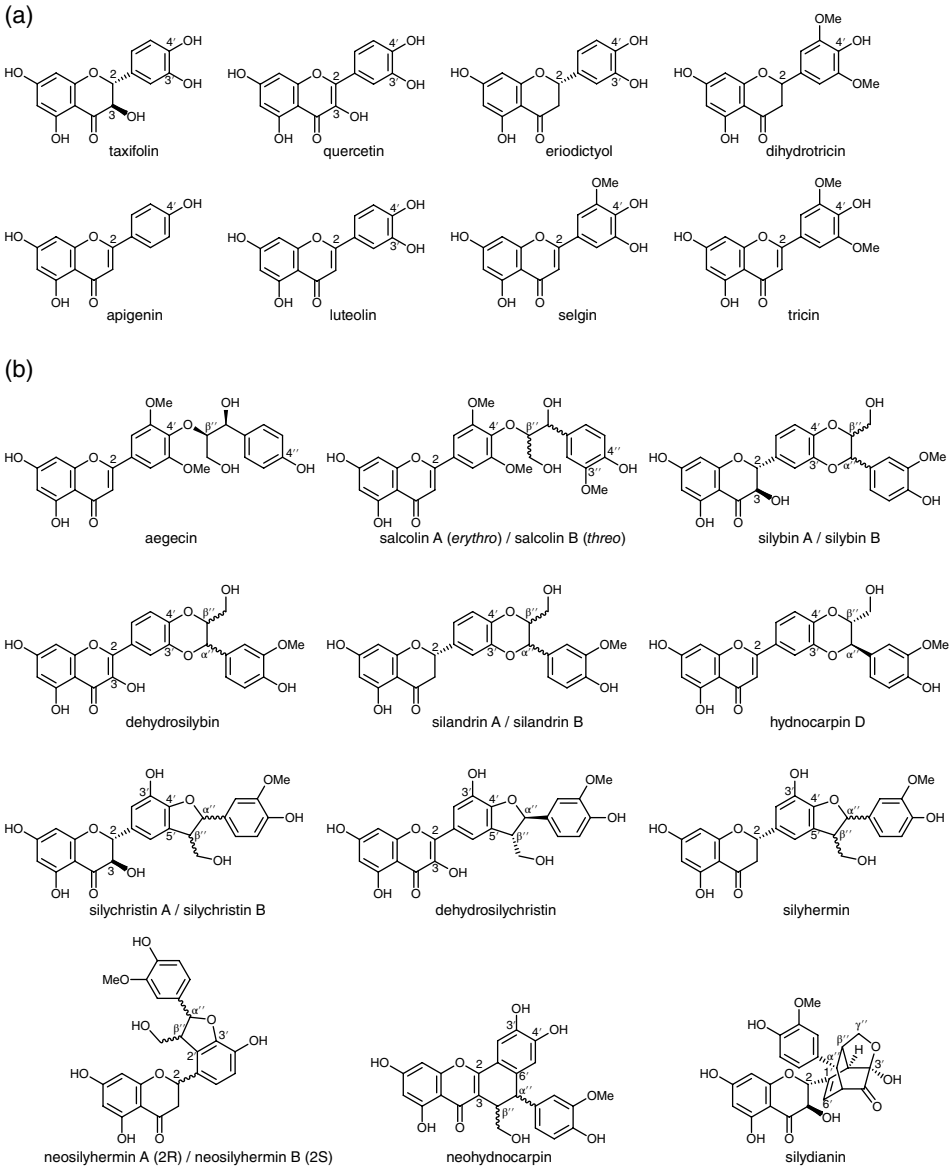

Figure 7.4 (a) Structures of the flavonoids that are known to form flavonolignans. (b) Examples of some flavonolignans identified in plants; all arise from putative radical coupling between the flavonoid and a monolignol.

monolignol conjugates in several kinds of grasses (Wenzig et al. 2005; Begum et al. 2010; Chang et al. 2010; Lee et al. 2015).

Flavonolignans are formed by oxidative radical coupling reactions generating a wide variety of regio- and stereoisomers and, like the lignans themselves, may involve proteins to direct stereospecific coupling (Davin et at., 1997; AbouZid et al. 2017). The coupling of flavonoids with monolignols may occur at different positions. Whereas tricin and dihydrotricin can only form 4–*O*–β linkages with monolignols, other flavonoids (eriodictyol,

taxifolin, quercetin, apigenin, luteolin, or selgin) can form additional linkages. For example, silybin is formed by oxidative radical coupling between the β-position of coniferyl alcohol and the 4′-OH of ring-B of taxifolin, followed by internal trapping of the quinone methide intermediate by the 3′-OH group of taxifolin forming a benzodioxane ring. Silychristin, on the other hand, is formed by radical coupling of coniferyl alcohol (at its β-position) with the C-5′ position of taxifolin followed by a subsequent α–*O*–4′ bonding during rearomatization producing a phenylcoumaran structure. These radical coupling reactions produce different pairs of diastereomers, such as silybin A and B, and silychristin A and B, and the same occur with other flavonolignans (Lee and Liu, 2003). All of these examples of flavonolignans illustrate the compatibility of flavonoids with lignification.

7.3.2 Stilbenolignans

Stilbenolignans are similarly produced from cross-coupling of monolignols with the phenylpropanoid moiety of hydroxystilbenes through different linkage types, as shown in Figure 7.5. These structure types commonly include benzodioxanes (as in aiphanol and 5-hydroxyaiphanol), phenylcoumarans (as in gnetofuran A and gnetucleistol F), or

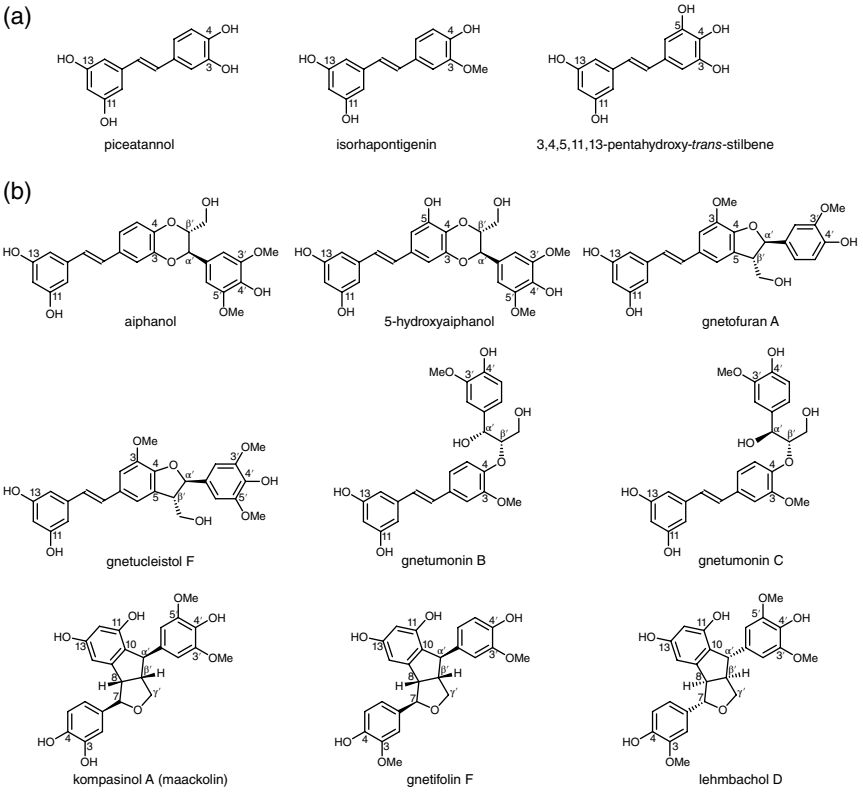

Figure 7.5 (a) Structures of the hydroxystilbenes that are known to form stilbenolignans. (b) Examples of some stilbenolignans identified in plants; all arise from putative radical coupling between the hydroxystilbene and a monolignol.

structures having a 3-oxabicyclo-[3.3.0]-octane ring system (as in kompasinol A, gnetifolin F, and lehmbachol D); stilbenolignans having simple 4—*O*—β′ ether structures (as in gnetumonins B and C) have recently been described in *Gnetum montanum* (Ma et al. 2017). Only a limited number of hydroxystilbenes (piceatannol, isorhapontigenin, and 3,4,5,11,13-pentahydroxy-*trans*-stilbene) have been found coupling with monolignols forming different stilbenolignans (Figure 7.5a). Piceatannol was found forming different stilbenolignans, such as aiphanol (Lee et al. 2001) and kompasinol A (Kobayahsi et al. 1996), from β–*O*–4′ and β–8′ couplings, respectively, of sinapyl alcohol with piceatannol. Isorhapontigenin was observed as part of the stilbenolignans gnetifolin F, gnetofuran A, lehmbachol D, gnetuclesitol F, and gnetumonins B and C, with different coupling types (Yao et al. 2006; Ma et al. 2017). Finally, the 3,4,5,11,13-pentahydroxy-*trans*-stilbene has also been found coupling with sinapyl alcohol in the stilbenolignan 5-hydroxyaiphanol (Lam and Lee, 2010) and 5-hydroxykompasinol A (Lam et al. 2008). As occurs with flavonolignans, all of these examples of stilbenolignans also illustrate the compatibility of some hydroxystilbenes with lignification because of the common radical coupling principles.

7.4 Lignin Monomers Derived from the Flavonoid and Hydroxystilbene Biosynthetic Pathways

Recent investigations have demonstrated that some members of the flavonoids (such as the flavone tricin) and hydroxystilbenes (particularly piceatannol), including also the respective hydroxystilbene glucosides (piceid, isorhapontin, and astringin), behave as authentic lignin monomers participating in radical coupling reactions to become integrally incorporated into the lignin polymer in some plants (del Río et al. 2012, 2017, 2020; Lan et al. 2015; Rencoret et al. 2018, 2019; Neiva et al. 2020), as is detailed in the following sections. These discoveries reveal the previously unknown interconnections between lignin biosynthesis and that of the flavonoids and hydroxystilbenes, and drastically expand, or even challenge, the traditional definition of lignin.

7.4.1 Lignin Monomers from the Flavonoid Biosynthetic Pathway – Tricin (and Naringenin and Apigenin in Rice Mutants)

The flavone tricin (5,7,4′-trihydroxy-3′,5′-dimethoxyflavone, Figure 7.2) was the first phenolic compound derived from outside the canonical monolignol biosynthetic pathway that was established as a true lignin monomer participating in cross-coupling reactions with monolignols (del Río et al. 2012). Tricin was first discovered in lignin preparations from wheat straw, and its structure was established by two-dimensional nuclear magnetic resonance (2D-NMR) (Figure 7.6). Previously unassigned signals in the heteronuclear single-quantum correlation (HSQC) spectra of grass lignins could be definitively assigned to tricin. The diagnostic signals corresponding to the C_8–H_8 and C_6–H_6 correlations appeared in the HSQC spectrum at δ_C/δ_H 94.1/6.56 and 98.8/6.20; the spectrum also showed the C_3–H_3 correlations at δ_C/δ_H 104.5/7.04, whereas the correlations for $C_{2'6'}$–$H_{2'6'}$ were observed at δ_C/δ_H 103.9/7.31 (del Río et al. 2012). The structure of tricin presents two phenolic hydroxyls at C-5 and C-7 of the chroman-4-one skeleton, with diagnostic phenolic

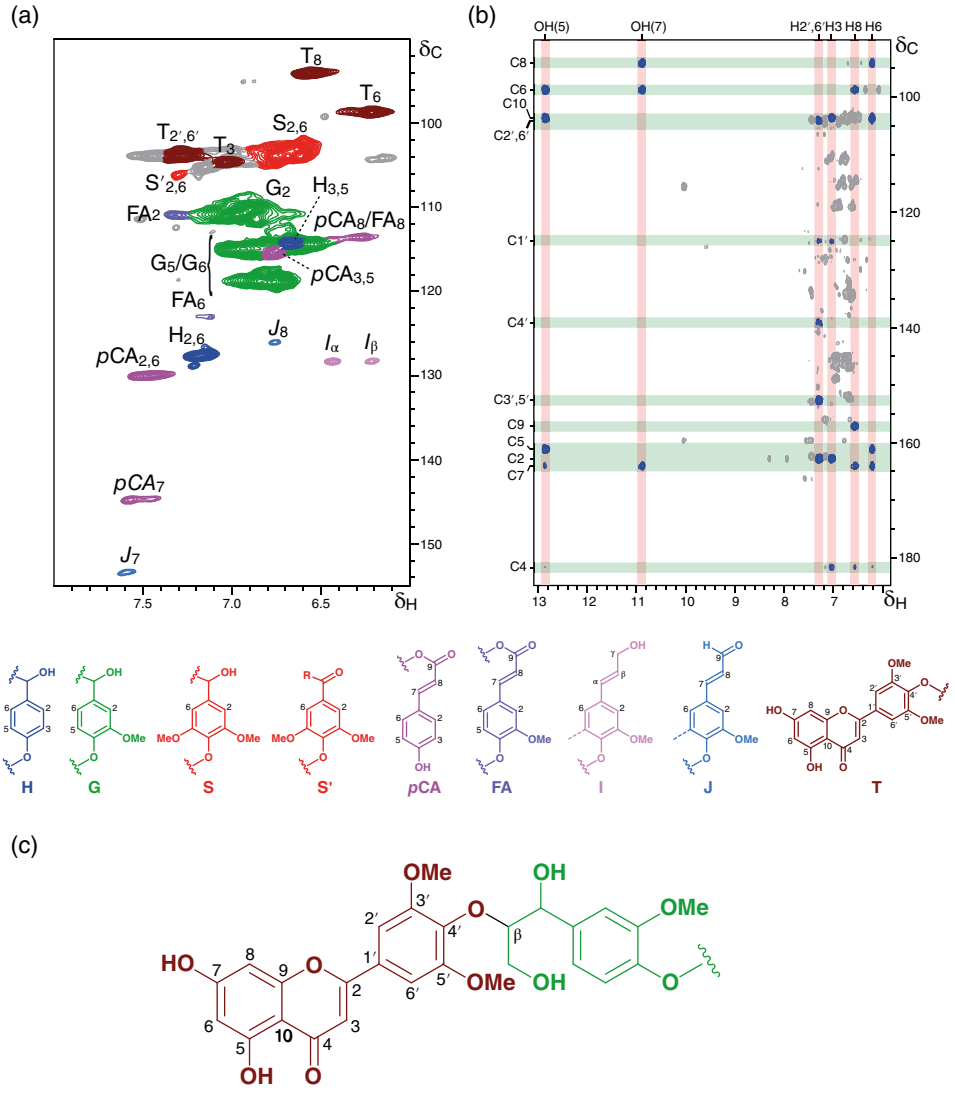

Figure 7.6 (a) Aromatic region of the 2D-HSQC-NMR spectrum of the lignin isolated from wheat straw showing the main correlation signals and their structural assignments to the color-coordinated structures below. (b) Partial HMBC spectrum (δ_C/δ_H 90–185/6.0–13.0) of wheat straw lignin that was crucial in establishing that the non-monolignol component of the lignin was the flavone tricin (5,7,4′-trihydroxy-3′,5′-dimethoxyflavone, **T**). (c) Mode of incorporation of tricin into the lignin structure through 4′–*O*–β ether linkages. *Source:* (a, b, c) Adapted from del Río et al. 2012. (*See insert for color representation of the figure.*)

proton chemical shifts that were readily apparent from their long-range correlations in the heteronuclear multiple-bond correlation (HMBC) spectrum at δ_H 12.86 and 10.88. More importantly, the HMBC spectrum revealed that the 4′-OH of ring-B was not free, suggesting that tricin was etherified to the lignin polymer through 4′–*O*–β ether linkages (as shown

in Figure 7.6c), as also occurred in the flavonolignans *threo*/*erythro* tricin 4′–*O*–β-guaiacylglycerol ethers, also known as salcolins A and B, that have been described in several plants (Wenzig et al. 2005; Chang et al. 2010; Lee et al. 2015). In fact, the signal for the correlation of the tricin C4′ carbon (at 139.5 ppm) and the proton at the β-position of a G-unit at 4.28 ppm was observed in the HMBC spectrum, providing evidence for this incorporation (del Río et al. 2012), which was later confirmed in separate studies (Lan et al. 2015, 2018). Additional biomimetic radical coupling reactions confirmed that tricin can only form 4′–*O*–β cross-coupled products with monolignols; no other type of cross-coupled products could be produced, nor were homodimeric coupling products seen (Lan et al. 2015).

Tricin cannot undergo radical dehydrodimerization and its only possible mode of incorporation into the lignin polymer is via 4′–*O*–β-coupling with a monolignol. Tricin can therefore only appear at the starting end of the lignin chain and this suggests a potential role as a nucleation site for lignification in grasses (as well as in other monocots), a role that was previously assigned to ferulates (Ralph et al. 1995). A detailed study of gel-permeation chromatography (GPC) fractions of maize lignin with different molecular weights showed that the percentage of tricin was slightly higher in lower-molecular-weight fractions, whereas the content of β–β resinol units was lower, adding evidence to the hypothesis that tricin acts as a nucleation site for lignification (Lan, Morreel et al. 2016).

A detailed analysis of the phenolic metabolites present in the lignifying zone of maize indicated the occurrence of a wide array of tricin-containing flavonolignan or low-molecular-weight flavonolignin (flavono-oligolignol) metabolites (Lan, Morreel et al. 2016). The compounds included a wide variety of products resulting from the coupling of all three monolignols (*p*-coumaryl, coniferyl, and sinapyl alcohols), with tricin (at its 4′–*O*- position) forming the respective tricin–4′–*O*–(β-arylglyceryl) ethers, along with the products arising from the coupling of tricin with the γ-acylated monolignols (the coniferyl and sinapyl acetates, and the *p*-coumarate conjugates). Trimeric compounds arising from the coupling of tricin-(4′–*O*–β)-monolignol with a further 4–*O*–β or 5–β linkage to another monolignol (including acylated monolignols) were also identified. In all cases, these tricin-containing metabolites were found to be fully racemic, further evidencing the combinatorial nature of the cross-coupling reactions between tricin and monolignols. It was therefore evident that the dimeric compounds resulting from the coupling of tricin with monolignols (and with their acetate and *p*-coumarate conjugates) should not be termed flavonolignans (that should be optically active); hence, these compounds should be considered to be oligomers that are destined for the lignin polymer, and the more appropriate term 'flavonolignols' or specifically 'tricin-oligolignols' was suggested for them (Lan, Morreel et al. 2016).

Soon after the discovery of the flavone tricin in the lignin of wheat straw, several other studies reported its occurrence in the lignins from other grasses, including giant reed (You et al. 2013), bamboo (Wen et al. 2013), barley (Rencoret et al. 2015), rice (Wu et al. 2013), maize (Lan et al. 2015), and sugarcane (del Río et al. 2015), as well as in other monocots such as in coconut coir (Rencoret et al. 2013). All these studies indicated that tricin occurred widely among lignins, particularly in grasses. A further survey of more than 50 plants from different origins (that included angiosperm monocots and eudicots, as well as gymnosperms) expanded the range of species with tricin incorporated into their lignins and

indicated that it was widely distributed in the lignins from species of the Poaceae family and that it also occurred in other monocots such as in the vanilla plant (*Vanilla planifolia* and *V. phalaenopsis*) from the Orchidaceae (Lan, Rencoret et al. 2016). Minor amounts of tricin were also found in the lignin from curaua (*Ananas erectifolius*) from the Bromeliaceae. In general terms, tricin was absent in the lignins from eudicotyledons, although some amounts could be detected in the lignins in alfalfa (*Medicago sativa*) and in *M. truncatula* from the Fabaceae (Bickoff et al. 1964; Lan, Rencoret et al. 2016; Lui et al. 2020; Ralph, 2020). The exact course of the evolution of tricin-lignins is therefore not yet clear but it appears to have occurred independently multiple times. No traces of tricin were observed in the lignins from gymnosperms, however. In the monocots, the content of tricin integrated into the lignin polymer was found to be higher than the content of extractable tricin, reaching up to 4841 and 5250 mg/kg in wheat and oat straws (in comparison to only 1014 and 1377 mg/kg of free tricin), which indicates that lignin, an abundant material that is usually regarded as a waste side-stream, can be an attractive and potential source for this valuable compound (Lan, Morreel et al. 2016).

As noted above, the entry into the biosynthesis of flavones is controlled by chalcone synthase (CHS), which converts *p*-coumaroyl-CoA into naringenin chalcone (Figure 7.7). The resulting naringenin chalcone is then isomerized by chalcone isomerase (CHI) to the flavanone naringenin, which is the common precursor for the biosynthesis of all major classes of flavonoids. The biosynthetic pathway leading from naringenin to tricin in grasses has only been recently elucidated (Lam et al. 2015, 2017). These investigations indicated that naringenin is converted into apigenin by a flavone synthase II (FNSII), and subsequent sequential hydroxylations and *O*-methylations at the flavone B-ring produce the respective luteolin, chrysoeriol, selgin, and finally the flavone tricin. It has also been recently demonstrated in several grasses (maize, rice, sorghum) that the caffeate *O*-methyltransferase (COMT) involved in the synthesis of sinapaldehyde or sinapyl alcohol in the monolignol biosynthetic pathway also participates in the biosynthesis of tricin *in planta* (Fornalé et al. 2017; Eudes et al. 2017; Lam et al. 2017; Lam, Tobimatsu et al. 2019). The interconnections of the monolignol and flavonoid biosynthetic pathways in the biosynthesis of grass lignins were clearly evidenced in a CHS-deficient mutant in maize, that lacked tricin and, obviously, its incorporation into the lignin polymer (Eloy et al. 2017). The study showed that the lignin for the mutant plant was enriched in β–β (resinol and tetrahydrofuran structures) and β–5 linkages (phenylcoumaran structures), further supporting the contention that tricin acts as an initiation site for lignin chains and that, in the absence of tricin, more monolignol dimerization reactions occur (Eloy et al. 2017).

Likewise, a recent study in rice showed that disruption of flavone synthase II (FNSII), the enzyme that catalyzes the direct conversion of the flavanone naringenin to the flavone apigenin, resulted in an altered cell wall lignin incorporating the intermediate naringenin, instead of tricin, into the lignin polymer (Lam et al. 2017). Naringenin, as tricin, can be incorporated into the lignin polymer via 4′–*O*–β cross-coupling with monolignols (as shown in Figure 7.8a). However, in contrast to tricin, naringenin has a nonsubstituted *p*-hydroxyphenyl B-ring, and thus can also couple with monolignols via 3′–β-type coupling, producing a phenylcoumaran structure, as depicted in Figure 7.8b. These phenylcoumaran structures were suggested to occur in the lignin of an *fnsII* rice mutant on the basis of the increase of signals for phenylcoumarans in the HSQC spectra (Lam et al. 2017). It is therefore evident that the flavanone naringenin can also be considered as an authentic lignin

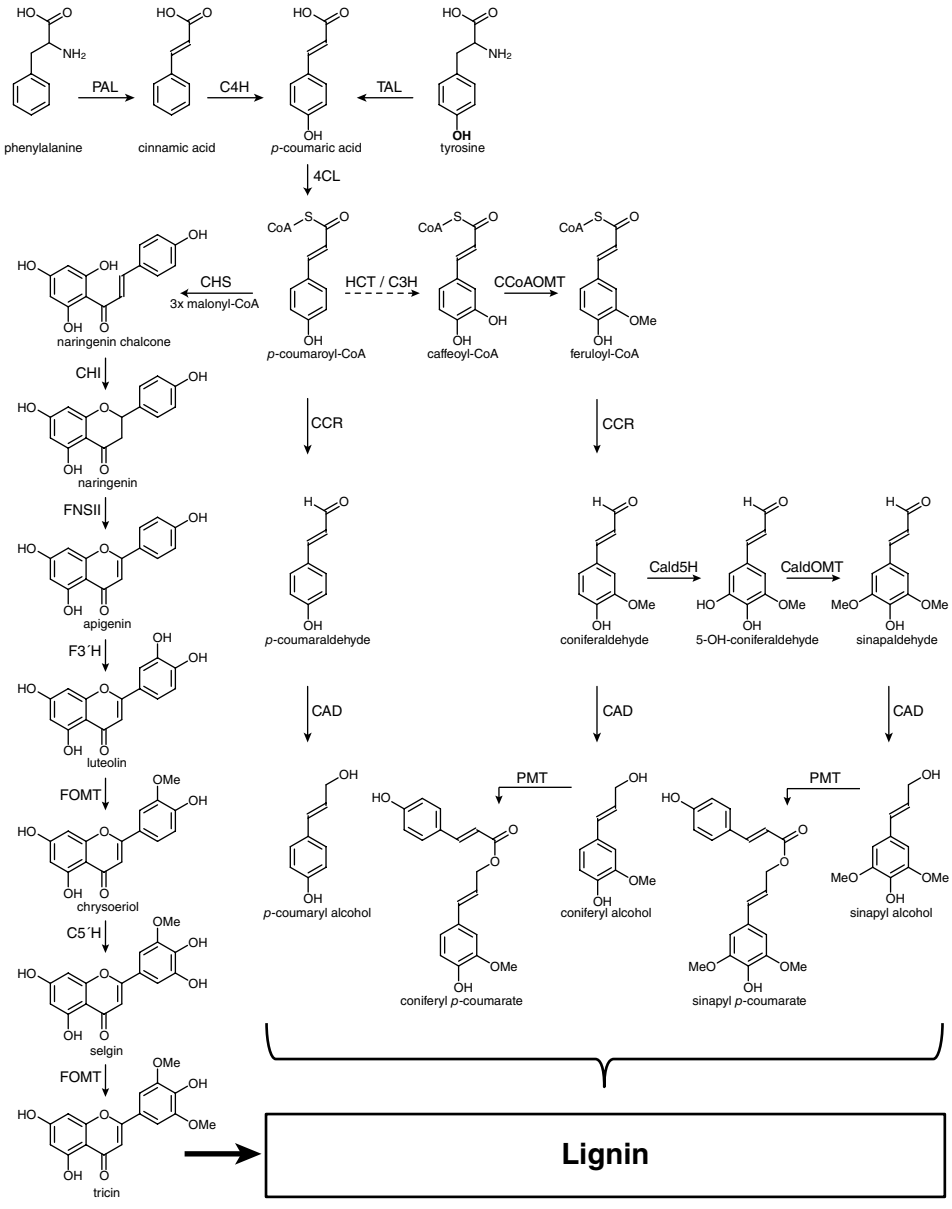

Figure 7.7 Lignin biosynthetic pathway in grasses (and other monocots) showing the interconnections of the monolignol and flavonoid biosynthetic pathways. PAL, phenylalanine ammonia-lyase; TAL, tyrosine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate:CoA ligase; HCT: *p*-hydroxycinnamoyl-CoA:quinate/shikimate *p*-hydroxycinnamoyltransferase; C3H: *p*-coumarate 3-hydroxylase; CCoAOMT: caffeoyl-CoA *O*-methyltransferase; CCR: cinnamoyl-CoA reductase; Cald5H (= F5H: ferulate 5-hydroxylase): coniferaldehyde 5-hydroxylase; CaldOMT (= COMT: caffeate *O*-methyltransferase): 5-hydroxyconiferaldehyde *O*-methyltransferase; CAD: cinnamyl alcohol dehydrogenase; PMT: *p*-coumaroyl-CoA:monolignol transferase (this step also requires *p*-coumaroyl-CoA from further up in the pathway as a co-substrate); CHS, chalcone synthase; CHI: chalcone isomerase; FNSII: flavone synthase II; F3′H: flavonoid 3′-hydroxylase; FOMT: flavonoid *O*-methyltransferase; C5′H: crysoeriol 5′-hydroxylase.

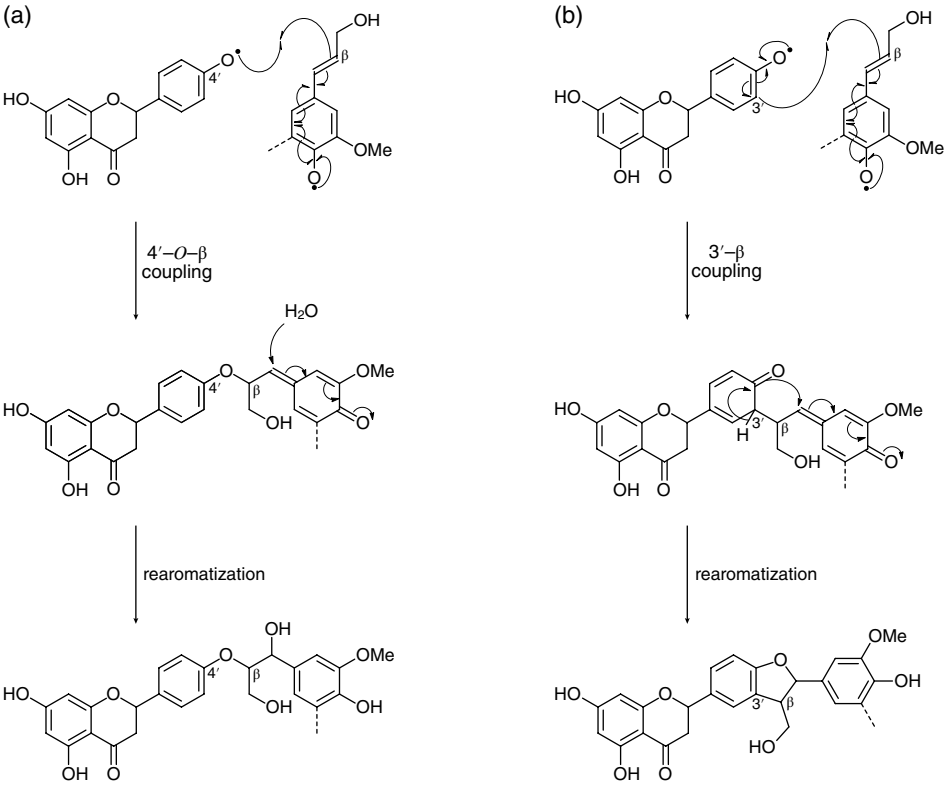

Figure 7.8 Naringenin cross-coupling modes with monolignols. (a) 4′–*O*–β coupling of naringenin with monolignols. (b) 3′–β coupling of naringenin with monolignols producing a phenylcoumaran structure. *Source:* (a, b) Adapted from Lam et al. 2017.

monomer in the *fnsII* rice mutant, participating in cross-coupling reactions with monolignols and being incorporated into the lignin polymer. Additional biomimetic radical coupling reactions of naringenin with monolignols further confirmed the compatibility of naringenin with lignification (Lam et al. 2017). Interestingly, the *fnsII* mutant plants presented normal growth performances, similar to the wild-type plants, suggesting that incorporating naringenin instead of tricin into the lignin structure did not affect normal growth and development; removing tricin altogether in the CHS mutant didn't appear to affect the stem biomass but did significantly increase leaf biomass (for largely unknown reasons) (Eloy et al. 2017).

More recently, it was claimed that the flavone apigenin can also be incorporated into the lignin polymer of culm tissues in a rice mutant lacking F3′H, the enzyme that hydroxylates the B-ring of apigenin to produce luteolin, an intermediate in the biosynthesis of tricin (Lam, Lui et al. 2019). This rice mutant cannot produce luteolin (and tricin in the last instance). Instead it will accumulate apigenin, which is the phenolic compound that is incorporated into the lignin polymer. Additional biomimetic radical coupling reactions of apigenin with monolignols further confirmed its compatibility with lignification. Thus, apigenin can also be considered as another noncanonical lignin monomer participating in

radical coupling reactions during lignification in this particular rice mutant (Lam, Lui et al. 2019).

As shown in Section 7.3.1, the wide diversity of flavonolignans identified in plants illustrates the compatibility of flavonoids with lignification. Although only the flavone tricin has been found in significant amounts in the lignins of a large number of plants, and the flavanone naringenin in the lignin of *fnsII* rice mutants and apigenin in rice mutants lacking F3′H, it is likely that other flavonoids, such as the flavanonol taxifolin, the flavonol quercetin, the flavanones eriodictyol and dihydrotricin, or the flavones luteolin and selgin, that are known to cross-couple with monolignols to form flavonolignans, could also be incorporated into the lignins of several plants through radical coupling reactions. Continuing lignin structural research and a broad survey of lignins from different plant origins is needed to discover other flavonoids that might be incorporated into the lignin polymers.

7.4.2 Lignin Monomers from the Hydroxystilbene Biosynthetic Pathway – Resveratrol, Isorhapontigenin, and Piceatannol, and Their *O*-glucosides Piceid, Isorhapontin, and Astringin

Recently, we also reported the occurrence of a second class of polyphenolic compounds, hydroxystilbenes (piceatannol, resveratrol, and isorhapontigenin), that behave 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 (del Río et al. 2017; Rencoret et al. 2018). Likewise, hydroxystilbene glucosides (piceid, isorhapontin, and astringin) were also found incorporated into the lignin polymer of Norway spruce bark, where they appear to act as genuine lignin monomers participating in radical coupling reactions during lignification (Rencoret et al. 2019; Neiva et al. 2020). Hydroxystilbenes are a class of nonflavonoid polyphenolics that, like the flavonoids, are metabolic hybrids resulting from a combination of the shikimate-derived phenylpropanoid and the acetate/malonate-derived polyketide pathways (Figure 7.3). However, and contrary to flavonoids that exhibit an enormous chemical diversity, simple hydroxystilbenes constitute a rather restricted class of compounds, the most important being resveratrol (3,11,13-trihydroxy-*trans*-stilbene), widely present in Vitaceae and Fabaceae, or pinosylvin (3,5-dihydroxy-*trans*-stilbene), which is present in Pinaceae. Hydroxystilbenes occur in a number of plant families where they play a role in plant protection and present antiviral, antibacterial, and antioxidant properties that are known to contribute to plant disease resistance (Roupe et al. 2006; Jeandet et al. 2010; Piotrowska et al. 2012).

The biosynthesis of hydroxystilbenes is controlled by stilbene synthase (STS) (Figure 7.9). STS catalyzes the formation of the stilbene backbone from three malonyl-CoA units and one CoA-ester of a cinnamic derivative; hence resveratrol is formed from *p*-coumaroyl-CoA whereas pinosylvin is formed from cinnamoyl-CoA. STS is a member of the CHS superfamily of type III polyketide synthases (Austin and Noel, 2003). STS enzymes are classified according to their preferred substrate into a *p*-coumaroyl-CoA-specific type such as resveratrol synthase (EC 2.3.1.95), which occurs primarily in angiosperms, or a cinnamoyl-CoA-specific type such as pinosylvin synthase (EC 2.3.1.146), which is typical for gymnosperms (Chong et al. 2009). The introduction of an additional hydroxyl group into the B-ring of the

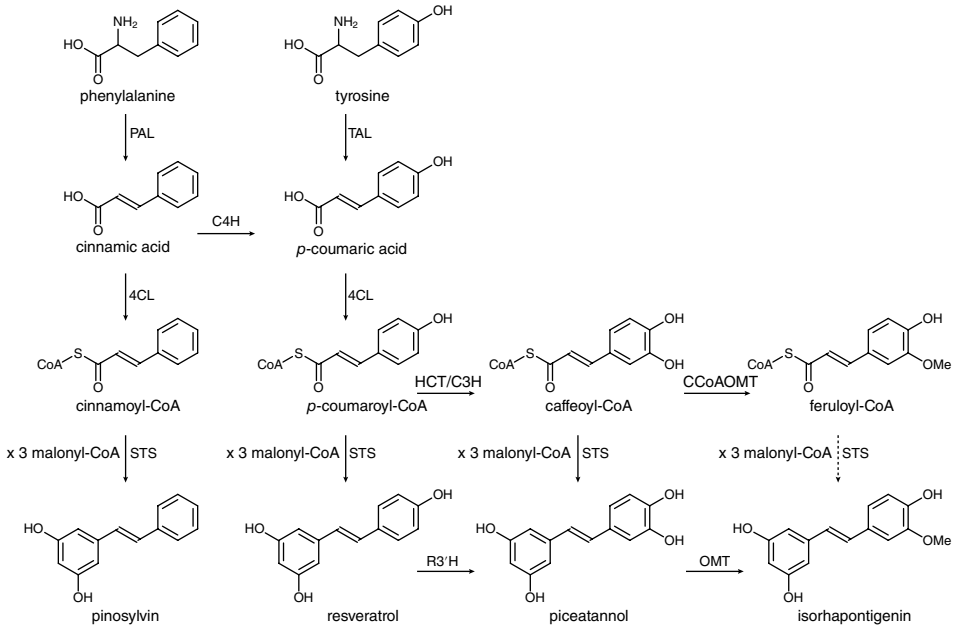

Figure 7.9 Biosynthetic pathway of simple hydroxystilbenes. PAL, phenylalanine ammonia-lyase; TAL, tyrosine ammonia-lyase; C4H, cinnamate 4-hydroxylase; 4CL, 4-coumarate:CoA ligase; HCT: *p*-hydroxycinnamoyl-CoA:quinate/shikimate *p*-hydroxycinnamoyltransferase; C3H: *p*-coumarate 3-hydroxylase; CCoAOMT: caffeoyl-CoA *O*-methyltransferase; R3′H: resveratrol 3′-hydroxylase; STS: stilbene synthase.

stilbene molecule, as in piceatannol (3,4,11,13-tetrahydroxy-*trans*-stilbene), seems to take place at the level of cinnamic acids. The specific STS responsible for the formation of piceatannol from caffeoyl-CoA has not been identified so far, although pinosylvin synthase was active with caffeoyl-CoA and was proposed to be responsible for the biosynthesis of piceatannol in *Picea* sp. (Raiber et al. 1995; Chong et al. 2009; Dubrovina and Kiselev, 2017); similarly, isorhapontigenin may presumably derive from feruloyl-CoA. However, it has also been proposed that resveratrol can undergo subsequent hydroxylation and methylation reactions by the corresponding stilbene 3-hydroxylase (S3H) and *O*-methyltransferase (OMT) to produce piceatannol and then isorhapontigenin (Hammerbacher et al. 2011).

Hydroxystilbenes, as the monolignols, can be oxidized to form radicals that are resonance-stabilized, as shown for piceatannol in Figure 7.10. Hydroxystilbene dimerization or cross-coupling with other hydroxystilbenes produces a wide variety of dimers (ε-viniferin, cassigarol E, scirpusin B, and others) and higher oligomers (Lin and Yao, 2006; Quideau et al. 2011; Rivière et al. 2012; Keylor et al. 2015). Hydroxystilbenes can also cross-couple with monolignols, and several stilbenolignans (see Figure 7.5) have been identified in a variety of plants from different families, as discussed in Section 7.3.2 (Kobayashi et al. 1996; Lee et al. 2001; Banwell et al. 2005; Yao et al. 2006; Lam and Lee, 2010; Begum et al. 2010; Ma et al. 2017), thus illustrating their chemical compatibility with lignification.

The first evidence for the occurrence of hydroxystilbenes incorporated into lignin polymers came from the release of significant amounts of piceatannol (together with minor

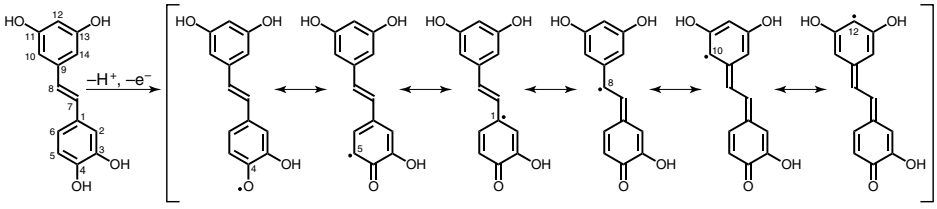

Figure 7.10 Piceatannol's phenolic radical and its different resonance forms. Although there are, in principle, different phenolic radicals that could be formed via the 3-, 11-, and 13-OH, all of the products formed are consistent with reactions only from the one arising from the 4-OH, presumably because 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.

amounts of resveratrol and isorhapontigenin) from lignin preparations isolated from several palm fruit (macaúba, carnauba, and coconut) endocarps by the Derivatization Followed by Reductive Cleavage (DFRC) degradation method (Figure 7.11) (del Río et al. 2017; Rencoret et al. 2018). DFRC is a chemical degradative method that selectively cleaves β–*O*–4 bonds in lignins, releasing the corresponding lignin monomers involved in those linkages (Lu and Ralph, 1997a, 1997b, 1998). This indicated that piceatannol (and to a lower extent resveratrol and isorhapontigenin) is an important component in the lignins of palm fruit endocarps, and that at least a part of them is present in the lignin polymer as β-ether linked structures (similar to the structures of gnetumonins B and C depicted in Figure 7.5), the linkages amenable to DFRC cleavage. However, and unlike tricin, which has only one possible mode of coupling with monolignols, piceatannol would be expected to couple and cross-couple with other piceatannols as well as with monolignols (and oligolignols) in a variety of ways, forming condensed structures that are not amenable to release by DFRC. It is therefore likely that the real amounts of piceatannol and other hydroxystilbene monomers incorporated into these lignins could be significantly higher than realized from the levels released by DFRC.

Useful information regarding the different linkages involving piceatannol in the lignin polymer was provided by 2D-NMR. Signals from piceatannol units were clearly observed in the 2D-HSQC-NMR spectra of the lignins isolated from several palm fruit endocarps, as shown for macaúba in Figure 7.12. The most striking feature in the aromatic region of the spectra was the presence of a previously unreported group of signals (labeled P_b and P_c) appearing at δ_C/δ_H 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, resinols C, or cinnamyl alcohol end-groups I). These new correlation signals, labeled P_b, P_c and V, corresponded to different structures involving piceatannol units that are depicted in Figure 7.12. Definitive identification of these structures was achieved by detailed HSQC-TOCSY and HMBC experiments and by comparing with piceatannol polymerization products and *in vitro* biomimetic cross-coupling reactions (del Río et al. 2017). Thus, the structure labeled P_b was identified as the dehydrodimerization product of two piceatannol units involving 8–*O*–4′/3′–*O*–7 linkages and producing a benzodioxane structure, similar to the stilbene dimer cassigarol E (Baba et al. 1994; Li et al. 2005; Morikawa et al. 2010); the structure labeled P_c was identified as another dehydrodimerization product of two

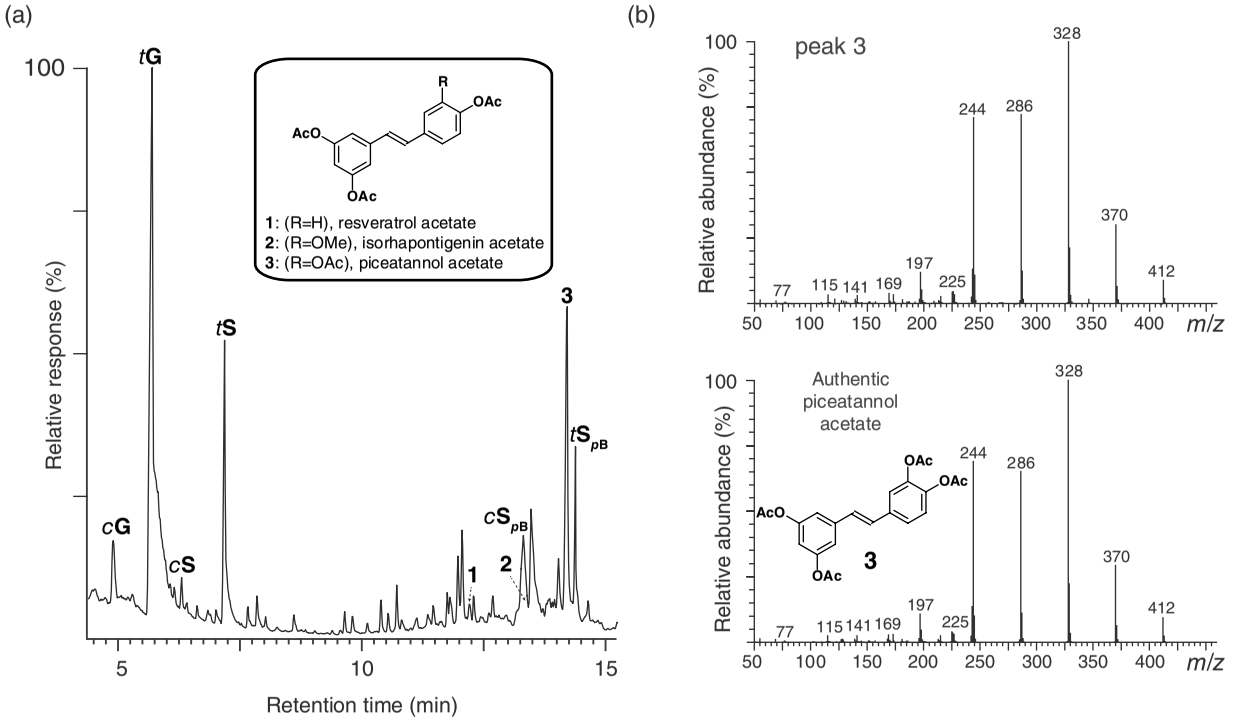

Figure 7.11 (a) Chromatogram of the DFRC degradation products released from the lignin from macaúba (*Acrocomia aculeata*) fruit endocarp showing the presence of hydroxystilbenes (**1**, resveratrol; **2**, isorhapontigenin; **3**, piceatannol, as their acetate derivatives). *c***G**, *t***G**, *c***S**, and *t***S** are the normal *cis*- and *trans*-coniferyl (**G**) and sinapyl (**S**) alcohol monomers (as their acetate derivatives). *c***S$_{pB}$** and *t***S$_{pB}$** are the *cis*- and *trans*-sinapyl *p*-hydroxybenzoates (as their acetate derivatives). (b) Comparison of the electron-impact (EI) mass spectra from peak **3** (acetylated) produced in the DFRC of the lignin from macaúba endocarp (top) and from an authentic standard of piceatannol **3** (acetylated) (bottom). *Source:* (a,b) Adapted from del Río et al. 2017.

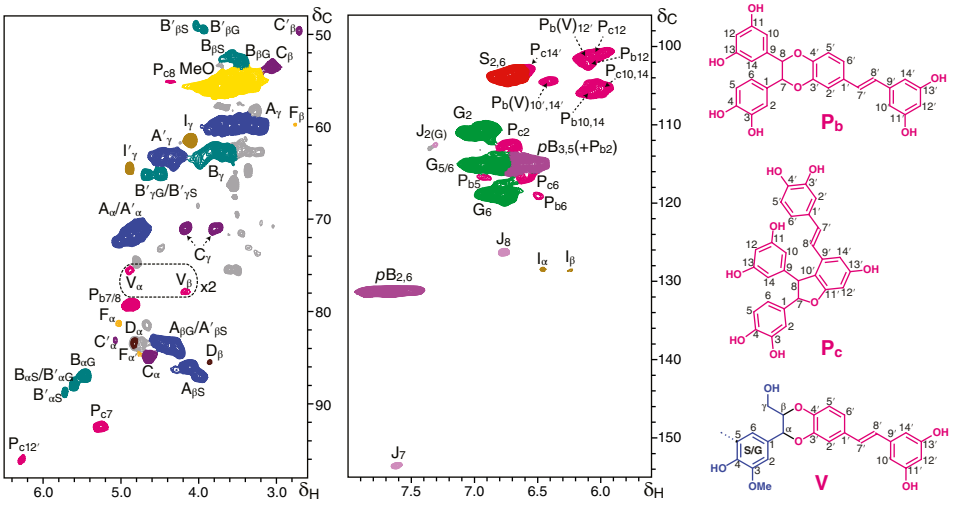

Figure 7.12 Side-chain (δ_C/δ_H 48-98/2.6-6.5) and aromatic (δ_C/δ_H 96-155/5.6-8.0 ppm) regions of the 2D-HSQC-NMR spectrum (in DMSO-d_6) of the lignin isolated from macaúba (*Acrocomia aculeata*) fruit endocarp showing the main signals from lignin and piceatannol (**P**) units. The structures of the main piceatannol homodimeric coupling (**P_b**, **P_c**) and piceatannol-lignin cross-coupling structures (**V**) are depicted. Signals from typical lignin structures are as follows: **A**: β–*O*–4′ alkyl-aryl ethers; **A′**: β-ether structures with *p*-hydroxybenzoates acylating the γ-OH; **B**: phenylcoumarans; **B′**: phenylcoumarans with *p*-hydroxybenzoates acylating the γ-OH; **C**: resinols; **C′**: tetrahydrofuran structures formed by β–β′-coupling of monolignols acylated at the γ-OH; **D**: dibenzodioxocins; **F**: spirodienones; **I**: cinnamyl alcohol end-groups; **I′**: cinnamyl alcohol end-groups acylated at the γ-OH; **J**: cinnamaldehyde end-groups; ***p*B**: *p*-hydroxybenzoates; **G**: guaiacyl units; **S**: syringyl units. *Source:* Adapted from del Río et al. 2017. (*See insert for color representation of the figure.*)

piceatannol units involving 8–10′/11′–*O*–7 linkages and producing a phenylcoumaran structure, similar to the stilbene dimer scirpusin B (Nakajima et al. 1978; Powell et al. 1987; Wang et al. 2011); structure V, on the other hand, was identified as a cross-coupling product of a piceatannol unit and a monolignol producing a benzodioxane bridge, similar to the stilbenolignan aiphanol (Lee et al. 2001).

The biosynthetic mechanisms of the different structures involving piceatannol are shown in Figure 7.13. Structure P_b was 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; structure P_c was 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, illustrating the importance of electron delocalization throughout both aromatic rings of the structure, as illustrated in Figure 7.10. 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. Biomimetic cross-coupling reactions between piceatannol and monolignols successfully proved that benzodioxane structures V could be easily formed

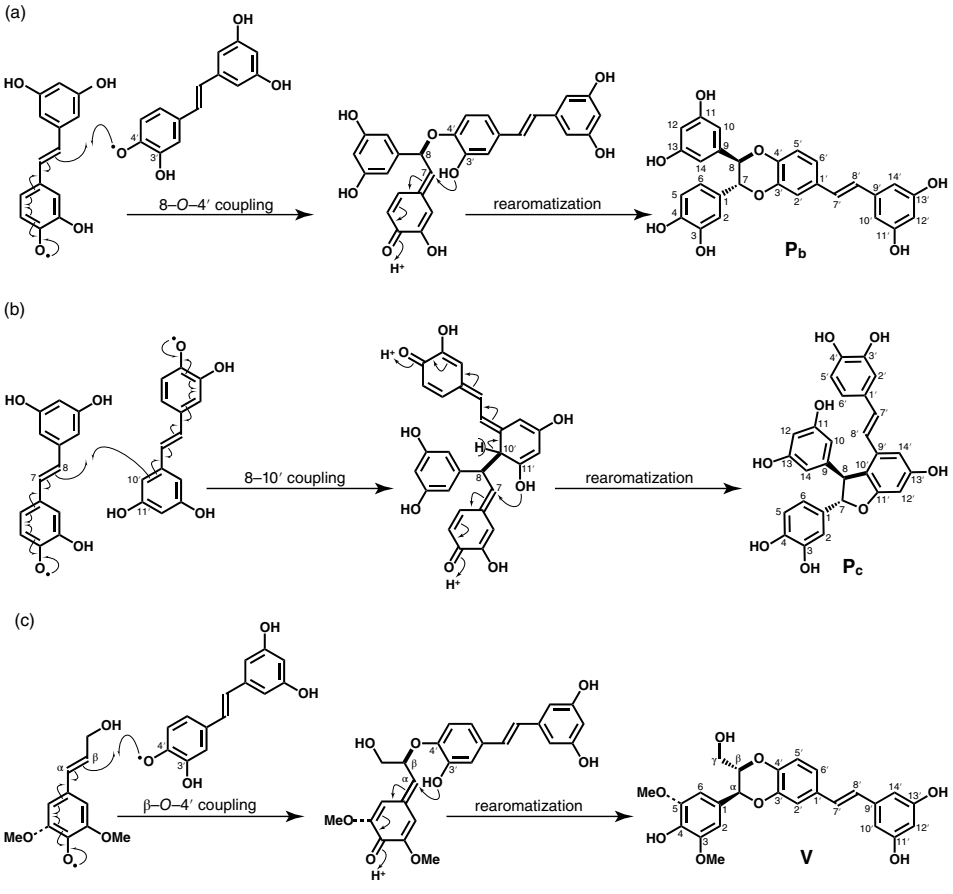

Figure 7.13 (a) Piceatannol dehydrodimerization by 8–*O*–4′ coupling to give the benzodioxane structure **P$_b$**. (b) Piceatannol dehydrodimerization by 8–10′ coupling to give the phenylcoumaran structure **P$_c$**. (c) Cross-coupling of monolignols M, sinapyl and coniferyl alcohol, with piceatannol 1 via β–*O*–4′ coupling to give the benzodioxane structure **V**. *Source:* (a, b, c) Adapted from del Río et al. 2017.

during the radical reaction (del Río et al. 2017). The occurrence of these benzodioxane structures in the lignins from palm fruit endocarps conclusively demonstrates that the hydroxystilbene piceatannol acts as an authentic lignin monomer, participating in coupling and cross-coupling reactions during lignification of these tissues and being integrally incorporated into the lignin polymer. Likewise, the study of the thermodynamics of these reactions using density functional theory (DFT) calculations indicated that, in general, the energetics of both homo-coupling and cross-coupling reactions are comparable with those of the monolignol coupling, demonstrating the compatibility of piceatannol with the lignification process (Elder et al. 2019, 2020).

As noted in Section 7.3.2, only a limited number of hydroxystilbenes (piceatannol, isorhapontigenin and 3,4,5,11,13-pentahydroxy-*trans*-stilbene) have been found coupling with monolignols and forming different stilbenolignans, indicating their compatibility with

lignification. In principle, all of these hydroxystilbenes can potentially occur as lignin monomers in plants. However, as far as we know, resveratrol has only been found forming dehydrodimers and higher oligomers but has not been found coupling with monolignols forming stilbenolignans (Begum et al. 2010; Rivière et al. 2012; Keylor et al. 2015), perhaps due to some type of chemical incompatibility, and this might explain why it has only been detected in minor amounts in the lignins of palm fruit endocarps. A broader survey of plant lignins is required to expand the range of plants with hydroxystilbenes incorporated into their lignins.

The incorporation of hydroxystilbenes into the lignins of palm fruit endocarps has been suggested to have a potential role in seed protection. The incorporation of piceatannol (and other hydroxystilbenes) into the lignins may allow the production of higher amounts of lignin by incorporating other phenolic compounds present in the cell wall into the lignin polymer (which indeed will form more condensed structures, as the phenylcoumarans and benzodioxanes shown in Figure 7.13), contributing to endocarp hardening. Hydroxystilbenes can also provide additional antioxidant, antifungal, and antiviral properties (Roupe et al. 2006; Jeandet et al. 2010; Piotrowska et al. 2012), thus further contributing to seed protection.

More recently, it has also been reported that the hydroxystilbene glucosides isorhapontin (isorhapontigenin-*O*-glucoside) and, at lower levels, astringin (piceatannol-*O*-glucoside) and piceid (resveratrol-*O*-glucoside), are incorporated into the lignin polymer in Norway spruce bark (Rencoret et al. 2019; Neiva et al. 2020). The corresponding aglycones isorhapontigenin, piceatannol, and resveratrol, along with glucose (as their peracetylated derivatives), were released by DFRC, a chemical degradative method that cleaves β-ether

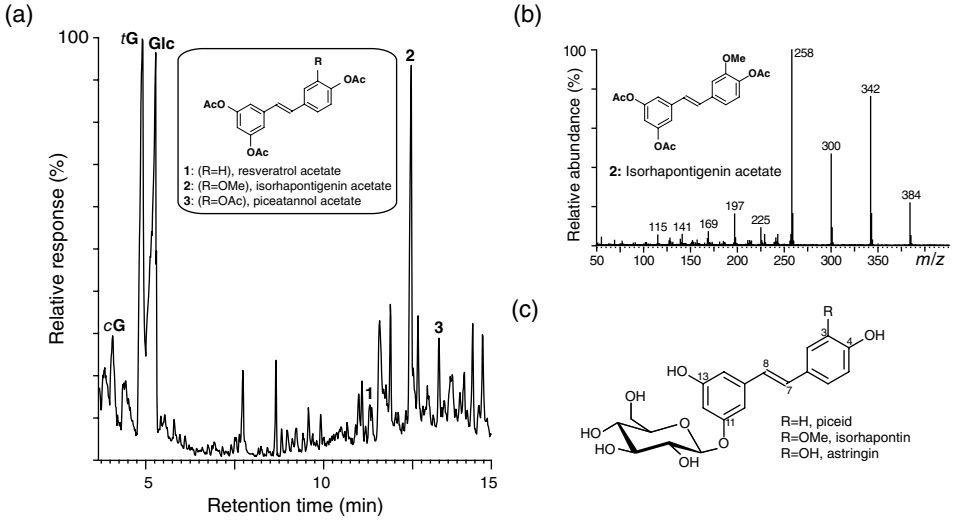

Figure 7.14 (a) Chromatogram of the DFRC degradation products released from the lignin of Norway spruce (*Picea abies*) bark showing the presence of hydroxystilbenes (**1**, resveratrol; **2**, isorhapontigenin; **3**, piceatannol, as their acetate derivatives). ***c*G** and ***t*G**, are the *cis*- and *trans*-coniferyl alcohol monomers (as their acetate derivatives). Note the occurrence of a peak from glucose **Glc** (as its peracetylated derivative). (b) Electron-impact mass spectrum of isorhapontigenin acetate (peak **2**), the major hydroxystilbene released upon DFRC. (c) Structures of the hydroxystilbene glucosides. *Source:* (a,b,c) Adapted from Rencoret et al. 2019.

bonds in lignin (Figure 7.14). The DFRC data, therefore, indicated that at least a part of the hydroxystilbene glucosides, particularly isorhapontin, was incorporated into the lignin of Norway spruce bark as β-ether linked structures, the ones cleaved by the DFRC degradation method. Additional information regarding the composition and structure of the lignin isolated from Norway spruce bark, including the mode of incorporation of the hydroxystilbene glucosides into the lignin polymer, was obtained from 2D-HSQC-NMR analysis (Figure 7.15). Signals from hydroxystilbenes were also clearly present in the aromatic

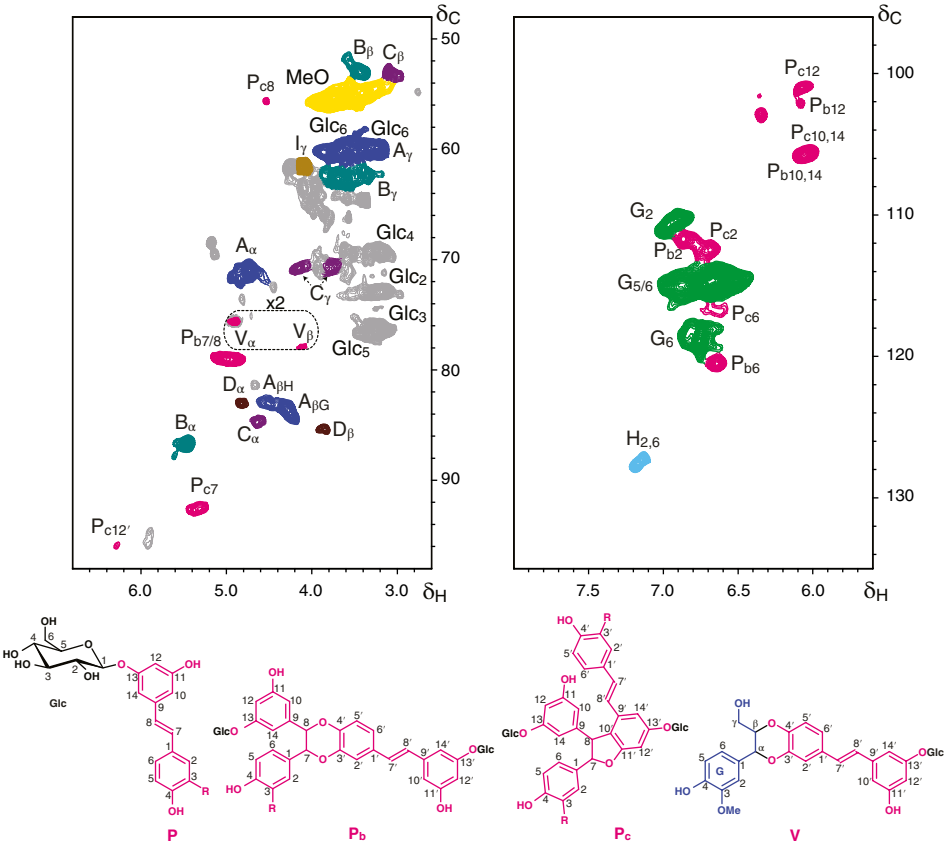

Figure 7.15 Aliphatic-oxygenated (δ_C/δ_H 48–98/2.6–6.8), and aromatic (δ_C/δ_H 96–135/5.6–8.0) regions of the 2D-HSQC-NMR spectrum (in DMSO-d_6) of the 'milled-wood' lignin preparation isolated from Norway spruce bark showing the main signals from lignin and hydroxystilbene glucoside units. The main signals from hydroxystilbene glucosides are: **P**: hydroxystilbene glucosides (isorhapontin, R= OMe; astringin, R = OH; piceid, R = H); $\mathbf{P_b}$: 8–*O*–4′/3′–*O*–7 benzodioxane structures involving isorhapontin (R = OMe), astringin (R = OH) or piceid (R = H) units; $\mathbf{P_c}$: 8–10′/11′–7 phenylcoumaran structures involving isorhapontin (R = OMe), astringin (R = OH) or piceid (R = H) units (please note that other phenylcoumaran structures arising from other linkages, such as 8–5′ and 8–12′, may also occur, but only the 8–10′ linkage has been authenticated due to the occurrence of signal $\mathbf{P_{c12'}}$); **V**: β–*O*–4′/3′–*O*–α benzodioxane structure formed by cross-coupling of astringin and coniferyl alcohol. Note that the signals from glucose (**Glc**) units appear in the aliphatic-oxygenated region. Signals from typical lignin structures are as follows: **A**: β–*O*–4′ alkyl-aryl ethers; **B**: phenylcoumarans; **C**: resinols; **D**: dibenzodioxocins; **I**: cinnamyl alcohol end-groups; **H**: *p*-hydroxyphenyl units; **G**: guaiacyl units. *Source:* Adapted from Rencoret et al. 2019. (*See insert for color representation of the figure.*)

region of the spectrum and were similar to those from the hydroxystilbenes observed in the 2D-HSQC-NMR spectra of lignins from palm fruit endocarps (del Río et al. 2017; Rencoret et al. 2018), including signals from isorhapontigenin and piceatannol, and confirming the results observed by DFRC. In addition, signals from the glucose moiety were clearly apparent in the aliphatic-oxygenated region of the spectrum, confirming the occurrence of the hydroxystilbene glucosides already advanced by DFRC. Most importantly, signals from structures involving the coupling of hydroxystilbene glucosides were clearly observed in the HSQC spectrum (Figure 7.15). Signals for benzodioxane ($\mathbf{P_b}$) and phenylcoumaran structures ($\mathbf{P_c}$) involving coupling of two hydroxystilbene glucosides, and signals for benzodioxane structures (**V**) formed by cross-coupling of astringin and coniferyl alcohol presented similar correlations to those observed for the incorporation of the hydroxystilbene piceatannol into the lignins of palm fruit endocarps (del Río et al. 2017). The occurrence of all of these coupled and cross-coupled structures involving hydroxystilbene glucosides indicates that these phenolic compounds, particularly isorhapontin and astringin, also behave as authentic lignin monomers that participate in radical coupling reactions during lignification in Norway spruce bark.

7.5 Conclusions and Future Prospects

Different phenolic compounds from outside the canonical monolignol biosynthetic pathway, namely the flavonoid and hydroxystilbenoid pathways, have been found to behave as authentic lignin monomers in several plants, particularly in monocots. The lignins from grasses and other monocots incorporate the flavone tricin (and naringenin and apigenin have been found incorporated into the lignins in some rice mutants) whereas the lignins from palm fruit endocarps incorporate the hydroxystilbene piceatannol (as well as resveratrol and isorhapontigenin at lower levels), and the lignin from Norway spruce bark incorporates hydroxystilbene glucosides, particularly isorhapontin. These discoveries broaden the definition of lignin monomers, and 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 'nonconventional' phenolic precursors, different from the three canonical monolignols, illustrates yet again 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, and subject exclusively to simple chemical compatibility (Ralph et al. 2008, 2019; Grabber et al. 2010, 2012, 2015; Vanholme et al. 2008, 2010, 2012, 2019; Mottiar et al. 2016; del Río et al. 2020). The incorporation of 'nonconventional' lignin monomers, not usually present in the lignins of other plants, as is the case for the tricin or the piceatannol described here (or any other flavonoid or hydroxystilbene that are compatible with lignification), can open up new ways to design and engineer the lignin structure to produce polymers and plant-based biomaterials with altered properties. On the other hand, valuable flavonoids and hydroxystilbenes could also be obtained from agricultural residues (cereal straws, palm fruit shells, or spruce bark) or from low-value lignin sidestreams in lignocellulose processing mills, which will open new opportunities for the valorization of these residues that are considered as wastes (Rinaldi et al. 2016).

7.6 Acknowledgments

This study has been 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). John Ralph, Wu Lan, and Hoon Kim were funded by the DOE Great Lakes Bioenergy Research Center (DOE BER Office of Science DE-FC02-07ER64494 and DE-SC0018409).

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