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Review

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Current understanding of lignan biosynthesis

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Dedicated to Professor Tien-Yau Luh of National Taiwan University.

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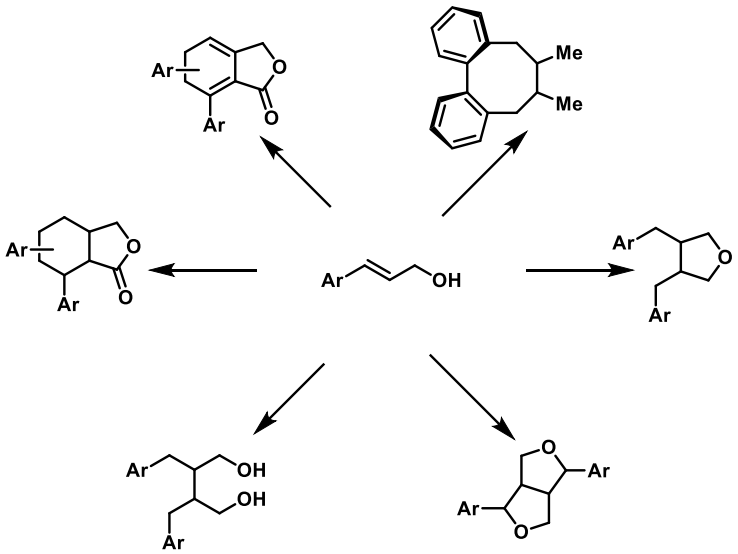
Abstract

Lignans constitute a large and diverse class of molecules widely distributed throughout the plant kingdom. They exhibit a wide range of pharmaceutically relevant biological activities and have attracted widespread research interests. This review covers the current understanding of lignan biosynthesis and aims to highlight key biosynthetic transformations responsible for their structural and biological diversity.



Keywords: Lignin, biosynthesis, podophyllotoxin, enzyme transformation

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1. Introduction

Lignans constitute a large and diverse class of natural products widely distributed in the plant kingdom and are found in plant roots, rhizomes, stems, leaves, seeds and fruits.[1] They are derived from the stereoselective oxidative coupling of two phenylpropanoid monomers, also termed monolignols, at the olefin of the propenyl moiety. The resulting β-β' (C8-C8') bond defines the substance class. Another defining characteristic of lignans is that oxygen represents the only heteroatom incorporated into the scaffold. Despite this rather narrow definition and common origin, lignans exhibit a vast structural diversity. A highly divergent biosynthesis leads to different patterns in cyclization, dividing lignans into structural subtypes: furofurans, furans, dibenzylbutanes, dibenzyllactones, dibenzylcyclooctadienes, aryltetralins and arylnaphthalenes.[2] An overview of the structural features, nomenclature and classification is provided in Figure 1.

Lignans exhibit a broad range of biological activities including antioxidant, antimicrobial, antiviral and antineoplastic properties.[3–6] While their specific functions *in planta* are largely unknown, lignans are thought to be involved in processes related to plant physiology and development. Furthermore, lignans are implicated to serve important functions in plant defense and survival against ecological stressors, as evidenced by their bioactive properties and distribution across numerous plant tissues. Many lignan-rich plants have been known for their therapeutic properties and potential health benefits to humans and have a long history of use in traditional medicine and diet.[7] Prominent examples include flax (*Linum usitatissimum*), sesame (*Sesamum indicum*), forsythia (*Forsythia suspensa*), olive (*Olea europaea*) and schisandra (*Schisandra chinensis*).

One of the most well-known lignans is podophyllotoxin from mayapple species (*Podophyllum peltatum, Sinopodophyllum hexandrum*). Podophyllotoxin is a potent anti-cancer and anti-viral agent that prevents cell division by destabilizing microtubules. It is a FDA-approved drug used for the treatment of external warts cause by human papillomavirus (HPV) since 1990, while it has been used since 1942.[8] Medicinal use of *Podophyllum* was reported as early as 900 AD in The Leech Book of Bald, an English pharmaceutical reference book.[9] Since the 1970s, dozens of podophyllotoxin derived drugs have entered clinical trials. Deoxypodophyllotoxin, a biosynthetic precursor to podophyllotoxin, was approved for phase I trials by the National Medical Products Administration in China in 2017.[10] Its semi-synthetic glycosylated congeners etoposide and teniposide were

approved for medical use in the United States in 1983 and 1992 respectively. Both of them are used for the treatment of several cancer types, including lung cancer, lymphoma, leukemia, neuroblastoma and testicular cancer. Etoposide is included in the World Health Organization's List of Essential Medicines.[11–13] Nordihydroguaiaretic acid (NDGA) has emerged as another promising lignan lead compound. It was isolated from the creosote bush (*Larrea tridentata*), which has been used in traditional medicine in northern Mexico and the southwestern United States for the treatment of more than 50 diseases.[14] It was shown to exhibit potent antioxidant, antiviral and antineoplastic activities. NDGA was used as a food antioxidant in the United States until the 1970s. Since then, it has been approved for treatment of actinic keratosis and its methylated derivative tetra-*O*-methyl NDGA has entered clinical trials for the treatment of HPV-linked cervical intraepithelial neoplasia. Both compounds have been identified to inhibit replication of dengue virus, hepatitis C virus, West Nile Virus and Zika Virus *in vitro*.[15,16] More than 150 dibenzylcyclooctadiene lignans, also termed *Schisandra chinensis* lignans, have been characterized to date and were all isolated from species in the *Schisandraceae* family.[17] Dibenzylcyclooctadienes make up the largest lignan subclass, however little is known about their biosynthesis. *Schisandra chinensis* has a long history of use in traditional Chinese medicine for the treatment of hepatitis and myocardial disorders among others. Notably, schizandrin A and B have been investigated for their antiviral activity against HIV, and schizandrin C was shown to possess potent hepatoprotectant properties.[18,19] Schizandrin C derivative was approved for the treatment of hepatotoxin-induced liver injury by the Chinese Food and Drug Administration in 2004.[20] Sesamin has been shown to exhibit potent hyaluronidase inhibition activity, thus attracting interest from the cosmetic industry.[21] It is the major lignan found in sesame seeds, a historical staple food in Africa and Asia and it has been popularized worldwide. Investigations about its health benefits have demonstrated anti-inflammatory and antioxidant properties.[22,23] Structures of several representative lignan natural products are shown in Figure 2. A comprehensive summary of bioactivities of lignan natural products can be found in recent reviews by Osmakov et al.[24] and Plaha et al.[25]

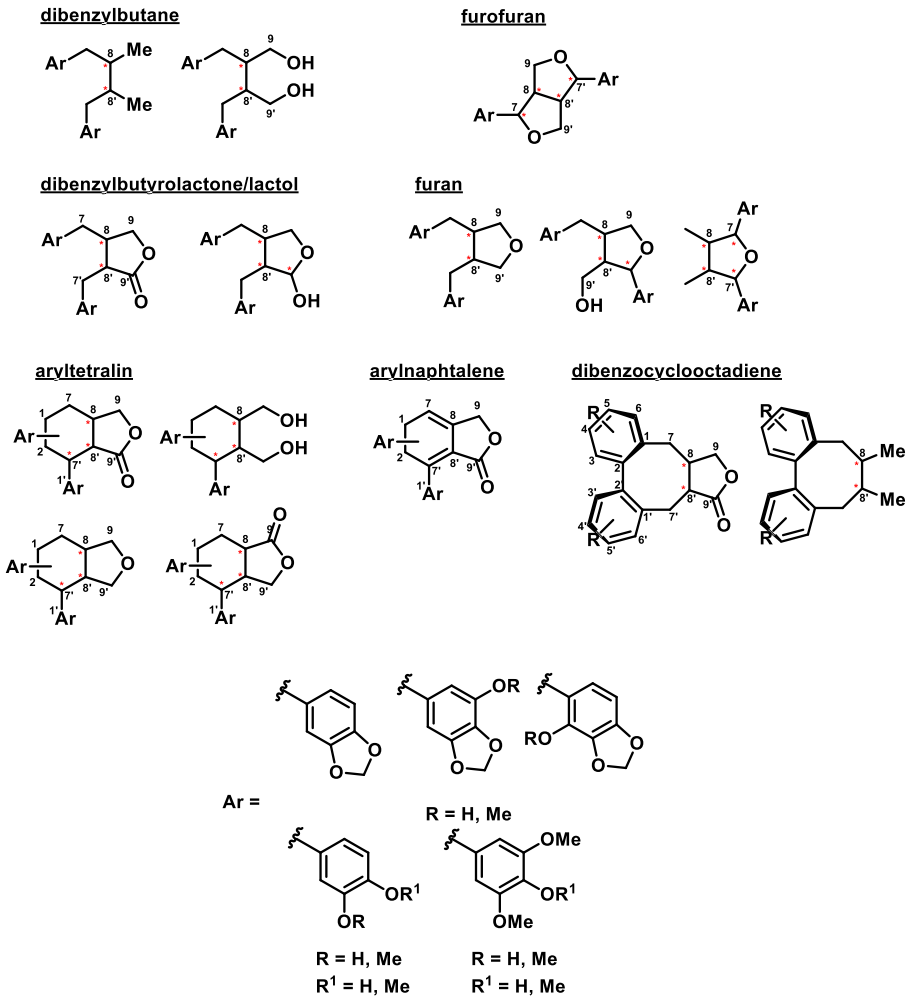

Figure 1. The structural features, nomenclature and classification of lignans.

Unsurprisingly, the sheer number (more than 750 lignans have been discovered since 2016) and diversity, along with substantial therapeutic potential have made lignan natural products a rich source of lead compounds for drug development.[26] However, natural occurrence in the producing organisms is often very low. For example, podophyllotoxin accounts for 0.3-1.0% (w/w) of rhizome mass, while sesamin constitutes 0.4-0.6% (w/w) of sesame oil, making extraction laborious and uneconomical.[27,28] An estimated 300,000 lbs. of mayapple roots are harvested per year to cover the demand of podophyllotoxin and epipodophyllotoxin, the precursor to etoposide and teniposide.[29] As mayapple species are not suitable to farm, wild *S. hexandrum* has become an endangered species.[30,31] While semi-synthetic and biotechnological approaches such as microbial cell factory, endophytic fungi and plant chassis have become promising alternatives, their economic viability is still limited.[32–37]

An ever-growing need for effective disease treatment and a recent advent of renewed interest in natural medicines and cosmetics have led to an increased global demand for lignan lead compounds. The lignan market is estimated to reach over 610 million USD by 2028.[38] It is therefore imperative to gain insight into the biosynthesis of lignan to aid in the identification of genes and elucidation of the corresponding enzyme mechanisms, providing a foundation for developing sustainable and economical methods of production. Nature effortlessly and efficiently accomplishes enormous structural diversity using a comparatively limited set of

enzymatic transformations. In this review, we provide an outline of a concerted pathway that is deployed to generate the scaffolds of lignans, which become branchpoints for the production of diverse natural products.

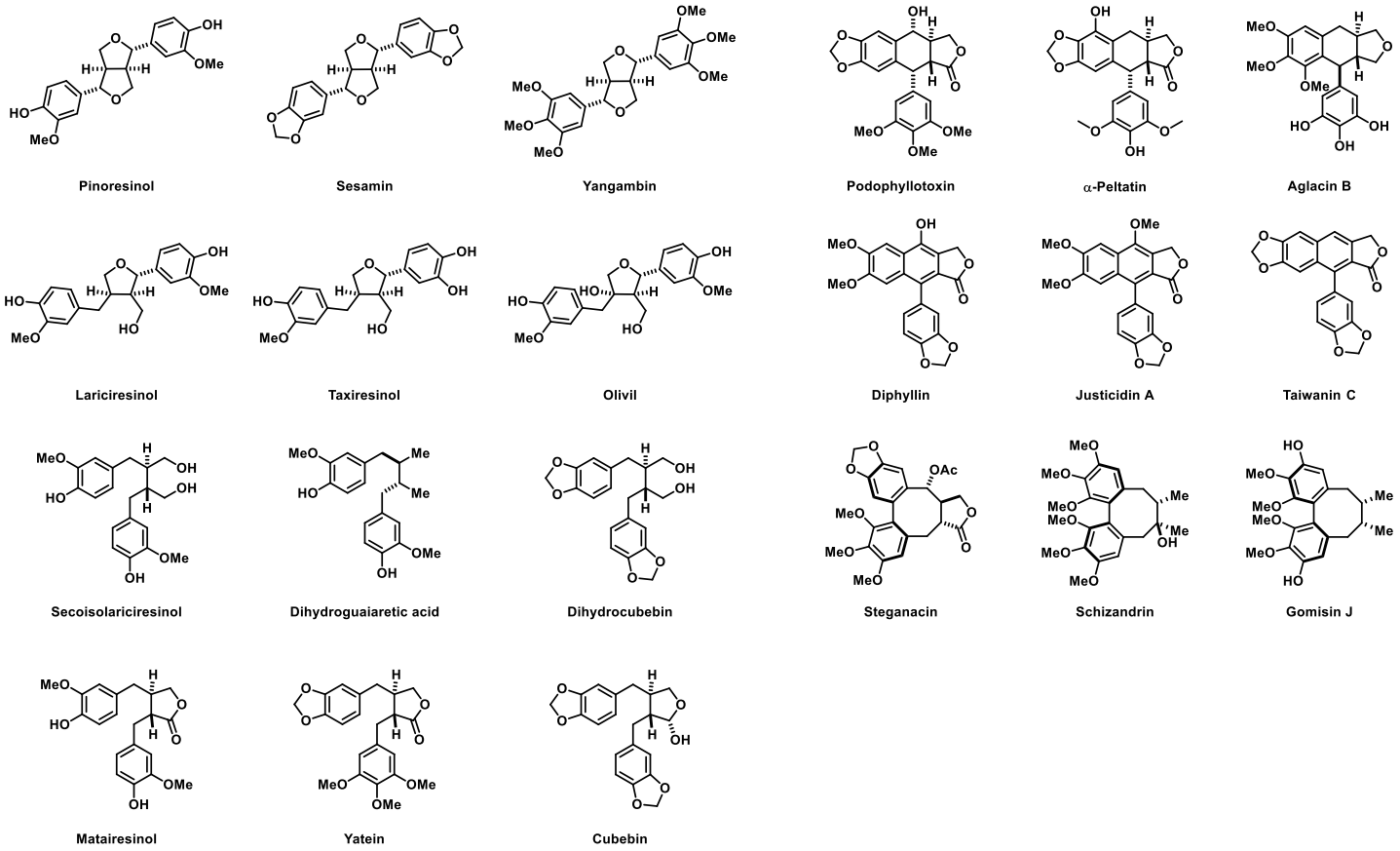

Figure 2. Representative examples of lignan natural prodcuts.

2. Formation of Coniferyl Alcohol

Before diving into the intricacies of lignan biosynthesis, it is important to discuss the biogenesis of their common precursor, coniferyl alcohol (CA) (Figure 3). In phenylpropanoid metabolism, as is the case for many other secondary metabolite pathways, amino acids, diverted from protein synthesis act as an entry point from which the natural product scaffold is built upon. For lignans, as well as flavanones, stilbenes, chalcones and other natural product families originating from the phenylpropanoid pathway, phenylalanine and tyrosine represent this entry point.[39,40] Phenylalanine ammonia-lyase (PAL) catalyzes elimination of ammonia to generate the cinnamic acid which is subsequently hydroxylated in the C4-position by cytochrome P450 monooxygenase cinnamate 4-hydroxylase (C4H).[41] *p*-OH-Coumaric acid is the skeleton for lignan natural products. Analogously, deamination of tyrosine is facilitated by tyrosine ammonia-lyase (TAL).[41,42] Cinnamic acid and *p*-OH-coumaric acid not only act as building blocks in lignans, but also serve as precursors to the structural polymer lignin, the second most abundant biopolymer on earth after cellulose.[43,44] PAL/TAL utilizes 5-methylidene-imidazolone (MIO) as the cofactor that is formed from three adjacent glycine, serine and alanine. Briefly, attack of the substrate's amino group onto the MIO cofactor generates a reactive intermediate, in which deprotonation followed by cleavage of the amine produces the C=C bond.[42]

From *p*-OH-coumaric acid, a common strategy is employed to generate CA, as well as the other two monolignols. This strategy involves an ATP-dependent 4-coumarate-CoA-ligase (4CL) to activate the C1-carboxylate of the *p*-OH-coumaric acid via CoA ligation.[45,46] With *p*-OH-coumaric acid also acting as an entry point for flavonoid biosynthesis, 4CL is indicated to channel the flux of *p*-OH-coumaric acid towards the respective pathways. In *Arabidopsis thaliana*, 4 isoforms of 4CL were identified, 2 of which were implicated in phenylpropanoid biosynthesis, 1 in flavonoid biosynthesis and 1 in piperine biosynthesis.[50-54] *p*-OH-Coumaroyl CoA undergoes shikimate/quinate group transfer by hydroxycinnamoyl-CoA: shikimate/quinate hydroxycinnamoyl transferase (HCT). HCT catalyzes the shikimation/quination of *p*-OH-coumaroyl CoA, which is hydroxylated by a cytochrome P450 monooxygenase C3'H and subsequently returned to the CoA ester by HCT.[49] HCT thus catalyzes a two-step reaction, yielding caffeoyl-CoA.[50] Additionally, feruloyl CoA production may also proceed via 4CL-mediated CoA ligation of ferulic acid. Furthermore, *O*-methylation catalyzed by SAM dependent caffeate 3-*O*-methyltransferase (COMT) or caffeoyl-CoA-*O*-methyltransferase (CCoAOMT) leads to feruloyl CoA formation (Figure 3).[51, 54]

With the production of *p*-OH-coumaroyl CoA, caffeoyl CoA and feruloyl CoA being clarified, subsequent steps toward CA follow a common pattern. The respective CoA-ester is reduced by NADPH-dependent cinnamoyl-CoA reductase (CCR).[52,53] Caffeoyl-CoA and feruloyl-CoA are readily reduced by CCR to generate the corresponding aldehyde intermediates: caffeyl aldehyde and coniferyl aldehyde, respectively. Caffeyl aldehyde may be converted to coniferyl aldehyde by COMT or further reduced to caffeyl alcohol by cinnamyl alcohol dehydrogenase (CAD) and subsequently *O*-methylated by COMT to generate CA. Reduction of coniferaldehyde by CAD naturally also results in the production of CA.[54] Additionally, reduction of *p*-OH-coumaroyl CoA yields *p*-OH-coumaraldehyde. C3-hydroxylation of *p*-coumaraldehyde has been shown in vitro, but there currently is no evidence supporting the results *in planta*. Therefore, *p*-OH-coumaraldehyde production is likely a shunt pathway.[50]

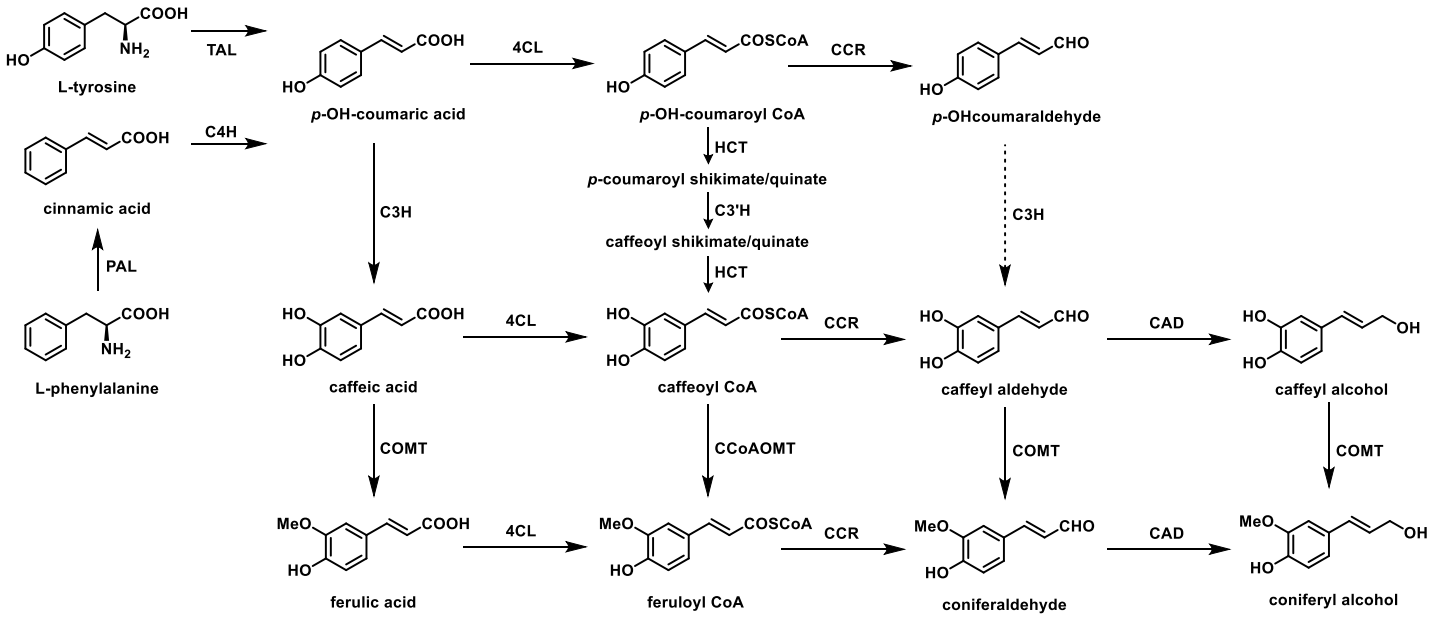

Figure 3. Formation of coniferyl alcohol (CA).

3. Scaffold Generation Overview

Dimerization of monolignols proceeds in a phenolic oxidative coupling reaction facilitated by an oxidase, e.g. laccase, and dirigent protein (DIR), in which the oxidase generates a phenoxy radical and DIR induces stereo- and regio-selective coupling of two monolignol radical species. Coupling of two coniferyl alcohol radicals gives rise to the primary lignan precursor, pinoresinol, other products including norlignans, neolignans are derived from coupling of other resonance forms of the phenoxy radical intermediates. The coupling mode leading to pinoresinol is depicted in Figure 4. Pinoresinol (the furofuran type) may then undergo consecutive reduction by pinoresinol lariciresinol reductase (PLR) to lariciresinol (furan-type) and secoisolariciresinol (dibenzylbutane-type). Secoisolariciresinol in turn is oxidized by secoisolariciresinol dehydrogenase (SDH) to form matairesinol. This pathway has been demonstrated in various lignan-producing plant species and can be described as the general lignan pathway. At each step, some intermediate flux is diverted from the general pathway and subjected to scaffold tailoring, which ultimately is the key to complexity generation in lignan biosynthesis.

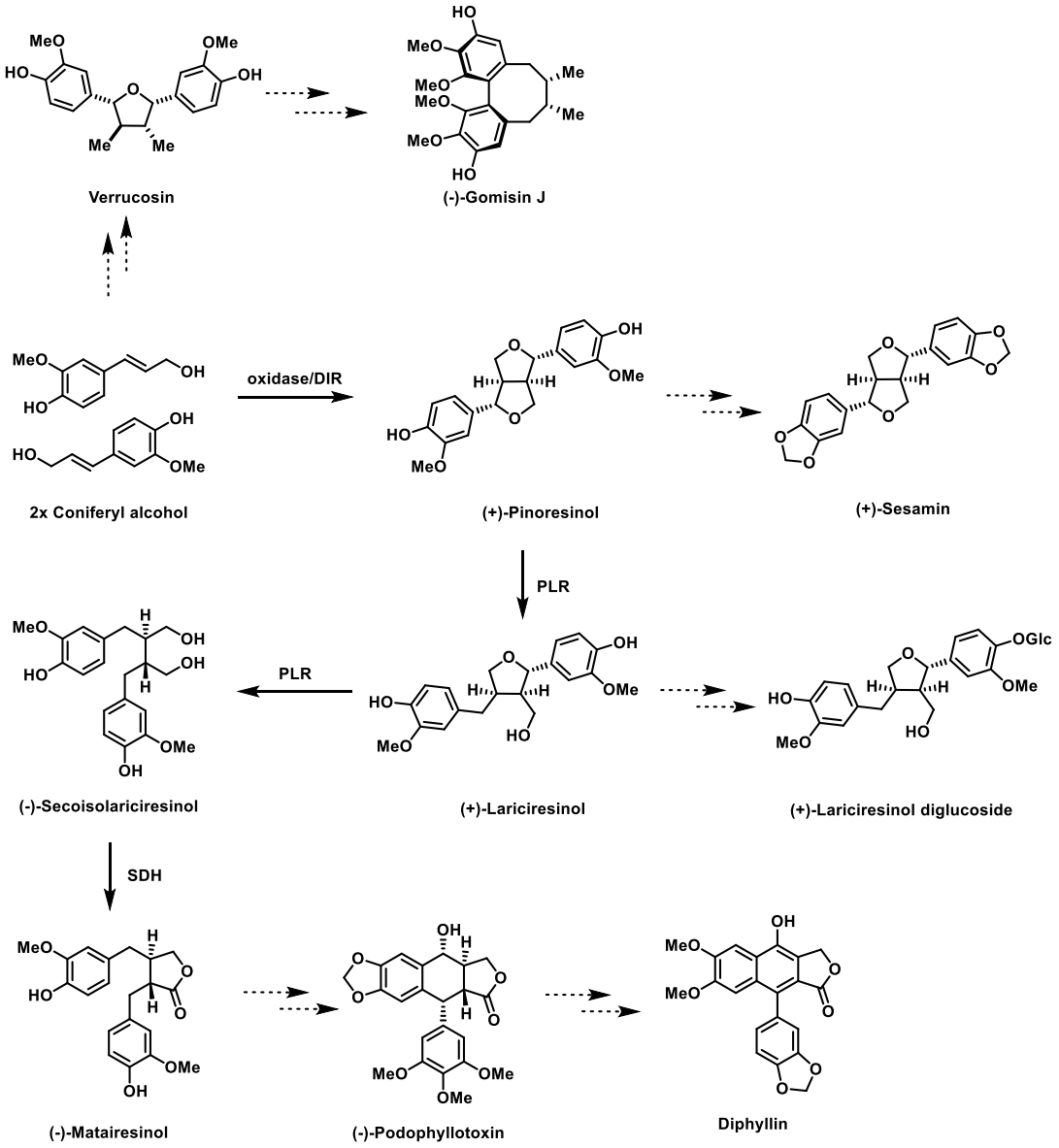

Figure 4. Formation of lignans: scaffold generation through coupling and other tailoring reactions.

4. Monolignol Dimerization

The dimerization process starts with the formation of the phenoxy radical intermediate. This process is enabled by oxidase enzymes, e.g, laccases and peroxidases.[55] While the involvement of DIR has been studied and several isoforms have been characterized, the detailed mechanism remain to be elucidated.[56] Studies on DIR-mediated coupling often utilize laccase isozymes from fungi.[57–59] In addition, the first plant laccase, *Zm*Lac3 from *Zea mays* was characterized by Xie et al. in 2020.[60] In the study, the structure of *Zm*Lac3 was solved and found to exhibit three cupredoxin-like domains stabilized by disulfide bridges and harboring four Cu centers at the interface of the trimer. *Zm*Lac3 was shown to exhibit substrate preference towards sinapyl alcohol, but it was able to use coniferyl alcohol and *p*-coumaryl alcohol. Similar findings regarding substrate promiscuity and preference have been reported for laccases.[61–63]

Coupling of the monolignol phenoxy radical intermediates is facilitated by DIRs (Figure 5). It is in this step that stereo- and site-selectivity is imparted. DIR was first identified by in *Forsythia suspensa*. In the presence of (*E*)-coniferyl alcohol, exclusively (+)-pinoresinol-formation was observed, while racemic mixtures of pinoresinol and other coupling products were obtained when only laccase was present.[56,64] The first crystal structure of DIR was characterized by Gasper et al. in 2016.[65] *At*DIR6 from *Arabidopsis thaliana* exhibited eight-stranded beta-barrel topology forming a trimeric structure with well-defined and spatially separated substrate binding cavities, each possessing two pockets for the binding of the proposed radical intermediates. *At*DIR6 was shown to exhibit enantio-complementary reactivity to *Fs*DIR, yielding (-)-pinoresinol exclusively. In a recent study by Sattely et al. structural comparison of DIRs, *Ps*DRR206 from *Pisum sativum* (for the production of (+)-pinoresinol) and AtDIR6 from *Arabidopsis thaliana* (for the production of (-)-pinoresinol), demonstrated previously postulated differential binding modes resulting in respective *si-si* or *re-re* coupling.[58] In the same study *Ph*DIR from *Podophyllum hexandrum* in conjunction with a fungal laccase *Tv*Lac from *Trametes versicolor* converted (*E*)-coniferyl alcohol and synthetic CA-analogs with varied *meta*-substitution to (+)-pinoresinol and analogues. The regioselective coupling mediated by DIR not only facilitates the formation of the lignan-defining C8-C8’ bond but is also the source of chirality in lignan biosynthesis. Overall, three new bonds (including one C-C and two C-O bonds) and four new stereocenters are generated in this single-step coupling – a feat unachievable by synthetic methods. While homotypic dimerization of coniferyl alcohol serves as the biosynthetic origin of the vast majority of lignan natural products, the scope of DIR-mediated coupling also includes other monolignols and likely extends to downstream intermediates of phenylpropanoid metabolism such as isoeugenol.[66,67] Regardless of substrate or coupling mode, the presence of *para*-hydroxyl moiety is a requirement of oxidase/DIR-enabled dimerization. It has been proposed that the hydroxyl group stabilizes the radical intermediate, therefore facilitating dimerization process.[56,57] The involvement of oxidase enzymes in radical generation and delivery, as well as the mechanism of radical capture by DIR remains poorly understood.

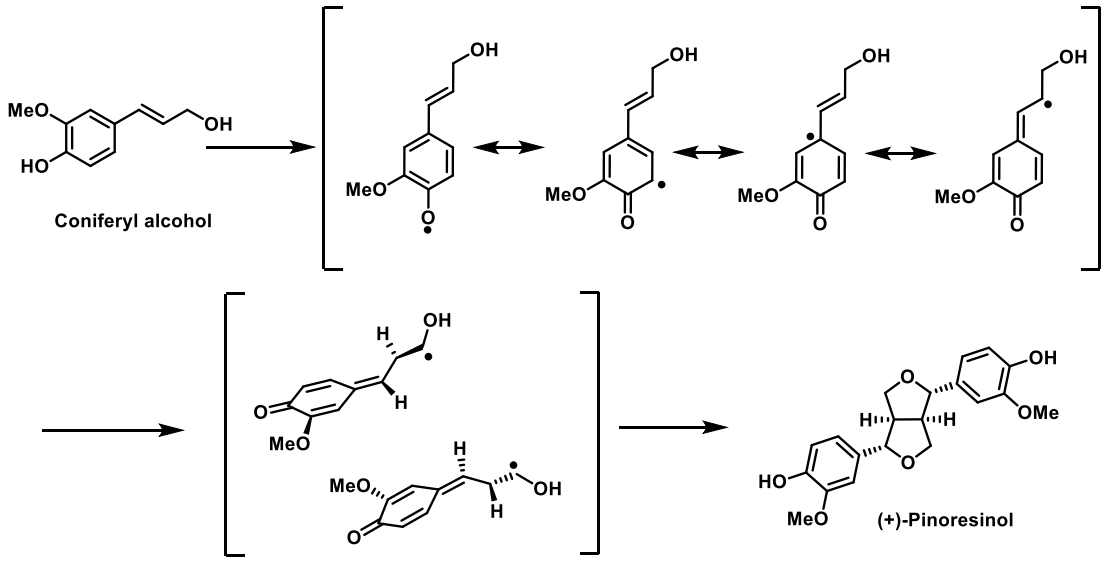

Figure 5. The proposed dimerization mechanism.

5. Reduction of Pinoresinol/Lariciresinol

Sequential reduction of the furan rings of pinoresinol to lariciresinol, then secoisolariciresinol is mediated by NADPH-dependent pinoresinol-lariciresinol-reductase (PLR) (Figure 6). Early investigations of PLR demonstrated the involvement of two isoforms of PLR, each carrying out one reduction in *Forsythia intermedia*.[68] Investigations of PLRs in lignan producing plant species, revealed varying degrees of substrate selectivity, with some PLRs can facilitate the reduction of both pinoresinol and lariciresinol. Therefore, the observed variations in substrate specificity of PLR likely play a role in controlling and diverting lignan flux in producing organisms.[69] The phenolic *para*-OH group is important for PLR activity, thus revealing a possible strategy for the reaction mechanism. The reaction is proposed to proceed via a quinone methide intermediate, ring opening, followed by a hydride provided from NADPH to restore aromaticity.[70] Biochemical characterization of PLR homologs using exogenously supplied racemic pinoresinol and lariciresinol has revealed that the PLR-catalyzed reduction is highly enantioselective. For example, PLR-Tp1 from *Thuja plicata* catalyzes stereoselective reduction of (+)-pinoresinol and (+)-lariciresinol, while PLR-Tp2 reduces (-)-pinoresinol and (-)-lariciresinol.[70] Though the observed enantiospecificity of PLR-Tp1 and PLR-Tp2 has been investigated using X-ray crystallographic analyses and site-directed mutagenesis approaches, the factors governing selectivity remain unclear.[71]

Additionally, the substrate specificity was investigated by Wu et al. via structural comparison of two PLR isoforms from *Camellia sinensis*.[72] A variable loop region consisting of 13 amino acid residues was found to be crucial to substrate recognition. Recently, Xiao et al. characterized the structures of PLR isoforms, *Ii*PLR1 (*Isatis indigotica*), *At*PrR1 (*Arabidopsis thaliana*) and *At*PrR2 (*Arabidopsis thaliana*), each with substrates pinoresinol and lariciresinol bound. In conjunction with biochemical assays, both substrate- and enantio-specificity were investigated. While *Ii*PLR1 exhibited comparable efficiency toward both pinoresinol and lariciresinol, *At*PrR1 showed significant preference for pinoresinol and *At*PrR2 exclusively converted pinoresinol.[73] By introducing mutations in the variable loop region, the substrate preference of *Ii*PLR1 and *At*PrRs could be switched. Though potential residues contributing to substrate enantiospecificity were proposed, their identity and the underlying mechanism could not be verified.[72,73]

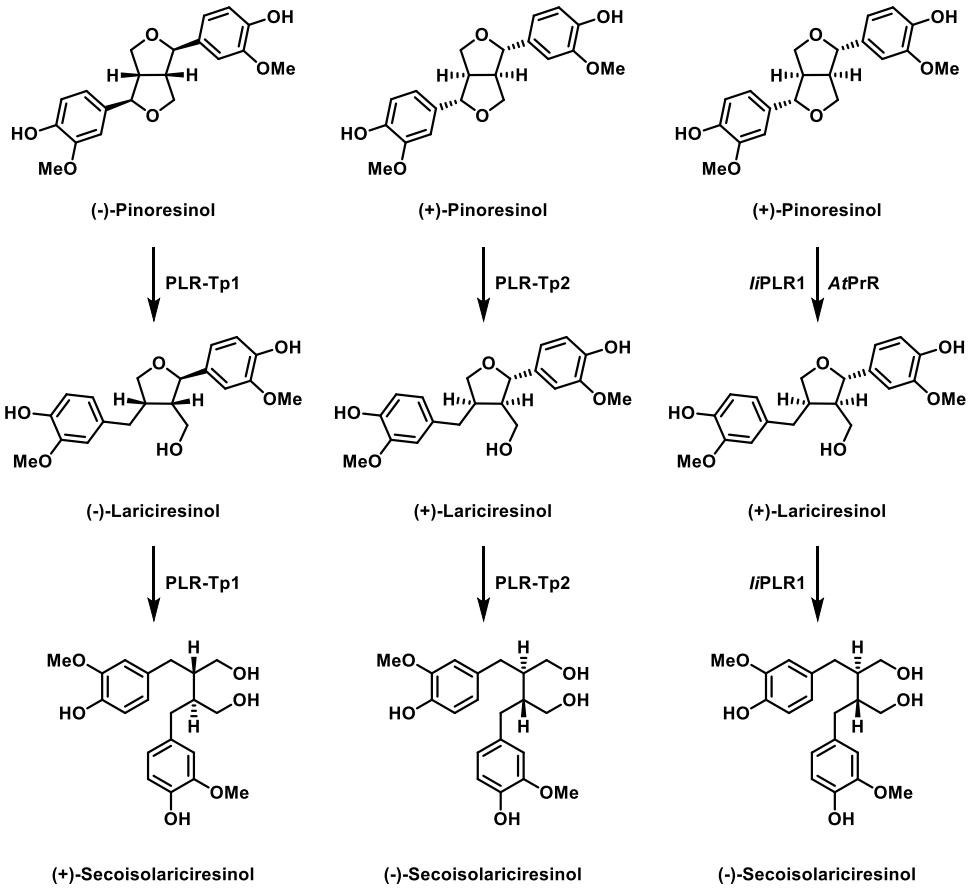

Figure 6. Reactions catalyzed by pinoresinol-lariciresinol-reductases (PLRs).

6. Dehydrogenation of Secoisolariciresinol

The next step in the lignan biosynthetic pathway involves the conversion of dibenzylbutane-type secoisolariciresinol to dibenzyllactone-type matairesinol. Selective sequential oxidation of one of the alcohols is facilitated by NAD(P)H dependent secoisolariciresinol-dehydrogenase (SDH) (Figure 7). The resulting carboxylic acid is then subjected to intramolecular cyclization resulting in lactone formation.[29] Structural analysis of SDH isolated from *Podophyllum peltatum* revealed a homotetrameric structure with a highly conserved catalytic triad consisting of Ser153, Tyr167 and Lys171.[74,75] *In vitro* biochemical assays with SDH from *Forsythia intermedia* and *Podophyllum peltatum* indicated higher activity in the presence of NADH instead of NADPH. Furthermore, incubation with racemic secoisolariciresinol resulted in selective formation of (-)-matairesinol.[75]

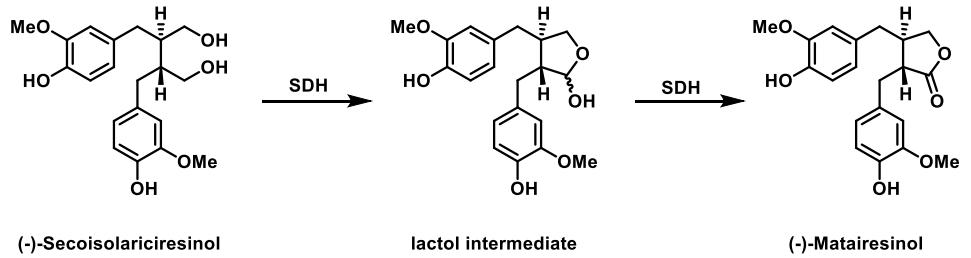

Figure 7. Reaction catalyzed by secoisolariciresinol-dehydrogenase (SDH).

Overall, the general lignan biosynthetic pathway utilizes sequential enzymatic transformations to generate four different lignan scaffolds: furofuran, furan, dibenzylbutane and dibenzyllactone, in which a vast majority of lignan natural products originate from either one of the four intermediates. Notably, varying degrees of enantio- and substrate-specificity divert the incoming flux of pinoresinol toward the diverse composition of lignan natural product observed in respective producer organisms.

7. Formation of Podophyllotoxin Core

Due to the pharmacological importance of podophyllotoxin, its biosynthesis has been the subject of extensive research and responsible genes have been largely identified. Herein, the biosynthetic pathway from matairesinol to deoxypodophyllotoxin will be detailed (Figure 8). The four enzymes responsible for the conversion of (-)-pluviatolide to (-)-deoxypodophyllotoxin in *Sinopodophyllum hexandrum* were first identified in 2015 by Lau and Sattely.[32] The first transformation involves the conversion of (-)-matairesinol to (-)-pluviatolide. Methylenedioxy (MDO) bridge formation in plants is associated with the cytochrome P450 monooxygenase family (CYP). CYPs compose a large and diverse enzyme superfamily, with members playing essential roles in plant secondary metabolism. For example, CYP719A1 from *Coptis japonica* catalzyed MDO formation in isoquinoline alkaloid biosynthesis.[76,77] Leveraging massively parallel sequencing of *Sinopodophyllum hexandrum* and *Podophyllum peltatum* transcriptomes, Marquez et al. identified two P450s involved in podophyllotoxin biosynthesis, *Sh*CYP719A23 and *Pp*CYP719A24.[78] In addition, other enzymes, e.g., non-heme iron oxygenases, have also been implicated in MDO bridge formation.[79] In the next step, (-)-pluviatolide is converted to (-)-bursehernin via *O*-methylation at C4'. It is followed by C5'-hydroxylation to yield (-)-5'-desmethyl-yatein and methylation to yield (-)-yatein.[80] The stereo-and regioselective C(sp^2)-C(sp^3) bond formation between the benzylic C7' and aryl C6 yields (-)-deoxypodophyllotoxin and completes the tetracyclic core of the aryltetralin class.

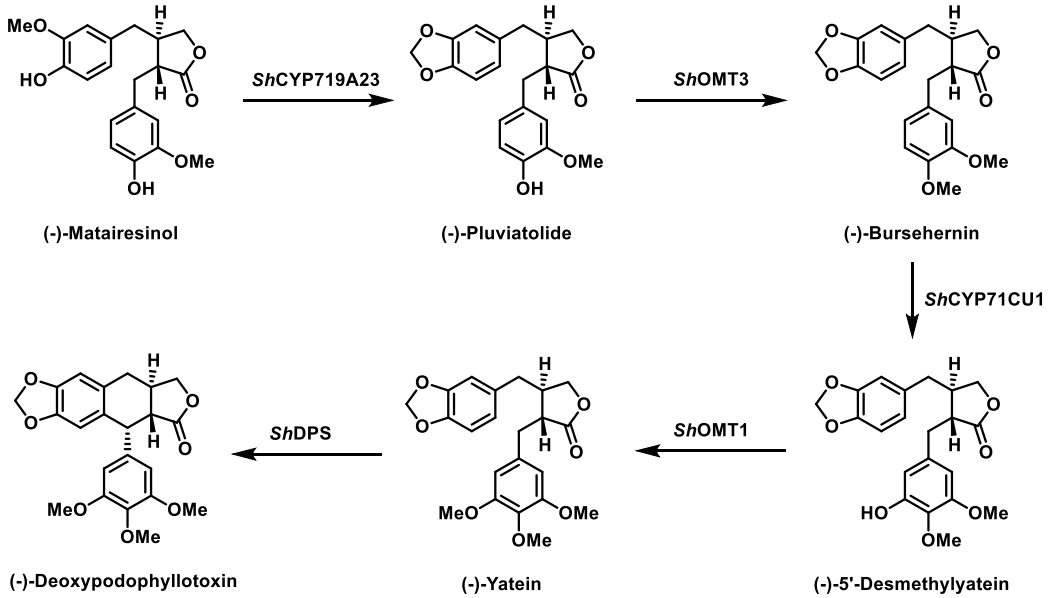

Figure 8. Biosynthesis of (-)-deoxypodophyllotoxin.

Iron and 2-oxoglutarate (Fe/2OG) dependent *Sh*2-ODD/*Sh*DPS from *Sinopodophyllum hexandrum* facilitates stereo- and regioselective C(sp^2)-C(sp^3) bond formation.[32]. The mechanism of this transformation was elucidated in 2022 by Tang et al. using substrate-bound protein structures, biochemical assays with substrate analogs and chemical model studies.[81] Briefly, the reaction is proposed to proceed via elimination of a hydrogen atom to generate a substrate radical. Following a carbocation at C7', C-C bond formation is likely induced by rotation of the benzodioxole moiety, positioning its C6 in close proximity to the C7'-centered carbocation (Figure 9). Furthermore, DPS was shown to convert both (+)- and (-)-yatein. Interestingly, the stereochemical conformation of the substrate does not impact the stereo-specificity of C-C bond formation.[81]

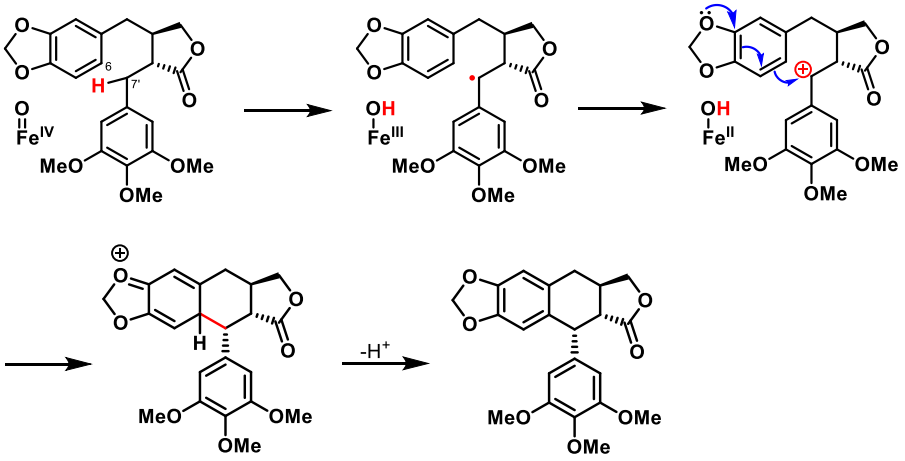

Figure 9. Proposed mechanism of an Fe/2OG enzyme catalyzed C-C bond formation.

8. Scaffold Tailoring in Podophyllotoxin and Derivatives

Following completion of the aryltetralin scaffold, the oxidative scaffold-diversification strategy resumes (Figure 10). The enzymes responsible for the conversion of (-)-deoxypodophyllotoxin to (-)-4'-desmethyl-epipodophyllotoxin, the aglycone of etoposide, were also identified in 2015.[32] CYP71BE54 was found to facilitate *O*-demethylation of (-)-deoxypodophyllotoxin at C4', yielding (-)-4'-desmethyl-deoxypodophyllotoxin. The production of the epimer of podophyllotoxin, epipodophyllotoxin, was found to be mediated by a cytochrome P450. *Sh*CYP82D61 in which it hydroxylates both deoxypodophyllotoxin and (-)-4'-desmethyl-deoxypodophyllotoxin, producing (-)-epipodophyllotoxin and (-)-4'-desmethylepipodophyllotoxin respectively. Interestingly, deoxypodophyllotoxin could also be converted to epipodophyllotoxin by human CYP3A4.[82] However, enzymes responsible for (-)-podophyllotoxin formation was not observed.

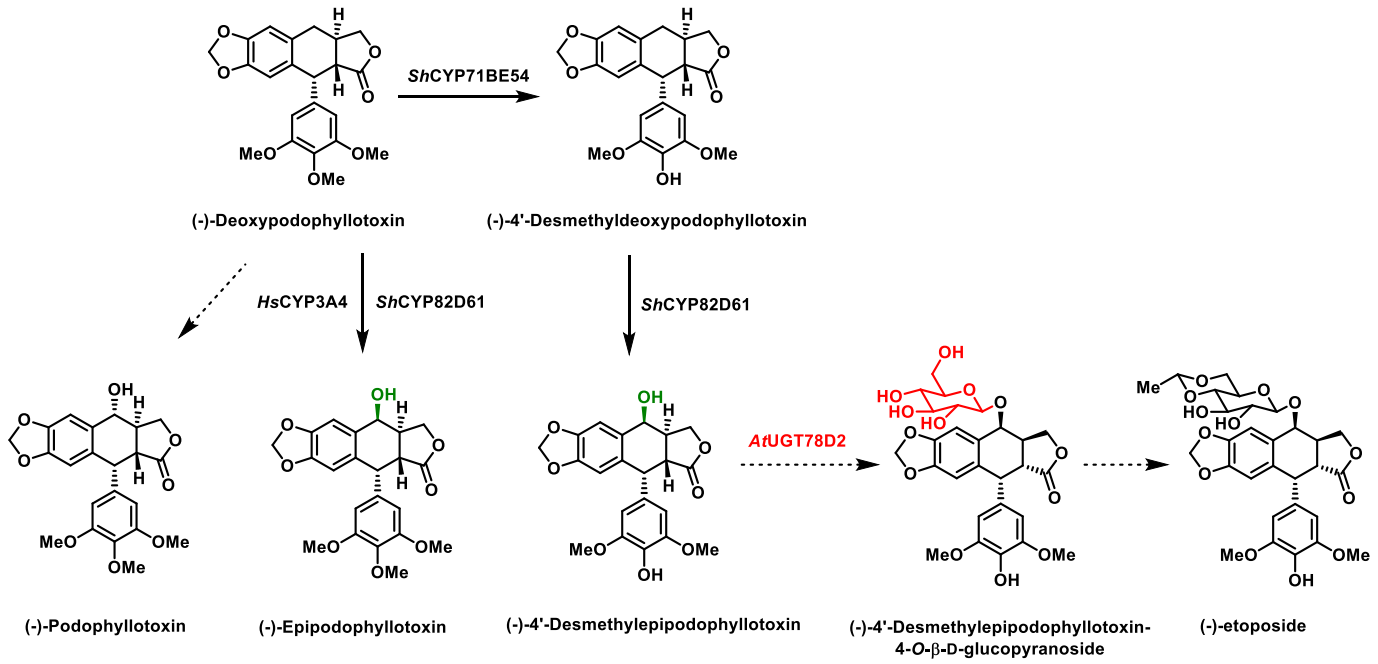

Figure 10. Tailoring enzymes in the formation of podophyllotoxin and derivatives.

9. Scaffold Tailoring in Sesamin Biosynthesis

A majority of furofuran lignans are derived from pinoresinol, as well as pinoresinol analogs stemming from related monolignol dimerization.[22,39,83] Among furofuran lignans, sesamin is one of the most well-known and commercially relevant. It is mainly produced by *Sesamum sp.,* e.g., *Sesamum indicum*.[23,84] Sesamin is produced from pinoresinol through formation of two methylenedioxy bridges (Figure 11). The pathway from pinoresinol to sesamin was first proposed in 1998 by Lewis et al.[84] In the study, a cytochrome P450 monooxygenase was shown to convert pinoresinol to piperitol. Further conversion to sesamin was not observed, hence the enzyme was termed piperitol synthase (PS) and the presence of a second enzyme, sesamin synthase (SS) was postulated.[84] Ono et al. later reported the identification and characterization of three *Si*P450 proteins, encoded by genes CYP81Q1 from *Sesamum indicum,* CYP81Q2 from *Sesamum radiatum* and CYP81Q3 from *Sesamum alatum.* Both recombinant proteins of CYP81Q1 and CYP81Q2 were shown to possess dual (+)-piperitol/(+)-sesamin forming activity, thus implicating them as (+)-piperitol/(+)-sesamin synthases (PSS).[85] Likely, (+)-piperitol is released from the enzyme active site after the first catalysis and is subsequently recaptured for the second catalysis (Figure 11). However, the mechanism of dual catalysis remains to be fully elucidated. On the other hand, CYP81Q3 showed no activity, coinciding with the absence of (+)-sesamin in *S. alatum.*

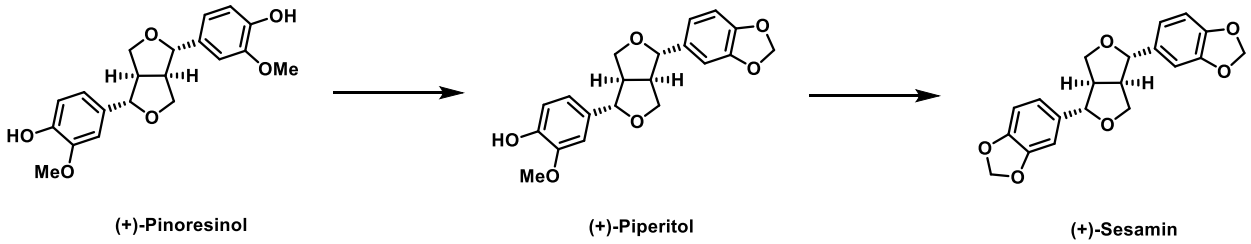

Figure 11. Formation of sesamin.

10. Glycosylation

Glycosylation represents one of the most common group transfer reactions in natural product biosynthesis to diversify natural product structures and introduction of a sugar moiety improves solubility and reactivity of the corresponding aglycone.[29,86,87] In plants, glycosylation is typically facilitated by uridine diphosphate (UDP)-dependent glycosyltransferases (UGTs). In lignan biosynthesis, glycosylation is also commonly used. *At*UGT71C1 from *Arabidopsis thaliana* was shown to perform monoglycosylation on both (+)-lariciresinol and (+)-pinoresinol with comparable efficiency. On the other hand, *Ii*UGT71B5 from *Isatis indigotica* facilitates mono- and di-glycosylation on pinoresinol only (Figure 12). Additionally, *Lu*UGT74S1 from *Linum usitatissimum* could utilize (-)-secoisolariciresinol as a substrate to catalyze mono- and di-glycosylation.[88]

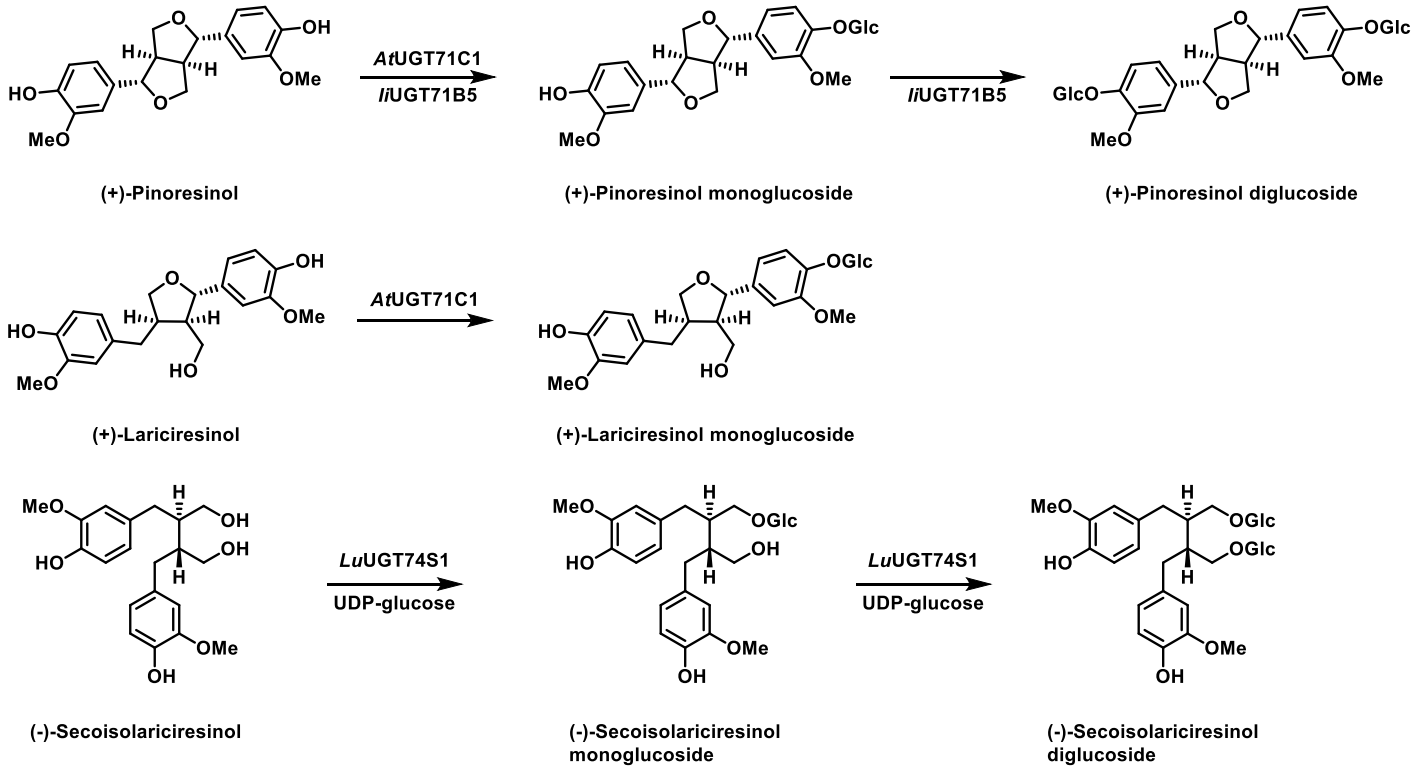

Figure 12. Examples of glycosylation in lignans.

Another prominent example of a lignan-glucoside is found in etoposide. The disaccharide moiety in etoposide is important to its topoisomerase II inhibition activity.[89,90] Glycosylation of the etoposide aglycone, (-)-4'-desmethylepipodophyllotoxin, has been achieved by leveraging the substrate promiscuity of non-native UGTs (Figure 10).[91]

11. Outlook for other Lignan Scaffolds

11.1. Arylnaphthalene Lignans

Arylnaphthalene lignans are produced by plant species of various genera, including *Haplophyllum, Justicia, Larrea* and *Phyllantus*. Much like other lignan-subtypes, arylnaphthalene lignans have been shown to exhibit a wide range of bioactivities.[92–94] Justicidins and diphyllin are prominent examples of the subtype and have been

investigated as putative remedies for topical inflammatory diseases such as psoriasis. Structurally, the arylnaphthalene scaffold is defined by a biaryl bond connecting naphthalene and phenyl moieties. Consequently, arylnaphthalene lignans lack the chiral centers characteristic of other lignan subclasses. Instead, the rotatory hindrance of the sp^2-sp^2 biaryl bond induces axial chirality and consequently the ability of forming atropisomers. Atropisomerism is rarely observed in nature, thus they have garnered considerable research interest in recent years.[95,96] To date, the biosynthetic formation of the arylnaphthalene-type lignan scaffold remains a mystery. From a biosynthetic viewpoint, it appears likely that arylnaphthalene lignans are derived from sequential dehydration of corresponding aryltetralin precursor.

In 2007, Hemmati et al. reported the presence of a PLR (PLR-Lp1) in arylnaphtalene-lignan producing *Linum perenne H*.[97] Leveraging an RNAi approach indicated PLR-Lp1 to be involved in arylnapthalene lignan biosynthesis. Likely, arylnapthalene lignan biogenesis follows the "general" lignan pathway to produce dibenzylbutyrolactone-type matairesinol as the key precursor.[92,95] Plausible intermediates toward justicidins and diphyllin have been isolated from *L. perenne*, including 7(8),7'(8')-tetrahydrojusticidin and dihydrojusticidin. This implies a biosynthetic strategy analogous to aryltetralin-lignan biosynthesis, supporting the hypothesis of tandem dehydration to yield the arylnaphthalene scaffold (Figure 13).[95,97]

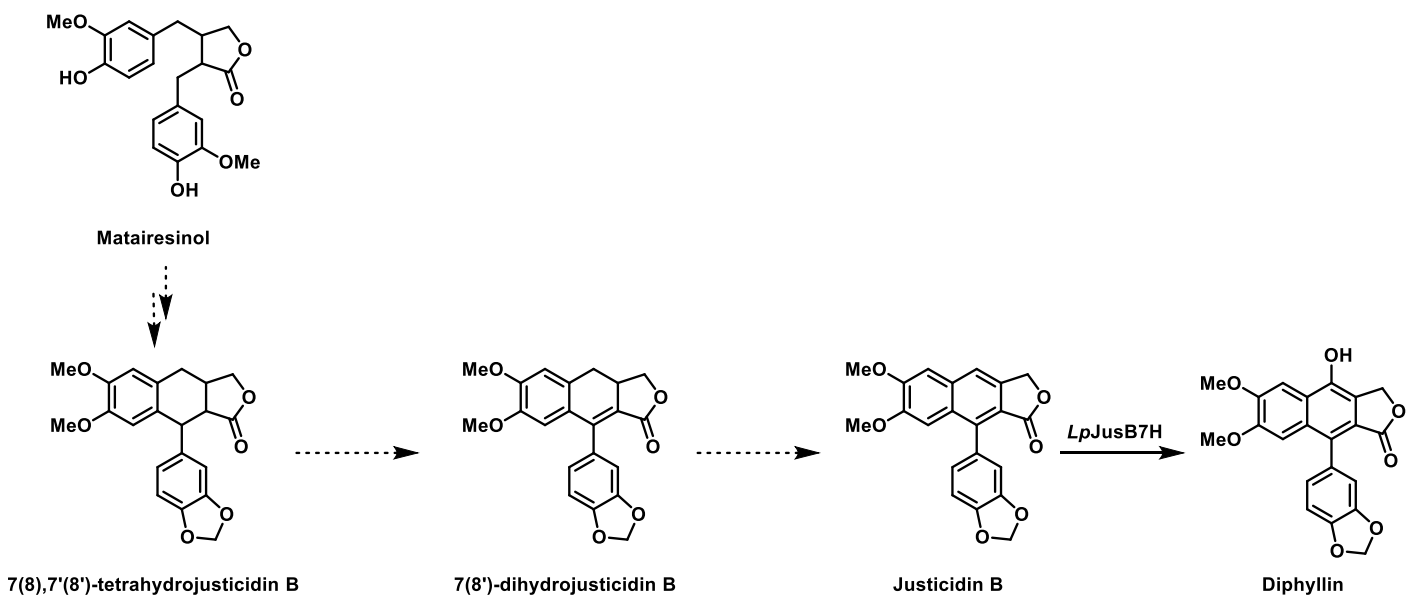

Figure 13. Possible biosynthetic pathway of diphyllin.

11.2. Dibenzylcyclooctadiene Lignans

A large number of dibenzylcyclooctadiene-type lignans have been isolated and characterized. The structural features and promising bioactivities of representative compounds have elicited significant research interest.[17,20,98,99] However, like arylnaphthalene lignans, the genes associated with the biosynthetic transformations leading to their formation remain unknown. However, several possible pathway intermediates have been proposed to provide some insight into early steps of Dibenzylcyclooctadiene Lignans (DBO) biosynthesis.[39,100]

Interestingly, all known and characterized DBO-lignans have been isolated from the *Schisandraceae* family.[18,19,68,101] The lack of genomic sequencing data of *Schizandera sp.* has made the identification of enzymes involved in their biosynthesis elusive. Recent advances in verrucosin biosynthesis in *Schizandera sp.*, have implicated it to be an on-pathway intermediate toward DBO-type lignans. In contrast to using coniferyl alcohol

as the precursor, verrucosin is likely resulting from dimerization of (*E*)-isoeugenol. Isotope labeling studies by Lopes et al. observed verrucosin formation in *Virola surinamensis* using labeled (*E)*-isoeugenol.[66] Furthermore, Dexter et al. identified an acyltransferase (*Ph*CFAT) in petunias responsible for the acetylation of coniferyl alcohol to yield coniferyl acetate.[67] A novel NADPH-dependent isoeugenol synthase (*Ph*IGS1) was shown to convert only coniferyl acetate, but not coniferyl alcohol, to (*E)*-isoeugenol. More recently, Dong et al. identified DIR and *Sc*CFAT genes in *S. chinensis*.[100,102] Taken together, a possible pathway for verrucosin formation was proposed (Figure 14). (*E)*-Isoeugenol is speculated to undergo dimerization to verrucosin facilitated by an auxiliary oxidase and *Sh*DIR, which in turn could be converted to dihydroguiaretic acid via a PLR-like reductase. Subsequently, cytochrome P450s and *O*-methyltransferases may then form pregomisin.[103]

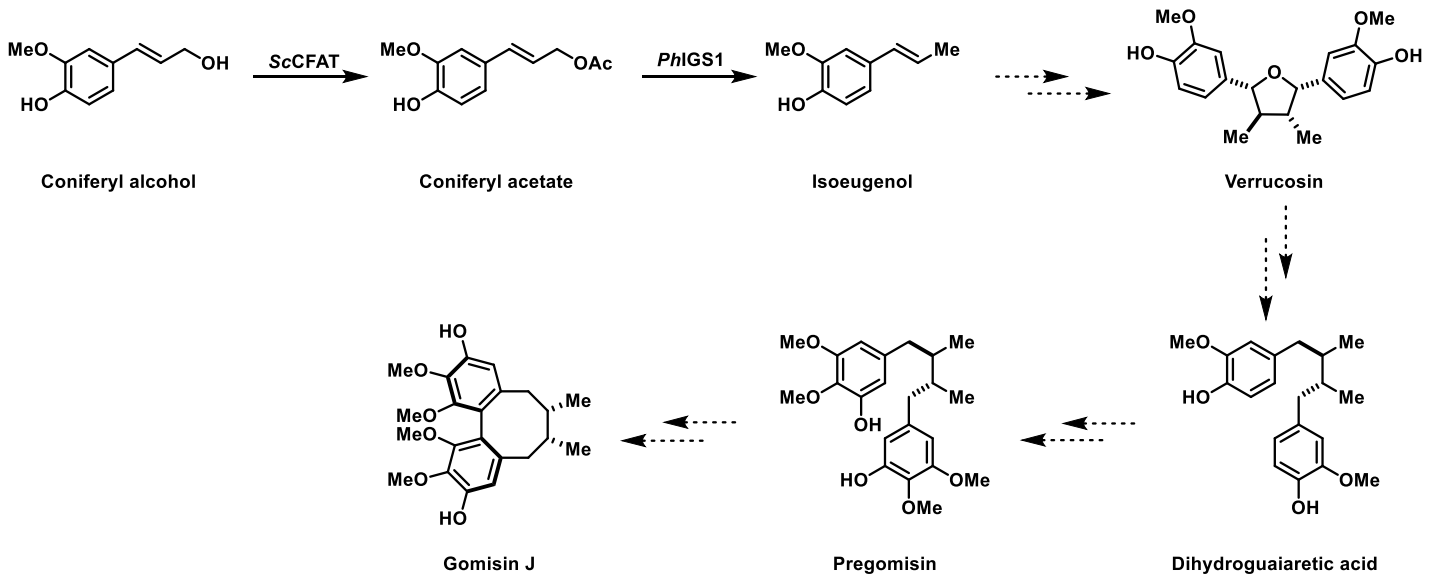

Figure 14. Possible biosynthetic pathway for dibenzylcyclooctadiene lignans.

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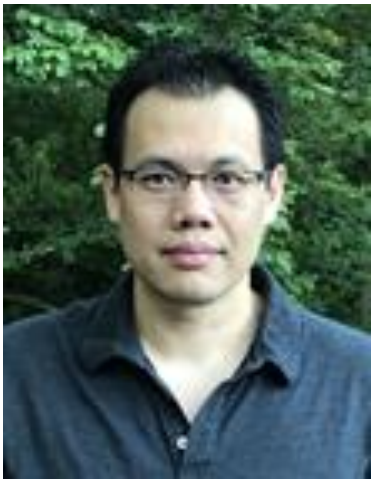
Authors' Biographies

Nicholas König Canty was born in 1997 in New York City. He received his B.S. degree in Chemistry and Biochemistry from Ludwig Maximilian University of Munich in 2020. In 2021, he joined the research group of Prof. Wei-chen Chang at North Carolina State University, where his current research interests focus on uncovering enzymatic mechanisms and studying their use for the synthesis of natural products.



Wei-chen Chang obtained his BS under Professor Tien-Yau Luh at National Taiwan University. He received his Ph.D. under Professor Hung-wen Liu at UT Austin in 2011. During 2011-2015, he worked with Professors J. Martin Bollinger and Carsten Krebs as postdoc fellow. In 2016, Dr. Chang started his independent career at North Carolina State University. His research focuses on mechanistic investigation of novel biological transformations found in natural product biosynthetic pathways.

