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•Review•

A review: biosynthesis of plant-derived labdane-related diterpenoids

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[ABSTRACT] Plant-derived labdane-related diterpenoids (LRDs) represent a large group of terpenoids. LRDs possess either a labdane-type bicyclic core structure or more complex ring systems derived from labdane-type skeletons, such as abietane, pimarane, kaurane, etc. Due to their various pharmaceutical activities and unique properties, many of LRDs have been widely used in pharmaceutical, food and perfume industries. Biosynthesis of various LRDs has been extensively studied, leading to characterization of a large number of new biosynthetic enzymes. The biosynthetic pathways of important LRDs and the relevant enzymes (especially diterpene synthases and cytochrome P450 enzymes) were summarized in this review.

[KEY WORDS] Biosynthesis; Cytochrome P450 enzyme; Diterpene synthase; Labdane-related diterpenoids
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Introduction

Diterpenoids harbor C20-carbon skeletons comprising four isoprene units. Labdane-related diterpenoids (LRDs) are a large group of diterpenoids, with over 10% contents of all terpenoids. This group of compounds possess either a labdane-type bicyclic core structure or more complex ring systems derived from labdane-type skeletons, such as abietane, pimarane, kaurane, beyerane, atisane, cassane, stemodane and manoyl oxide (Fig. 1) [1]. Labdane is named after labdanum, an oleoresin of rockrose plant from which labdane-type diterpenoids were isolated for the first time [2]. LRDs have been widely used in perfume and food industries for centuries. And many of them possess various bioactivities, such as anti-microbial, anti-viral, anti-inflammatory and antitumor activities [3-10], and therefore play critical roles in effectiveness of the corresponding medicinal herbs or even used as commercial drugs. For instances, andrographolide from *Andrographis paniculata* is used as an inflammatory agent [11]; triptolide from *Tripterygium wilfordii* is an antitumor agent [12]; tanshinones are the main anti-inflammatory and antibacterial constituents of the traditional Chinese medicinal herb Danshen (*Salvia miltiorrhiza*) [13].

Reviews concerning the biosynthesis of multiple types of diterpenoids have been published [1, 14-16]. As mentioned above, LRDs comprise the largest group of diterpenoids and possess potent bioactivities, and their biosynthesis has been extensively studied, leading to characterization of a large number of new biosynthetic enzymes. Therefore, LRD biosynthesis is easily the sole topic of a review. Indeed, LRD biosynthesis was specifically reviewed by Reuben J. Peters in 2010 [17], which mainly focused on diterpene synthases and their mechanisms. After that, great advances on LRD biosynthesis have been achieved, especially structure elucidation of class I, class II and class I/II diterpene synthases (diTPSs) and characterization of a large number of cytochrome P450 enzymes (CYPs) which play critical roles in oxidative modification of terpene olefins. The present review updates the advances on biosynthesis of LRDs and comprehensively summarizes the enzymes related to LRDs biosynthesis in plants, mainly including diTPSs, CYPs, glycosyltransferases and acetyltransferases.

General Biosynthetic Routes of LRDs

LRDs originate from two common precursors, isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) which are synthesized through two pathways, i.e.

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Dedicated to the 90th Anniversary of the Founding of Shenyang Pharmaceutical University

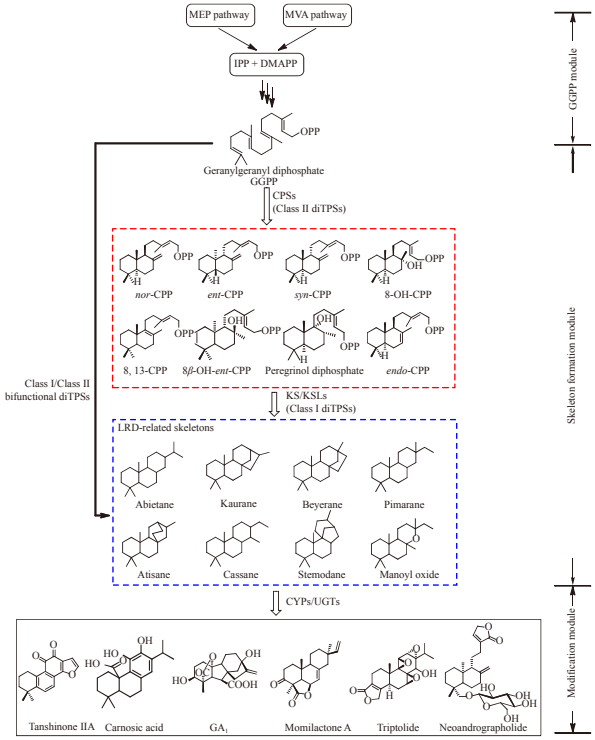

Fig. 1 General biosynthetic pathway of LRDs. CPS: copalyl pyrophosphate synthase; KS: kaurene synthase; KSL: kaurene synthase-like enzyme; CYP: cytochrome P450 enzyme; UGTs: uridine pyrophosphate (UDP)-dependent glucosyltransferases

the mevalonate pathway (MVA pathway) in the cytosol [18] and the 2-methyl-D-erythritol-4-phosphate (MEP) pathway in the plastids [19].

The biosynthesis of LRDs is usually divided into three modules. First, three IPP molecules are successively tethered to one DMAPP molecule to yield the common diterpenoid precursor *E*, *E*, *E*-geranylgeranyl pyrophosphate (GGPP) under the catalysis of geranyl pyrophosphate synthase, franesyl pyrophosphate synthase and GGPP synthase or via three sequential IPP condensation steps to DMAPP under the catalysis of sole GGPP synthase [20], which is called the GGPP module (Fig. 1). Second, the GGPP is cyclized to form the corresponding cyclic skeletons of LRDs under the catalysis of diTPSs, which is called the skeleton formation module (Fig. 1). Finally, the LRD-related skeletons are further modified by oxidation, methylation, acylation, and glycosylation, etc., to produce LRDs with great diversity in structure and bioactivity [21]; that is the modification module (Fig. 1).

DiTPSs Invovled in LRD Biosynthesis

The structural diversity of LRDs can be primarily attributed to their various skeletons which are formed under the catalysis of diTPSs involved in LRD biosynthesis. In the biosynthetic pathways, various copalyl pyrophosphate (CPP) synthases (CPSs) belonging to class II diTPSs, catalyze the primary cyclization of GGPP to afford various bicyclic CPPs or their alcoholic derivatives, such as *nor*-CPP [22-35], *ent*-CPP [27-29, 34, 36-43], *syn*-CPP [34, 36, 44, 45], 8-OH-CPP [46-48], 8, 13-CPP [27], 8*β*-OH-*ent*-CPP [49], peregrinol diphosphate [50], and *endo*-CPP [51] (Fig. 1 and Table S1). Then, these pyrophosphate intermediates may be further converted into more complex ring systems including kaurane-, abietane-, pimarane-, beyerane-, atisane-, canssane-, stemodane- and manoyl oxide-type skeletons by class I diTPSs, such as kaurene synthase (KS) and kaurene synthase-like enzymes (KSLs) (Figs. 1 and 2) [52]. In addtion, these two-step cyclizationreactionscanbeachievedbysomebifunctionalclassI/IIdiTPSs (Fig. 1), such as AgAS (abietadiene synthase from *Abies grandis*). The structural features and catalytic mechanisms of distinct diTPSs are listed in detail as below.

CPSs

The structure of CPS was initially reported in 2011 [53] (Fig. 3), which facilitated a deeper understanding of the catalytic mechanism of this group of enzymes. CPSs belong to class II diTPSs, harboring three domains (*α*, *β* and *γ*) and a conserved catalytic motif DXDD located in *β* domain which can protonate the double bond between C-14 and C-15 of GGPP to generate geranylgeranyl cation, and thereby initiate cyclization. The carbocations are eventually quenched by either elimination of protons or nucleophile attack of water molecule to yield CPPs or their alcoholic derivatives with distinct stereochemistry. Among these bicyclic intermediates, *nor*-CPP, *ent*-CPP and *syn*-CPP are predominant ones and formed under the catalysis of *nor*-CPS, *ent*-CPS and *syn*-CPS, respectively (Fig. 2 and Table S1). Other CPSs are reported to be capable of cyclizing GGPP into unusual CPPs or alcoholic derivatives of CPPs. For instances, NtCPS2 from *Nicotiana tabacum* [46], CcCLS from *Cistus creticus* [47] and GrTPS1 from *Grindelia robusta* [48] converted GGPP into 8-hydroxy-*nor*-CPP (also called LPP) as their sole product. ZmCPS4 from *Zea Mays* produceed labda-8, 13-dien-15-yl pyrophosphate (8, 13-CPP) as its major product and LPP as its minor product [27]. SmCPS4 from *S. miltiorrhiza* produced 8*β*-hydroxy-*ent*-CPP (*ent*-LDPP) [49]. VacTPS1 from *Vitex agnus-castus* L. produced peregrinol pyrophosphate [50]. In addition, a bifunctional diTPS SmCPSKSL1 was characterized from *Selaginella moellendorffii*, with a rare function of producing labdan-7-13*E*-dienyl pyrophosphate (*endo*-CPP) [51] (Fig. 2).

KS and KSLs

CPPs and their derivatives can be further cyclized under the catalysis of KS or KSLs (Fig. 2). KS and KSLs belong to class I diTPSs. The structure of a class I diTPS taxadiene synthase involved in taxol biosynthesis has been reported, harboring three domains (*α*, *β* and *γ*) (Fig. 4) [54]. Although KS and most KSLs possess three domains like taxadiene synthase, an *α*/*β* bi-domain diTPS SmKSL for synthesis of miltiradiene was identified from *S. miltiorrhiza* [26]. Identification of SmKSL indicated that domain-loss events might independently occur multiple times during the evolution of plant TPSs [55]. Like all the class I TPSs, KS and KSLs pos-

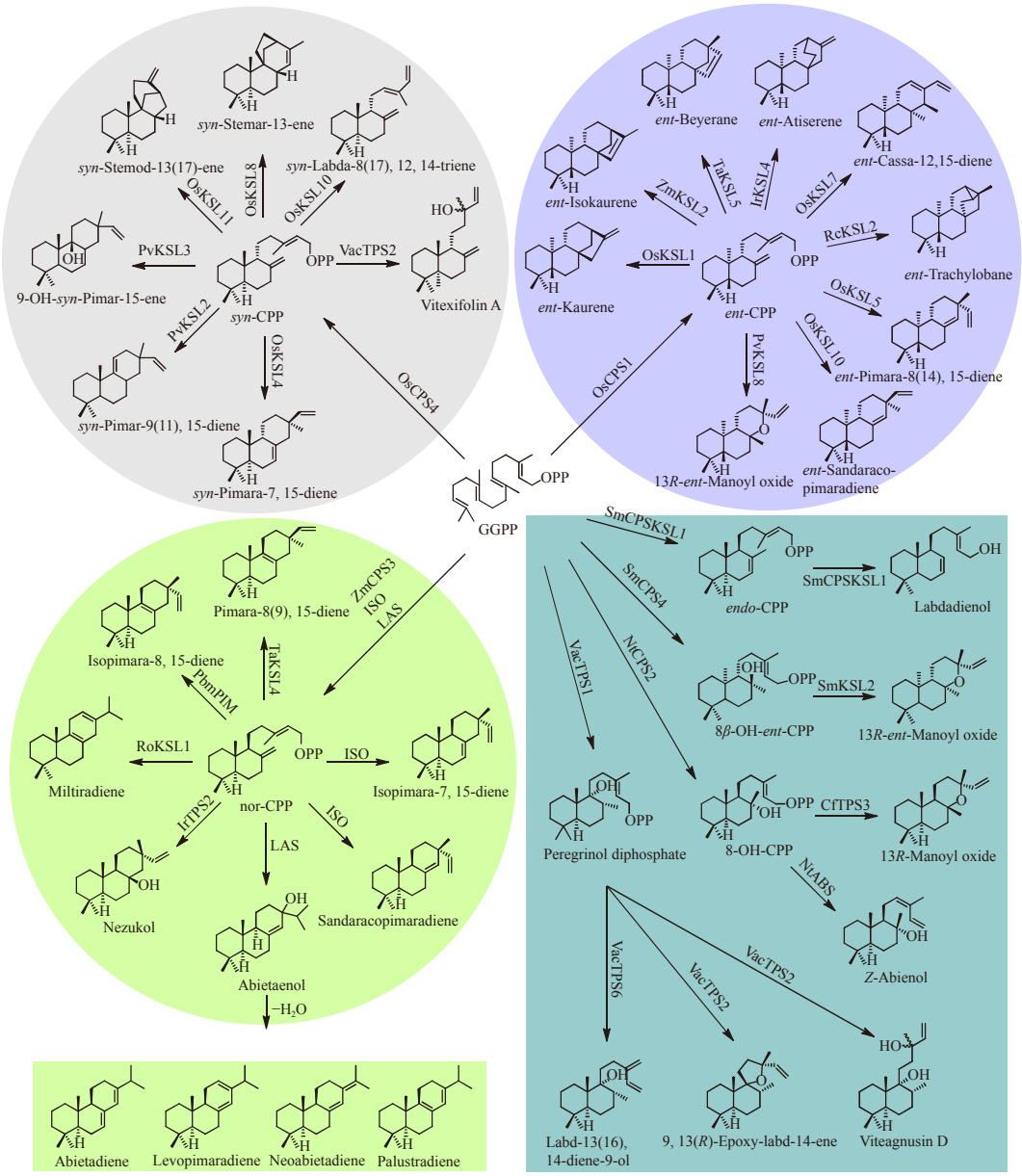

Fig. 2 Formation of LRD-related skeletons under the catalysis of diTPSs

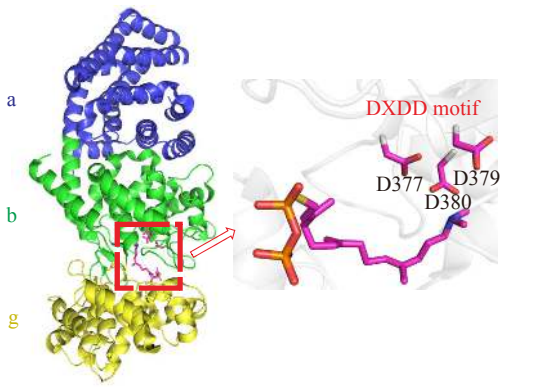

Fig. 3 AtCPS1 structure (*α*, *β* and *γ* domains are colored in blue, green and yellow, respectively)

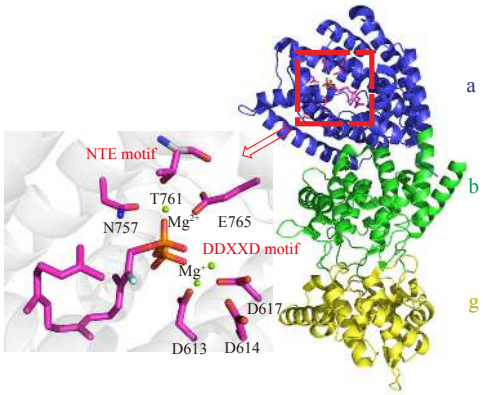

Fig. 4 Taxadiene synthase structure (*α*, *β* and *γ* domains are colored in blue, green and yellow, respectively)

sess two conserved motifs **DD**XX**D** and (**N**, **D**)XX(**S**, **T**)XXX (**E**, **D**) in their *α* domains. Residues in bold coordinate with three Mg^{2+} ions which ionize CPPs by eliminating pyrophosphate anion to yield carbocation intermediates and thereby trigger further cyclization. KS and KSLs, together with their substrates and products, are summarized in Fig. 2.

In the catalytic process, KS or KSLs usually interact with their CPS partners. Accordingly, KSL/CPS fusion might significantly increase catalytic efficiency [56]. For instance, in *S. miltiorrhiza* SmCPS (a *nor*-CPS) and SmKSL were responsible for conversion of GGPP into miltiradiene, a diterpene olefin intermediate in the biosynthesis of tanshinones, and the fusion of SmKSL and SmCPS caused a 2.9-fold increase in miltiradiene production in the engineering yeast [56].

Class I/class II bifunctional diTPSs

From lycophytes and gymnosperms, some bifunctional diTPSs were identified [22]. All of these enzymes are also tridomain TPSs [57] (Fig. 5), including AgAS (abietadiene synthase from *A. grandis*), Iso (isopimaradiene synthase from *Picea abies*) [58], GbLSP (levopimaradiene synthase from *Ginkgo biloba*) [23], SmMDS [59] and SmCPSKSL1 [51] (miltiradiene synthase and labda-7, 13*E*-dien-15-ol synthase from *Selaginella. moellendorffii*) and AbCAS (*cis*-abienol synthase from *A. balsamea*) [60]. These diTPSs possess both the class I catalytic motifs DDXXD and (N, D)XX(S, T)XXX (E, D) and the class II motif DXDD. Their class I motifs are located in the *C*-terminal *α* domain, while their class II motifs in the *N*-terminal *β* and *γ* domains. Therefore, both class I (ionization-initiated) and class II (protonation-initiated) cyclizations of GGPP are performed. For instance, AgAS protonatesd the double bond between C-14 and C-15 of GGPP to yield (+)-CPP and then removed the pyrophosphate anion to trigger formation of the third ring to afford abiet-8(14)-ene carbocation which was subsequently attacked by a molecule of water to yield 13-hydroxyl-(8)14-abietene. 13-Hydroxyl-(8)14-abietene was not stable and subject to spontaneous water elimination to generate four stable compounds, namly (−)-abietadiene, (−)-levopimaradiene, (−)-neoabietadiene and (−)-palustradiene [61] (Fig. 2).

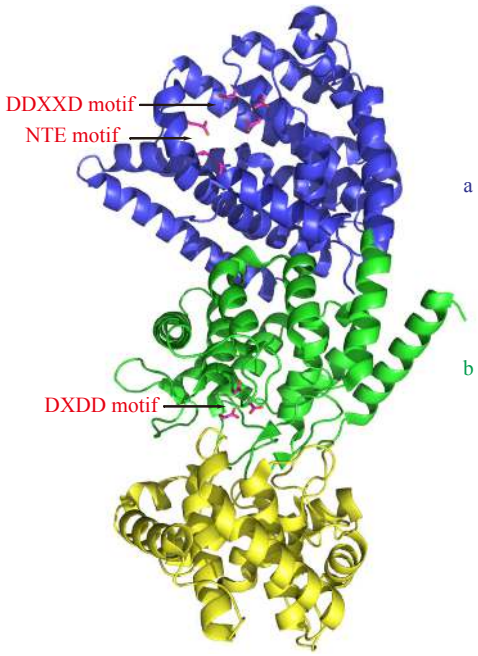

Fig. 5 AgAS structure (*α*, *β* and *γ* domains are colored in blue, green and yellow, respectively)

CYPs Involved in LRD Modifications

CYPs involved in the biosynthesis of kaurene-derived diterpenoids

Gibberellins, with a 6/5/6/5 ring system derived from *ent*-kaurene (Fig. S1), are a group of phytohormones which are essential for plant growth and development [62]. Moreover, many kaurane-type diterpenoids possess pharmaceutical bioactivities. Oridonin from *Rabdosia rubescens* exhibited a broad range of biological effects such as anticancer and anti-inflammatory activities [63, 64]. 11*β*-Hydroxy-*ent*-16-kaurene-15-one from *Jungermannia tetragona* showed potent inhibitory activities against several cancer cell lines [65]. Although a large number of kaurane-type diterpenoids have been isolated from plants, the number of CYPs related to their biosynthesis is limited except for the ones involved in gibberellin biosynthesis.

The CYPs responsible for oxidation of C-3, C-13 and C-19 of kaurene have been characterized (Table S2). In *Stevia rebaudiana*, C-19 of *ent*-kaurene was oxidized by *ent*-kaurene oxiase (KO) [66-69] to kaurenoic acid which was hydroxylated at its C-13 by SrKAH to afford steviol, the precursor of the natural sweeteners steviol glycosides, such as stevioside and rebaudioside A (Reb A) [70] (Fig. S2). In rice, OsKOL4, also named CYP701A8, is responsible for hydroxylation of C-3 of *ent*-kaurene to yield 3*α*-hydroxy-*ent*-kaurene [71].

In the biosynthetic routes of gibberellins (GAs) [62, 72-76] (Fig. S1), kaurenoic acid oxidase (KAO) catalyzed the oxidation of C-7 of kaurenoic acid and formation of the GA skeleton (GA_{12}) [62, 77]. C-13 of GA_{12} was hydroxylated by gibberellin 13-hydroxylase (13ox) to afford GA_{53} [78]. Gibberellin 20-hydroxylase (20ox) [73, 79-81] catalyzed the oxidation of C-20 of GA_{12} and GA_{53} to yield GA_{15}, GA_{24}, GA_9, GA_{25}, GA_{44}, GA_{19}, GA_{17} and GA_{20} (Fig. S1). Then, conversions from GA_9 to GA_4, 2,3-didehydro GA_9 and GA_7 and from GA_{20} to GA_1, GA_5 and GA_3 were achieved under the catalysis of a sole CYP gibberellin 3-hydroxylase (3ox) (Fig. S1) [74, 82-84]. Among these GAs, GA_1, GA_3, GA_4 and GA_7 are potent phytohormone molecules, which were subsequently inactivated to generate GA_8, GA_{34} and their catabolites through further oxidation catalyzed by gibberellin 2-hydroxylase (2ox) (Fig. S1) [85-92]. All the CYPs were identified from *Arabidopsis thaliana*, while some of their isoenzymes were found in other plants, e.g. two 13ox CYP714B1 and CYP714B2 from rice [93], and two 2ox from *Haseolus coccineus* L. [76] and *Z. mays* [94], respectively.

In addition, some CYPs were found to be capable of oxidizing *ent*-isokaurene where a double bond between C-15 and C-16 was replaced by a double bond between C-16 and C-17 in *ent*-kaurene. A CYP MtKO from the medicinal herb *Montanoa tomentosa* oxidized *ent*-isokaurene to isokaurenoic acid [95]. CYP71Z6 from rice was able to hydroxylate C-2 and C-3 of *ent*-isokaurene, which is considered to be a crucial step in the biosynthesis of oryzadione, a phytoalexin [96]. The CYPs involved in the biosynthesis of kaurene-derived diterpenoids are summarized in Table S2.

CYPs involved in the biosynthesis of abietane-type diterpenoids

Plants belonging to the family Lamiaceae are rich in phenolic diterpenoids. For instance, the medicinal herb rosemary (*Rosmarinus officinalis*), *S. pomifera* and *S. fruticose* contain a large amount of carnosol and carnosic acid, and *S. miltiorrhiza* is rich in tanshinones. These compounds possess various bioactivities, such as antioxidant, anti-inflammatory and antibacterial activities, because they are widely used in pharmaceutical, food and cosmetics industries. Biosynthesis of carnosic acid and tanshinones has been intensively studied [97, 98] (Fig. S3). They share the same the early steps from GGPP to 11, 20-dihydroxy ferruginol. The olefin precursor miltiradiene was converted into abietatriene through spontaneous oxidation [99]. A handful of CYPs including CYP76AH1 [100] and CYP76AH3 [98, 101] from *S. miltiorrhiza*, CYP76AH4 [99, 102], CYP76AH22 and CYP76AH23 from rosemary and CYP76AH24 from *S. pomifera* and *S. Fruticose* [103], were able to successively hydroxylate C-12 and C-11 of abietatriene to yield 11-hydroxy ferruginol, which was subsequently oxidized into 11, 20-dihydroxy ferruginol by CYP76AK subfamily enzymes, including CYP76AK1 from *S. miltiorrhiza*, CYP76AK7 and CYP76AK8 from rosemary and CYP76AK6 from *S. pomifera* and *S. fruticose* [103, 104]. Furthermore, CYP76AK6, CYP76AK7 and CYP76AK8 oxidized C-20 to yield carnosic acid. 11, 20-Dihydroxy ferruginol was spontaneously oxidized into 10-hydroxymethyl tetrahydromiltirone. Recently, CYP71D373 and CYP71D375 were identified from *S. miltiorrhiza*, with a function of catalyzing formation of D ring of tanshinones to yield cryptotanshinone, methylenedihydrotanshinquinone and 15, 16-dihydrotanshinone respectively, where miltirone, 4-methylenemiltirone and Ro acted as the substrates [105]. In addition, CYP71BE52 from *S. pomifera* was able to oxidize C-2 of ferruginol to produce salviol [104] (Fig. S3). Moreover, many of these CYPs are substrate-promiscuity enzymes, which often results in significant diversity of phenolic diterpenoids in planta. For instance, CYP76AK6, CYP76AK7 and CYP76AK8 can also successively oxidize C-20 of ferruginol to produce pisiferol, pisiferal and pisiferic acid (Fig. S3).

T. wilfordii is a traditional Chinese medicinal herb used for treatment of rheumatoid arthritis. Triptolide is the main pharmaceutical constituent of *T. wilfordii*, belonging to abietane-type diterpenoid. CYP728B70 identified from *T. wilfordii* has been proved to catalyze carboxylation at C-19 of abietatriene to produce dehydroabietic acid which is supposed to be the precursor of triptolide [106] (Fig. S3).

The abietane-type diterpenoid acids are the major components of conifers oleoresins which play crucial roles in plant defenses against pests and pathogens. As mentioned above, in *A. grandis* and *P. abies* (Norway spruce), abietadiene synthases convert GGPP into an unstable product 13-hydroxy-8(14)-abietene which spontaneously transforms to abietadiene, levopimaradiene, neoabietadiene and palustradiene. These four olefins were oxidized into the corresponding acid products abietic acid, levopimaric acid, neoabietic acid and palustric acid by CYP720B subfamily enzymes, such as CYP720B1 from *Pinus taeda* (loblolly pine) [107] and CYP720B4 from *Picea. sitchensis* (Sitka spruce) [108]. In addition, CYP720B2 and CYP720B12 cloned from *P. banksiana* (jack pine), *P. contorta* (lodgepole pine) and Sitka sprucecan directly oxidized C-18 of 13-hydroxy-8(14)-abietene into carboxyl group, and abietic acid, levopimaric acid, neoabietic acid and palustric acid were subsequently obtained through elimination of a molecule of water [109] (Fig. S4). The CYPs involved in abietane-type diterpenoids are summarized in Table S3.

CYPs involved in the biosynthesis of pimarane-type diterpenoids

Momilactones serve as allelopathic substances in land plants to inhibit the growth of competing plants. The biosynthetic pathway of momilactone A and momilactone B has been completely elucidated in rice [110] (Fig. S5). OsCPS4 and OsKSL4 converted GGPP into s*yn*-pimara-7, 15-diene, the precursor of momilactone A and B [111]. CYP76M8 and CYP99A3 oxidizes C-6 and C-19 of s*yn*-pimara-7, 15-diene into a ketone and a carboxyl groups, respectively, and OsMAS (a short-chain dehydrogenase reductase, SDR) subsequently catalyzed the formation of the five-membered lactone. Then, CYP701A8 oxidizes C-3 into a ketone group to yield momilactone A. Momilactone A can be converted into momilactone B through hydroxylation of C-20 followed by formation of the acetal group at C-3 under the catalysis of CYP76M14. In the conversion of momilactone A to momilactone B, CYP76M14 may function before CYP701A8.

In addition, the CYPs responsible for oxidation of *ent*-sandaracopimaradiene have also been characterized. For instance, CYP701A8 in rice, also named OsKOL4, hydroxylated C-3 of *ent*-sandaracopimaradiene to 3α-hydroxy-*ent*-sandaracopimaradien (Fig. S5) [71]. Then, CYP76M6 and CYP76M8 catalyzed hydroxylation at C-7 and C-9 of 3α-hydroxy-*ent*-sandaracopimaradien to yield oryzalexins D and E, respectively [112, 113] (Fig. S5). The CYPs involved in piamarane-type diterpenoids are summarized in Table S4.

CYPs involved in the biosynthesis of cassane-type diterpenoids

Phytocassanes belonging to cassane-type diterpenoids, serve as phytoalexins in plants, and their biosynthesis can be induced under biotic and abiotic stress. In rice, *ent*-cassa-12, 15-diene is considered as the precursor of phytocassanes A−E. Although the biosynthetic pathway of phytocassanes is not completely elucidated, a handful of CYPs responsible for

oxidation of C-2, C-3 and C-11 of *ent*-cassa-12, 15-diene have been characterized [114] (Fig. S6). CYP701A8 and CYP71Z7 successively catalyzed the hydroxylation of C-3 and oxidation of C-2 into a ketone group to produce 3*α*-hydroxyl-*ent*-cassadiene and 3*α*-hydroxyl-*ent*-cassadiene-2-one [71, 114]. 3*α*-Hydroxyl-*ent*-cassadiene and 3*α*-hydroxyl-*ent*-cassadiene-2-one were converted into 1-deoxyphytocassane C and phytocassane D, respectively, through oxidation of C-11 into a ketone group by CYP76M7 and CYP76M8 [113]. In addition, CYP71Z7 can catalyzes the oxidation of C-2 of phytocassane C, 2-deoxyphytocassane A and 1-deoxyphytocassane C to produce phytocassane B, phytocassane A and phytocassane D, respectively (Fig. S6). The CYPs involved in cassane-type diterpenoids are summarized in Table S5.

CYPs involved in the biosynthesis of manoyl oxide-type diterpenoids

Forskolin isolated from *Coleus forskohlii* (Lamiaceae) is a cyclic AMP booster, which was potentially used to treat glaucoma and heart failure. Forskolin is derived from 13*R*-manoyl-oxide [115]. All the CYPs involved in biosynthesis of forskolin have been characterized, which belong to CYP76 subfamily [116] (Fig. S7). Among them, CYP76AH8, CYP76AH11, CYP76AH15 and CYP76AH17 are responsible for oxidation of C-11 to produce 11-oxo-13*R*-manoyl-oxide, while CYP76AH11 also catalyzes the hydroxylation at C-1, C-6 and C-7 to yield 9-deoxy-7-deacetylforskolin. Then, CYP76AH16 hydroxylates C-9 of 9-deoxy-7-deacetylforskolin to afford 7-deacetylforskolin which is converted into forskolin through acetylation of the hydroxy group of C-7 by CfACT1-8 (an acetyltransferase). In addition, CYP76AH24 from *S. pomifera* was found to be capable of catalyzing the hydroxylation at C-11 of 13*R*-manoyl-oxide to generate 11-*β*-hydroxy-13*R*-manoyl oxide [117]. The CYPs involved in manoyl oxide-type diterpenoids are summarized in Table S6.

Glycosylation and Acylation of LRDs

Glycosylation and acylation are also crucial modification steps in LRD biosynthesis, and sugar moieties and acyl groups often substantially contribute to their unique properties or pharmaceutical activities. Several uridine pyrophosphate (UDP)-dependent glucosyltransferases (UGTs) are characterized in the biosynthetic pathway of the natural no-calorie sweeteners steviol glycosides. As stevioside and rebaudiosides A, D and M are the four most important sweetener substances, we only introduce four UGTs responsible for synthesis of these four compounds [118, 119] (Fig. S2). For example, SrUGT85C2 catalyzed glycosylation of 13-OH of steviol to yield steviolmonoside (Sm). SrUGT91D2 introduced a glucosyl group at position C-2 of the sugar moiety of Sm to generate steviolbioside (Sb). SrUGT74G1 glucosylated the carboxyl group (C-19) of Sb to afford stevioside. SrUGT76G1 added a glucosyl group at position 3-OH of 13-*O*-glucosyl to rebaudioside A (Reb A). Then, SrUGT91D2 and SrUGT76G1 successively catalyzed glucosylation at 2-OH and 3-OH of the glucosyl group at C-19 to respectively produce Reb D and Reb M.

Andrographolide and neoandrographolide obtained from *A. paniculata* belong to *ent*-labdane-type diterpenoid glycosides. Due to their potent anti-inflammatory activities, they have potentials to be developed into the next generation of natural anti-inflammatory drugs. Andrograpanin is the aglycone of neoandrographolide. It has been reported that ApUGT can convert andrograpanin to neoandrographolide through glycosylation of 19-OH [120] (Fig. S8).

In addition, acyl moieties are often found in the structures of LRDs. As mentioned above, in the biosynthetic pathway of forskolin, CfACT1-8 was responsible for acetylation of 7-OH of deacetylforskolin to form forskolin [116] (Fig. S7).

Summary and Perspectives

Plant-derived LRDs represent a large group of terpenoids. Due to their various pharmaceutical activities and unique properties, many of LRDs have been widely used in pharmaceutical, food and perfume industries. The studies on LRD biosynthesis were extensively conducted, leading to characterization of a large number of new biosynthetic enzymes, especially diTPSs and CYPs. However, few LRDs biosynthetic pathways have been completely revealed. Identification of the candidate biosynthetic genes is a big challenge in the studies on plant metabolite biosynthesis. Advances in next-generation sequencing and bioinformatics are helpful to overcome the challenge of identifying candidate biosynthetic genes from plants. An increasing number of well-qualified plant genomes have been obtained, which leads to discovery of biosynthetic gene clusters of LRDs, such as the biosynthetic gene clusters of tanshinones [105] and rice-derived diterpenoid phytoalexins [121]. Transcriptome, together with new bioinformatics techniques (e. g. self-organizing maps [122]), may facilitate the discovery of relevant biosynthetic genes which are not clustered. Characterization of biosynthetic genes is another challenge. Synthetic biology approach is a powerful tool to overcome this challenge. Compared with *in vitro* reaction assay, synthetic biology approach can circumvent large-scale expression of enzymes for establishment of *in vitro* reactions and utilization of expensive or even inaccessible substrates [99, 102]. Genome editing techniques (e. g. CRISPR-Cas9) and RNA interference methods enable investigation of the roles of candidate genes in planta [106]. Further development in relevant technologies will lead to elucidation of more complete biosynthetic pathways of LRDs and characterization of more new enzymes, which will enable the construction of platforms for large-scale production of natural and unnatural LRDs by synthetic biology approaches or combinatorial biosynthesis.

Supporting Information

Supporting information of this paper can be requested by sending E-mails to the corresponding authors.

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