

SPECIAL ISSUE REVIEW

Plant terpene specialized metabolism: complex networks or simple linear pathways?

Emily R. Lanier[†] , Trine Bundgaard Andersen[†]  and Bjørn Hamberger^{*} 

Department of Biochemistry and Molecular Biology, Michigan State University, Molecular Plant Sciences Building, 1066 Bogue Street, East Lansing, Michigan, 48824, USA

Received 14 December 2022; revised 28 February 2023; accepted 6 March 2023; published online 9 March 2023.

^{*}For correspondence (e-mail hamberge@msu.edu).[†]These authors are contributed equally.

SUMMARY

From the perspectives of pathway evolution, discovery and engineering of plant specialized metabolism, the nature of the biosynthetic routes represents a critical aspect. Classical models depict biosynthesis typically from an end-point angle and as linear, for example, connecting central and specialized metabolism. As the number of functionally elucidated routes increased, the enzymatic foundation of complex plant chemistries became increasingly well understood. The perception of linear pathway models has been severely challenged. With a focus on plant terpenoid specialized metabolism, we review here illustrative examples supporting that plants have evolved complex networks driving chemical diversification. The completion of several diterpene, sesquiterpene and monoterpene routes shows complex formation of scaffolds and their subsequent functionalization. These networks show that branch points, including multiple sub-routes, mean that metabolic grids are the rule rather than the exception. This concept presents significant implications for biotechnological production.

Keywords: biosynthetic gene cluster, cytochrome P450, enzyme promiscuity, metabolic network, terpene specialized metabolism, terpene synthase, UDP-dependent glycosyl-transferases.

INTRODUCTION

Reasoning and scope of this review

In the wider metabolic network that contains the entirety of plant metabolism, specialized metabolism has generally been viewed as a separate branch of enzymatic reactions producing metabolites that are not critical for the 'central' functions needed for plant survival. The main roles of specialized metabolites appear to be adaptation to the environment, that is, biotic and abiotic interactions. These include on the one side, plant defense against herbivores and pathogens or attraction of pollinators, natural predators of herbivores, seed dispersers and microbial symbionts. On the other side, they also play critical roles in abiotic interactions such as drought stress and protection from UV light (Weng et al., 2021). The importance and nature of these traits was already recognized in the second half of the 19th century, long before the beginning of what we now know as plant chemical ecology (Hartmann, 2008). In modern times, the boundaries between specialized

metabolites and hormones, signaling molecules required for regulation and lastly metabolites vital for growth and development are becoming increasingly hazy. Specialized metabolites are emerging as multifunctional, that is, playing roles in regulation of growth (flavonoids) or defense (callose deposition, glucosinolates, benzoxazinoids), or have examples of catabolism and re-integration into central metabolism (Erb & Kliebenstein, 2020).

Humans have recognized the bioactive nature of medicinal plants for thousands of years, extracting and combining numerous plant components into medicines (Leroi-Gourhan, 1975). With the advent of modern chemical techniques, it became possible to isolate and characterize single bioactive components (Patridge et al., 2016). This idea of single-compound medicine underpins the modern pharmaceutical paradigm, and thus it has also driven the initial approach to plant biosynthetic pathway discovery. In this approach, a linear pathway is often presumed, or at least sought, with the goal of producing a single molecule of interest with high specificity. Yet the past few decades

of biosynthetic pathway research have shown that many compounds are in fact the result of metabolic grids governed by promiscuous enzymes leading to complex mixtures of related natural products. As a result, it appears plausible that many plant biosynthetic pathways are geared towards chemical diversity rather than singular end-products. Likewise, pharmaceutical research has now recognized the limits of single-molecule treatments in the face of complex, multi-target human diseases, and multi-component treatments such as those designed based on traditional Chinese medicinal recipes are now finding traction in modern combination therapy (Li & Weng, 2017; Zhao, Luan, et al., 2020). This network hypothesis is also supported by the sheer diversity of specialized metabolites reported from plants, notwithstanding the many thousands of compounds yet to be reported. Recent work investigating the ecological underpinnings of phytochemical diversity suggests that plants generate a chemical array to target the variety of generalist and specialist pests in their environment (Salazar et al., 2018; Volf et al., 2018; Whitehead et al., 2021). Linear pathways would require separate enzymatic and regulatory structures for each compound, but metabolic grids of promiscuous enzymes can generate tens of products per enzyme. This is far more efficient both in terms of metabolic cost as well as the ability to tweak chemistry quickly on an evolutionary time scale. In stark contrast to this strategy generating chemical diversity stands the general, that is, primary metabolism, where networks of often functionally redundant but differentially expressed genes lead to single end-products. The principle is exemplified by the shikimate pathway yielding the amino acid phenylalanine and the subsequent core phenylpropanoid pathway gate keeper of routes to lignin, soluble and wall-bound phenolics and flavonoids (Hamberger et al., 2006; Hamberger & Hahlbrock, 2004; Leong & Last, 2017). In this review, we will focus on terpenoids, the largest class of plant specialized metabolites. We give a brief overview of the history of terpene biosynthetic pathway discovery and how rapidly evolving technologies have enabled probing of metabolic networks to an extent never before possible. Examination of select examples from the field of terpenoid pathway discovery provides evidence that generally, plant specialized metabolism is composed of complex interacting networks and not simple linear pathways.

Biochemistry of terpene biosynthesis, early pathways to the scaffolds

Several excellent reviews and studies cover the nature and evolution of plant terpene biosynthesis, and the rise of chemical diversity, so we will cover this only briefly (Chen et al., 2011; Jia et al., 2022; Karunanithi & Zerbe, 2019; Weng et al., 2012; Zhou & Pichersky, 2020; Zi et al., 2014). Terpenoids (terpenes or isoprenoids) are derived from the

five-carbon building blocks isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). These are synthesized through the mevalonate (MVA) pathway and the more recently discovered methylerythritol phosphate (MEP) pathway. The MVA pathway is localized to the cytosol and shares common ancestry with the animal, fungal and archaeal kingdoms (Yoshida et al., 2020). The MEP pathway is localized to the plastid and thus is of prokaryotic origin. Prenyl transferases are enzymes that condense IPP and/or DMAPP into longer prenyl diphosphates utilized by terpene synthases (TPSs). Typically, this is a head to tail condensation with double-bonds retaining trans-configuration, but there are examples of irregular and cis-prenyl transferases (Akhtar et al., 2013; Demissie et al., 2013; Miller et al., 2020; Rivera et al., 2001). TPSs then catalyze complex carbocation cascade reactions on the prenyl diphosphate substrate, resulting in cyclic or linear terpene backbones. The carbocation cascade is initiated by either removal of the diphosphate (class I TPSs), or by protonation of the distal double-bond (class II TPSs). The shape (dictating substrate and intermediate conformations) and electronics (stabilization of carbocations) of the active site residues then control the sequence of the carbocation cascade. The final product is determined by which potential energy minima is reached on the reaction path as well as the method of carbocation quenching, which may be through water addition, protonation or deprotonation (Tantillo, 2011). The nature of terpene formation relying on this delicate energy control has great importance to the evolution of terpene biosynthesis. The control of reaction product can be altered by few or even a single amino acid switch (Jia & Peters, 2016; Wilderman & Peters, 2007; M. Xu et al., 2007; J. Xu et al., 2017). There are also many examples of multi-product TPSs, where TPS-directed carbocation cascades may be biased towards one product but allow for several minor products to result. This phenomenon is outlined in more detail in the following sections.

Increasing complexity, functionalization and decoration of terpenes

The nature of terpene biosynthesis lends itself naturally to biosynthetic networks. A single or few changes in the amino acid sequence of a TPS can profoundly change its product profile (Irmisch et al., 2015; K  llner et al., 2020; M. Xu et al., 2007). TPSs may also produce arrays of dozens of products (Steele et al., 1998). After cyclization, the terpene backbone is often further decorated by other biosynthetic enzymes, such as cytochrome P450 monooxygenases (P450s), 2-oxoglutarate-dependent dioxygenases (2-ODDs), acyl transferases and glycosyl transferases (Wang et al., 2019). This increases the polarity of the molecules and often adds the most potent bioactive moieties. Specifically, P450s, drivers of the evolution of terpene

specialized metabolites, are known to be promiscuous and may oxidize their substrates in multiple positions as well as oxidizing multiple related backbones (for review, see Bathe & Tissier, 2019; Hamberger & Bak, 2013). This promiscuity with related intermediates in the same pathway is key to creating complex chemical networks. The ability for one enzyme to accept multiple related substrates increases flexibility of the network and exponentially expands the number of products that can be made by just a few enzymes. P450s are also recognized for their role as gatekeepers in pathway bifurcations of industrially relevant bioproducts (Banerjee & Hamberger, 2018). Thus, it is common in phytochemical studies for many related compounds to be identified from a single plant.

History of terpene pathway discovery

Recognizing their nutritional value, the biosynthesis of carotenoid pigments were among the first terpenes that attracted attention in the mid-1950s (James & Hollinger, 1954; Kramer & Tarjan, 1959). Early studies relied on labeling studies, cell-free extracts and purified enzymes (Modi & Patwa, 1959) in a field that gained traction. The terpenes investigated expanded significantly, including mint oil terpenes, insecticidal pyrethrins, and remarkably the identification of the early committed steps in cyclization and oxidation of the growth hormone gibberellin (Battaille et al., 1961; Crowley et al., 1962; Dennis & West, 1967; Upper & West, 1967). The mint family (Lamiaceae), exceptionally rich in terpenes, represents an illustrative example of how the terpene research in plants evolved over the next two decades, with citation records increasing from 48 annual articles in 1968 to 365 in 1999 (<https://pubmed.ncbi.nlm.nih.gov/>; terpene, biosynthesis, plant). Pioneered by the lab of Rodney Croteau, this period saw the enzymatic cyclization from common acyclic precursors to key terpenes in peppermint, sage and thyme (Croteau et al., 1973; Croteau & Karp, 1977; Poulou & Croteau, 1978), elucidation of multistep routes to more complex products (Kjonaas et al., 1985; Kjonaas & Croteau, 1983), and demonstration of the function of a cytochrome P450 in a multistep pathway (Karp et al., 1987). This emerging theme of multistep pathways was applied to the discovery of the first committed step in the biosynthesis of paclitaxel (taxol) through isolation of a cDNA clone from a cDNA library and heterologous expression of the encoded taxadiene synthase and demonstration of a P450 activity involved in the first hydroxylation (Hefner et al., 1996; Wildung & Croteau, 1996). With that, the groundbreaking work of Croteau's lab arguably gave birth to the new field of genomics-driven terpene pathway discovery and bioengineering of isoprenoid biosynthesis, including modification of the mint oil composition (Lange & Croteau, 1999; Mahmoud & Croteau, 2001; McCaskill & Croteau, 1997).

Technologies accelerating pathway discovery

Sequencing and the first genome set the stage

The next two decades in pathway discovery that are in focus of this review are driven by emerging genomic technologies, notably the early cloning and sequencing of cDNA libraries (or their fragments, expressed sequence tag libraries), hybridization of labeled cDNA to arrayed sequences (i.e. microarrays), whole-genome shotgun sequencing and recently new high-throughput sequencing technologies (i.e. 454, illumina, nanopore, pacbio). The vast amount of data generated creates two pillars for the discovery of pathways: (1) the genetic underpinning and composition of genomes and transcriptomes; and (2) organ, cell-type and condition-specific expression. Sequencing, assembly and release of the Arabidopsis genome in 2000 (The Arabidopsis Genome Initiative, 2000) was a harbinger of the explosion in plant genome and transcriptome sequencing capabilities that exponentially followed. The emerging framework of the genome led already in 1997 to the first report of a TPS-like gene with similarity to cDNA sequences from gymnosperms, Lamiaceae and Solanaceae on a 23.9-kb fragment from the long arm of chromosome IV (Aubourg et al., 1997). Cloning and heterologous expression of an identified cDNA clone showed activity as multiproduct monoterpene synthase (Bohlmann et al., 2000). Mapping of TPS sequences in the genome identified a small family in Arabidopsis and allowed to propose a model for the evolutionary history of plant TPSs from general to specialized metabolism (Trapp & Croteau, 2001). The complement of 40 TPS genes was reported briefly thereafter, including their classification into six phylogenetic subfamilies TPS-a to TPS-f, following an earlier proposed scheme (Aubourg et al., 2002; Bohlmann et al., 1998). This work also reported the first gene clusters in terpene specialized metabolism. The next year showed the dramatic advances with two milestones achieved, a functional link between biosynthesis and floral emission of terpenes from Arabidopsis flowers, and the first metabolically engineered transgenic Arabidopsis lines (Aharoni et al., 2003; Chen et al., 2003). Findings in these early studies deploying functional genomics remained instructive for the coming decades.

Co-expression

A functional linkage between non-homologous genes that together form metabolic pathways can be inferred from co-expression across tissues and cell-types, in time course experiments, or under other conditions that elicit pathway activity. An important tool accelerating the discovery of pathways and establishing these links between genes with unknown function became the Affymetrix Arabidopsis thaliana ATH1 microarray. Thousands of global transcript datasets were deposited in public databases and

represented a treasure trove. The large family of P450s represented an ideal target, as they are highly diverse and at that time only a fraction was functionally assigned. Ehltling et al. (2008) re-annotated over 4000 genes, and built and mined the extensive 'CYPedia' database for co-expressed pathways. This revealed, among other findings, highly coordinated expression of P450s from the CYP71 clan under pathogen stress and elicitation and in triterpene pathways. The team led by Anne Osbourn fully validated the bioinformatic predictions with characterization of the thalianol pathway and the report of the corresponding biosynthetic gene cluster (BGC), while the above manuscript was under evaluation (Field & Osbourn, 2008). The well-cited database provided a highly valuable tool in the next decade for identification of cryptic and highly complex monoterpene pathways, with an example for formation of flower volatiles described in detail in the respective section below (Ginglinger et al., 2013). It is intriguing that while co-expression analyses corroborated numerous instances of genomically clustered functionally characterized pathways, such as those in *A. thaliana* or *Oryza sativa* (Shimura et al., 2007; Swaminathan et al., 2009), it did not support the inverse, pathway discovery based on genomic clustering alone, highlighting that the combination of both technologies is critical (Wisecaver et al., 2017). This early strategy of global co-expression analyses also provided the blueprint for similar work approaching non-model plants with increasingly accessible transcriptome panels, but without accessible genomes (Wisecaver et al., 2017). Together, genomic technologies and co-expression strategies have enabled characterization of numerous terpenoid biosynthetic pathway enzymes over the past decade. In the following sections we explore some examples of these discoveries to evaluate the evidence for network-like organization of specialized terpenoid pathways.

DITERPENOIDS

Diterpenoids are derived from an acyclic 20-carbon prenyl diphosphate precursor. This is most commonly geranylgeranyl diphosphate (GGPP), though an all-cis precursor, nerylneryl diphosphate, has been identified in a few plants (Gericke et al., 2020; Matsuba et al., 2015; Miller et al., 2020). Both the prenyl diphosphate synthase and the diterpene synthase (diTPS) contain N-terminal targeting peptides for the chloroplast, so the precursors for these compounds are derived primarily from the MEP pathway. Some diterpenes are made by a single class I diTPS. However, the bicyclic labdane-type diterpenes require the sequential action of a class II followed by a class I diTPS (Peters, 2010). This is exemplified by the gibberellins, central metabolites that proceed from GGPP via ent-copalyl diphosphate (ent-CPP) to ent-kaurene. Labdane-type diTPSs in specialized metabolism have evolved from this role in central metabolism through duplication and

neofunctionalization. There are now 20 diterpene diphosphates identified (Wang et al., 2022) made by the class II or copalyl diphosphate synthase (CPS)-type diTPSs (TPS-c family). These can be converted to over 7000 individual scaffolds through the action of the class I or kaurene synthase-like (KSL) diTPSs (TPS e/f family; Peters, 2010). Class I diTPSs introduce the first layer of promiscuity into diterpene biosynthesis, as several have been shown to accept multiple diterpene backbones (Andersen-Ranberg et al., 2016; Jia et al., 2019; Johnson et al., 2019). This can contribute to network-type biosynthesis within a single plant, though sometimes specific diterpene pathways are separated by tissue-specific expression. This promiscuity has also enabled production of new-to-nature diterpene backbones by combining class II and class I diTPSs from different plants together in a heterologous host (Andersen-Ranberg et al., 2016; Jia et al., 2019). Though the genetic sequencing advances of the past two decades have enabled a surge in diterpene biosynthesis studies, only a tiny fraction of the over 23 000 diterpenes so far identified from plants (Dictionary of Natural Products, March 2022 | CRC Press, Taylor & Francis Group) have known pathways.

Tanshinones

The abietanes are a widely present class of tricyclic diterpenoids with significant and numerous bioactivities reported (Gonzalez, 2015). One of the most well-studied pathways is for the tanshinones, a class of bioactive diterpenoid components of the Chinese medicinal herb *Dan-shen* (*Salvia miltiorrhiza*). Because tanshinone biosynthesis has been recently reviewed (Wang & Peters, 2022), we will not detail this body of work in-depth here. However, the network aspects of key enzymes identified so far are notable (Figure 1). The biosynthesis of tanshinones begins with a classic labdane pathway. A class II diTPS catalyzes formation of (+)-copalyl diphosphate [(+)-CPP], and a class I diTPS cyclizes this into miltiradiene, a tricyclic abietane-type backbone (Gao et al., 2009). This backbone is capable of auto-oxidation to the aromatic abietatriene. From there, three P450s (CYP76AH1, CYP76AH3 and CYP76AK1) have been identified that together catalyze oxidations on four different carbons. Initially, CYP76AH1 catalyzes hydroxylation of C12 on abietatriene to afford feruginol (Zi & Peters, 2013). This step is required for additional oxidations. Subsequently, CYP76AH3 and CYP76AK1 represent bifurcations and can oxidize their substrate either in various positions, including multiple oxidation steps, or accept a range of different substrates, yielding an array of oxidation products (Guo et al., 2016). Another set of enzymes, CYP71D375 (and CYP71D373) and a 2-oxoglutarate dehydrogenase (Sm2ODD14), has been shown to catalyze furan ring formation for the classic tetracyclic tanshinone backbone (Ma et al., 2021; Song et al., 2022). Additional oxidation and demethylation steps

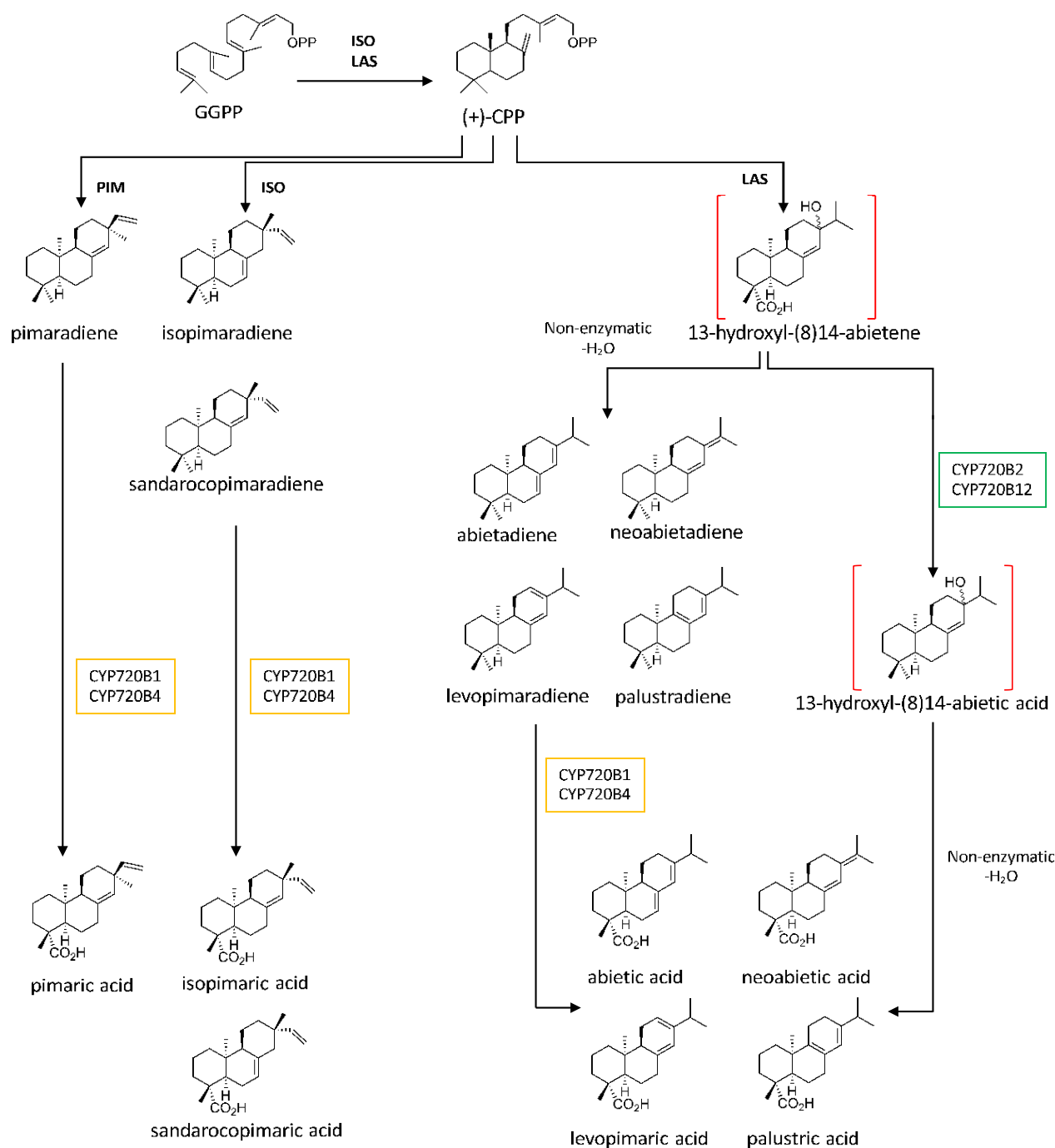


Figure 1. Overview of tanshinone, carnosic acid and triptolide biosynthesis.

All three of these angiosperm abietane pathways begin with a class II diTPS [(+)-CPS] that converts GGPP to (+)-CPP followed by a mitratriene synthase (MS). Sequential steps are represented by a single arrow. Curved double arrows indicate steps where multiple enzymes can interact with multiple substrates in a net-work fashion, that is, a P450 may oxidize multiple positions and/or the same position on multiple substrates. For clarity, not all enzyme products from network steps are shown. Question marks represent steps that have not yet been elucidated. Red boxes indicate tanshinone biosynthesis in *Salvia miltiorrhiza*, blue boxes indicate carnosic acid biosynthesis, and purple boxes indicate triptolide/triptonide biosynthesis in *Tripterygium wilfordii*. Species names are included where the pathway enzymes are from multiple species (Sfp, *Salvia fruticosa/pomifera*; Ro, *Rosemary officinalis*). Inset shows standard numbering for the abie-tane backbone. Not all side-products and intermediates are shown. CPP, copalyl diphosphate; CPS, copalyl diphosphate synthase; diTPS, diterpene synthase; GGPP, geranylgeranyl diphosphate.

have not yet been elucidated. Decades of phytochemical studies have shown that there are at least 40 different tanshinone diterpenoids present in *S. miltiorrhiza* (Pang et al., 2016). Many of these contain the oxidations and ring closures so far elucidated, but there is additional variation as well. Based on the work so far presented, the most likely explanation is that a sizeable fraction of the hundreds of P450s and other biosynthetic-type enzymes predicted in the *S. miltiorrhiza* genome are involved in a larger network (Song et al., 2020). While the pathway proceeds in a linear fashion up to ferruginol, it afterwards winds circuitously towards many end-products, facilitated by the promiscuity of the P450s. Some steps mingle while others require specific substrates. The most abundant and/or bioactive tanshinones are the goal of biosynthetic elucidation, but minor products, as well as specific metabolic intermediates along the way, may represent a core part of the plant's defense strategy.

The earlier stage of the oxidation network in tanshinone biosynthesis is present in similar form in other species of the mint (Lamiaceae) family. Another important abietane diterpenoid, carnosic acid, is found in high abundance in rosemary (*Rosmarinus officinalis*) and sage (*Salvia* spp., Birtic et al., 2015; Loussouarn et al., 2017). An orthologous CYP76AH enzyme produces ferruginol in carnosic acid biosynthesis (*R. officinalis*, *Salvia pomifera*, *Salvia fruticosa*). Additional CYP76AH and CYP76AK family members catalyze promiscuous oxidations leading towards a network of carnosic acid and related compounds (Ignea et al., 2016; Scheler et al., 2016; Figure 1). Carnosol is another abundant and related compound in these plant species, but the biosynthetic link between carnosic acid and carnosol has not yet been elucidated.

Resin acids

Another subset of bioactive abietane diterpenoids are conifer diterpene resin acids (Figure 2). Although the product array is less complex than observed in the tanshinone and carnosic acid chemical families, the network has been more completely mapped and demonstrates a key example of a multi-product diterpenoid pathway. In gymnosperms, the branch of labdane scaffolds is primarily synthesized by bi-functional diTPSs that contain both the class II and class I active sites (TPS-d family). The grand fir (*Abies grandis*) abietadiene synthase was the first of the diTPSs identified over 25 years ago, together with a proposed pathway to the carboxylic acid, and remains a model enzyme for a broad range of studies with focus on structure–function relationship, catalytic mechanism and is notably one of the very limited number of plant diTPSs crystallized to date (Vogel et al., 1996; Zhou et al., 2012). As the biochemistry of the pathway has been reviewed extensively, we will focus here on two noteworthy milestones defining the metabolic grid of resin acid biosynthesis. The enzyme from

Norway spruce (*Picea abies*) received attention for its capacity (among other orthologs) to afford a mixture of abietadiene, levopimaradiene, neoabietadiene and palustradiene, all double-bond isomers of the shared labdane skeleton (Keeling et al., 2011). Through an extensive series of careful examinations, Keeling et al. demonstrated that the reaction mechanism of PaLAS proceeds via water quenching of the abieta-8(14)-en-13-yl carbocation, resulting in 13-hydroxy-8(14)-abietene, detected by cold on-column injection and a lower final oven temperature. Dehydration of this product during work-up, or conventional GC–MS analysis then results in the non-enzymatically derived spectrum of products. P450s of the CYP720B subfamily (CYP720B1 and CYP720B4) had been shown to catalyze the three-step oxidation of the diterpene olefins to their carboxylic acids (Hamberger et al., 2011; Ro et al., 2005). However, a fascinating facet of the biological relevance of the unstable intermediates was discovered later, when a different clade of CYP720Bs (containing CYP720B2 and CYP720B12) was found inactive with the olefin scaffolds. These were ultimately shown to accept the unstable diTPS product 13-hydroxy-8(14)-abietene, affording the same diterpene resin acids characteristic for the profiles in conifers (Geisler et al., 2016). In this case a network is created in two ways: in the first, two P450s promiscuously oxidize the known diterpene olefins; whereas in the second, a single unstable intermediate is oxidized before decomposing into a similar set of diterpene resin acids. These findings emphasize the modularity and combinatorial nature of this diterpene specialized pathway.

Forskolin

The biochemistry leading to the cyclic AMP activator and epoxy-labdane type forskolin has been reviewed (Banerjee & Hamberger, 2018). Hence, we will here only briefly summarize the route, and focus instead on the relevance of promiscuous enzymes in creating chemical diversity through a metabolic grid, with significant implications for biotechnological applications using those plant enzymes. Early signs for more complex, rather than linear, routes were found with the complement of enzymes catalyzing formation of key diterpene scaffolds in Indian coleus (*Coleus forskohlii*). Conspicuously, the epoxy-labdane manoyl oxide and its derivative forskolin accumulate in the roots of the plant, while diterpenoids derived from the common olefin miltiradiene are detected in aerial parts of the plant. This was shown to be seemingly controlled by differently expressed modules, consisting of two distinct and tissue-specific class II diTPSs coupled with a pair of broadly expressed class I diTPS homologs. Swapping out the enzyme catalyzing the initial cyclization can then give rise to the different terpene scaffolds (Pateraki et al., 2014). In the roots, the subsequent conversion of manoyl oxide to forskolin requires six regio- and stereospecific

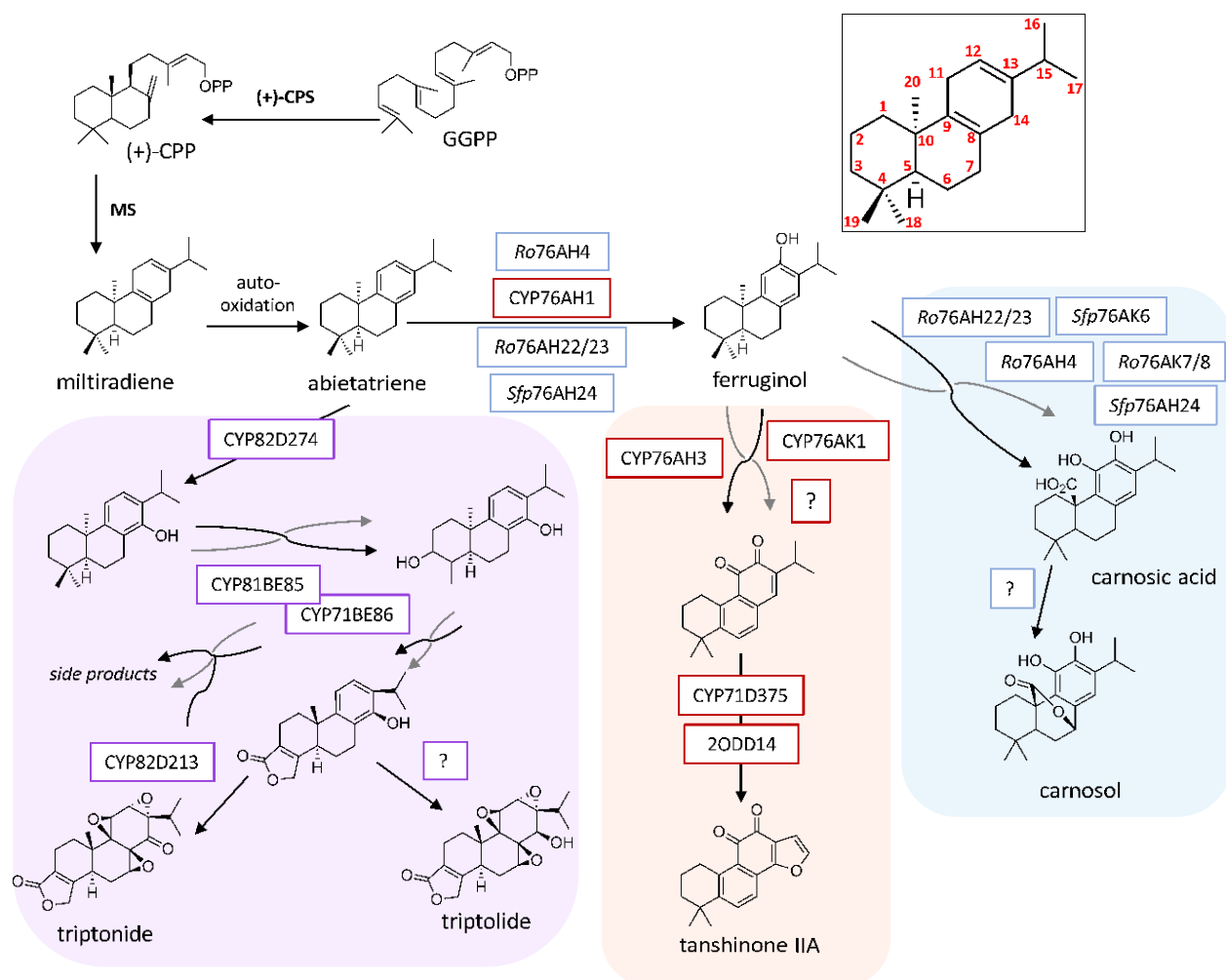


Figure 2. Overview of diterpene resin acid biosynthesis. Adapted from Geisler et al. (2016).

The bifunctional diTPSs isopimaradiene synthase (ISO), pimaradiene synthase (PIM) and levopimaradiene/abietadiene synthase (LAS) convert GGPP via the intermediate (+)-CPP into the various diterpene olefins. In the case of LAS, the initial and unstable diTPS product is 13-hydroxy-8(14)-abietene (indicated by red brackets). Studies of the CYP720B subfamily showed that one clade, CYP720B1 and CYP720B4 (outlined in orange), converts a range of diterpene olefins to the corresponding acid. However, another clade, CYP720B2 and CYP720B12 (outlined in green), can only accept 13-hydroxy-8(14)-abietene to the corresponding 13-hydroxy-8(14)-abietic acid, which then undergoes dehydration to form abietic acid, levopimaric acid, neoabietic acid and palustric acid. CPP, copalyl diphosphate; diTPS, diterpene synthase; GGPP, geranylgeranyl diphosphate.

monooxygenations and a single regiospecific acetylation. Following the hypothesis that the corresponding enzymes catalyzing formation of forskolin should be co-expressed in the root, a panel of seven P450s of the CYP76AH-subfamily and two BAHD-type acyltransferases were found (Pateraki et al., 2017). Upon testing of the P450s, nearly all were found to oxidize manoyl oxide, together giving rise to a broad spectrum of products, including multi-oxygenated forms clearly not intermediates of the hypothesized (linear) route to forskolin. One prolific P450 yielded 11 discrete products, including three hydroxyl groups and a keto group. In biotechnological applications, such promiscuity would translate to near-complete loss of control, yet in plants this may be an important contribution to chemical

diversity. In contrast, a second P450 catalyzed regiospecific formation of keto-manoyl oxide in a configuration present in both forskolin and many forskolin/manoyl oxide-derived diterpenoids. Using this P450 as an anchor, all other P450s were tested first in pairs, then in triplets in all permutations. Again, up to 19 different products were detected in these assays, indicating that in planta diterpene biosynthesis is anything but linear. However, and at this stage unexpected, a single combination of three P450s yielded a small and single peak of deacetyl-forskolin, the second last intermediate towards forskolin. Of 10 acyltransferases identified and tested, two resulted in acetylation of deacetyl-forskolin. These enzymes are notoriously promiscuous, so it was again not surprising to see a broad

range of acetylated products with one, forskolin representing a minor fraction. In contrast, the second enzyme exhibited high activity and specificity with efficient conversion of the intermediate to forskolin and lacking detectable side-products. With that, a minimal set of enzymes was reported constituting the entire and specific linear route from GGPP to forskolin, and opening the door for biotechnological production (Figure 3a; Pateraki et al., 2017).

Triptolide

A final example of abietane diterpenoids is triptolide (Figure 1). The suggested biosynthetic routes to rearranged (4 ? 3) abeo-abietane type diterpenes in the root and root cultures of *Tripterygium wilfordii* represent an instructive case leading to a diverse portfolio of complex decorated related products (Inabuy et al., 2017; Kutney et al., 1981). A hallmark of this group is the bioactive triepoxide triptolide (Kupchan et al., 1972). While a linear route was early suggested, a metabolic grid appeared plausible (Inabuy et al., 2017; Kutney et al., 1981). Functional characterization of all candidate diTPSs from the TPS-e/f subfamily afforded specific combinations of class II/I diTPSs giving access to the kaurane, manoyl-oxide and kolavenyl scaffolds, characteristic for *T. wilfordii*. Markedly, functional

redundancy was detected in both class II and class I diTPSs, which together form a metabolic grid to the diterpene scaffolds. The lack of functional combinations yielding abietane-type diterpenes prompted analysis of TPSs outside the canonical TPS-e/f subfamily. This led ultimately to the discovery of TwTPS27, a class I TPS of the TPS-b subfamily, typically harboring monoterpene synthase activity (Hansen et al., 2017). Like its other class I counterparts, this enzyme was shown to accept multiple substrates, and may contribute abietane and manoyl-oxide scaffolds in specific stereochemistry to the biosynthetic capacity of the *T. wilfordii* diTPSs (Hansen et al., 2017). Even though production of the postulated triptolide intermediate dehydroabietic acid was demonstrated in yeast, using the proxy CYP720B4 from Sitka spruce (Forman et al., 2017), it took another 3 years until the corresponding gene encoding CYP728B70 was identified from the *T. wilfordii* genome (Tu et al., 2020). The breakthrough came with the elegant demonstration of a cascade of four different P450s of the two different subfamilies, 82D and CYP71BE, identified from over 60 candidates with activity on the abietane-type miltiradiene. Drawing on parallels with the earlier reported forskolin pathway, the team now led by Hansen and Andersen-Ranberg who identified the

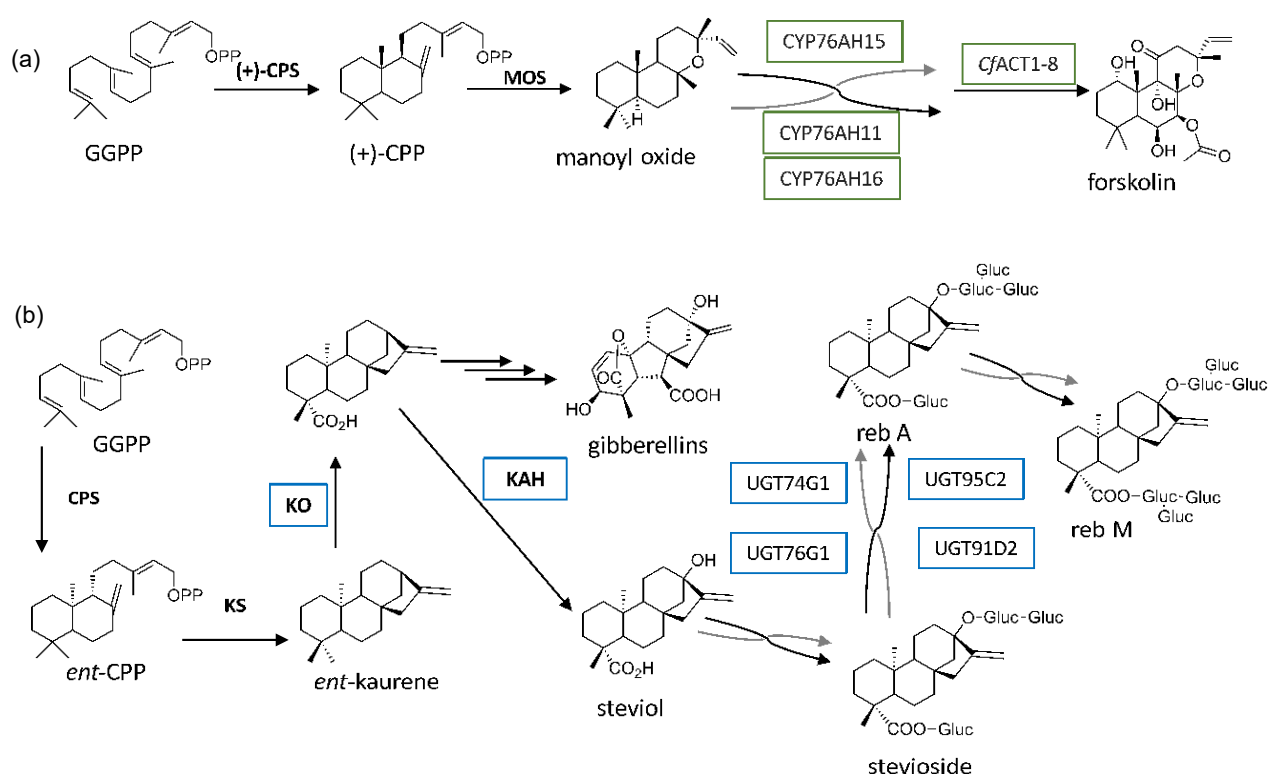


Figure 3. Overview of (a) forskolin and (b) steviol glycosides biosynthesis.

Sequential steps are represented by a single arrow. Curved double arrows indicate steps where multiple enzymes can interact with multiple substrates in a network fashion, that is, a P450 may oxidize multiple positions and/or the same position on multiple substrates. For clarity, only a few representative enzyme products from network steps are shown. CPS, ent-CPP synthase; KAH, kaurenoic acid 13-hydroxylase; KO, kaurene oxidase.

diterpene cyclases in *T. wilfordii* focused on an anchor P450 (TwCYP82D274) catalyzing the first step in the pathway, which afforded the aromatic hydroxylated intermediate 14-hydroxy-dehydroabietadiene (Hansen et al., 2022). Guided by promiscuous activities of *C. forskohlii* enzymes, homology and the recognition that most P450s oxidizing diterpene specialized metabolites reside in the CYP71 clan, they identified two P450s (TwCYP71BE85 and TwCYP71BE86) to oxygenate the A-ring of 14-hydroxy-dehydroabietadiene resulting in a C4 ? C3 methyl shift and lactone formation. Notably, both were found to additionally produce a broad panel of oxygenated and polyoxygenated diterpenes with the starting diterpene scaffold miltiradiene or the first oxidized intermediate. Testing of orthologs of the first P450 established TwCYP82D213 as the last step in the route giving access to the triptonide derivative triptonide, next to a plethora of oxidized miltiradiene products. Indeed, the authors acknowledge that the striking substrate and product promiscuity of the TwP450s could suggest that instead of a simplistic model of a linear pathway, triptonide may be one of the many products of a metabolic grid in *T. wilfordii* and engineered biotechnological hosts (Hansen et al., 2022).

Macrocyclic diterpenes

In addition to the two-step class II/class I diTPS pathways leading to the labdane-type diterpenes, there are single-step class I diTPSs capable of catalyzing the transformation of GGPP into unique diterpene scaffolds (Karunanithi & Zerbe, 2019). These are often members of the TPS-a family, which are typically sesquiterpene synthases (sesquiTPSs), but can acquire a transit peptide and evolve to convert GGPP in the plastid (Johnson, Bhat, Sadre, et al., 2019). One important example of this phenomenon are the macrocyclic diterpenes found extensively in the Euphorbiaceae plant family. Several of these compounds have strong bioactivity and pharmaceutical applications (Kemboi et al., 2021). Investigations into the diTPSs across multiple Euphorbiaceae species determined that casbene synthase likely produces the precursor to most macrocyclic diterpenes in this plant family (Kirby et al., 2010; Mau & West, 1994; Zerbe et al., 2013). Casbene is a 14:3 bicyclic diterpene that was hypothesized to proceed through the 5:11:3 lathyrane skeleton to other ring configurations that require additional oxidations and ring closures (Kirby et al., 2010). The highly oxidized nature of the macrocyclic diterpenoids found in Euphorbiaceae suggested involvement of P450s. With a combination of genomic and transcriptomic sequencing, the CYP726 subfamily was found to be key in conversion of casbene to jolkinol C, the first lathyrane intermediate, demonstrating the ability of a P450 to catalyze carbon-carbon bond formation in a ring closure (Figure 4). King et al. (2014) found the first active P450s in a BGC containing diTPSs, P450s and other terpene

associated genes in castor bean (*Ricinus communis*), later confirmed by Boutanaev et al. (2015). In characterizing these enzymes, they found that multiple P450s (CYP726A14, CYP726A17 and CYP726A18) could oxidize the 5-position of casbene to either a hydroxy or keto group. CYP726A16 could then add a 7,8-epoxy group to 5-keto casbene, but not casbene, requiring linear and sequential oxidation. Later, this group investigated a similar BGC in another Euphorbiaceae species, *Jatropha curcas*. In this BGC, they found orthologous CYP726As capable of oxidation at the 5- and 6-positions, and another P450, CYP71D495, that catalyzed production of 9-keto casbene (King et al., 2016). The combination of the 5,6- and 9-hydroxylases led to the production of jolkinol C, likely through non-enzymatic tautomerization of the enol group followed by an intramolecular aldol reaction that creates the key C-C bond. Similarly, Luo et al. (2016) found in the same year a CYP71D/CYP726A pair from *Euphorbia peplus* that catalyzed the same oxidation reactions. However, in this instance a short-chain alcohol dehydrogenase (ADH) was also required for formation of jolkinol C. Careful in vitro experiments in this study demonstrated that the hydroxylations and oxidations from hydroxy to ketone could proceed in any order and combination of P450s and the ADH, network fashion. However, the presence of all three invariably led to jolkinol C production when expressed in planta, though not in yeast, ostensibly due to the different pH than plant cells (Nguyen et al., 2012). Wong et al. optimized expression of both the *E. peplus* and *J. curcas* P450s in yeast, and demonstrated production of jolkinol C in titers up to ~800 mg L⁻¹, and validated that while the ADH was not critical for jolkinol C formation, it did significantly increase the titer (Wong et al., 2018). Fattahian et al. (2020) recently reviewed the extensive literature on jatrophone and other cembrene-derived diterpenoids, which contains numerous hypotheses from synthetic studies as to how subsequent ring transformations may occur. The heavily oxidized structures isolated from Euphorbiaceae species indicate that these biosynthetic pathways likely rely on additional oxidative enzymes, such as ADHs, to attain these configurations. Further variation exists in the acylation of these compounds, as the polyester form is typically isolated from the plant. With over 500 structures reportedly isolated, it is apparent that some degree of network exists in the biosynthesis. Like other pathways examined thus far, certain steps require exact precursors while others may occur in any order before crossing another committed step. A single plant species often accumulates multiple end-products based on the same diterpene backbone, such as casbene in the case of the Euphorbiaceae (Esposito et al., 2016; Kemboi et al., 2021). In this case, the pathway is linear through the biosynthesis of casbene, the common precursor, and then differs in progressively divergent and complex oxidations.

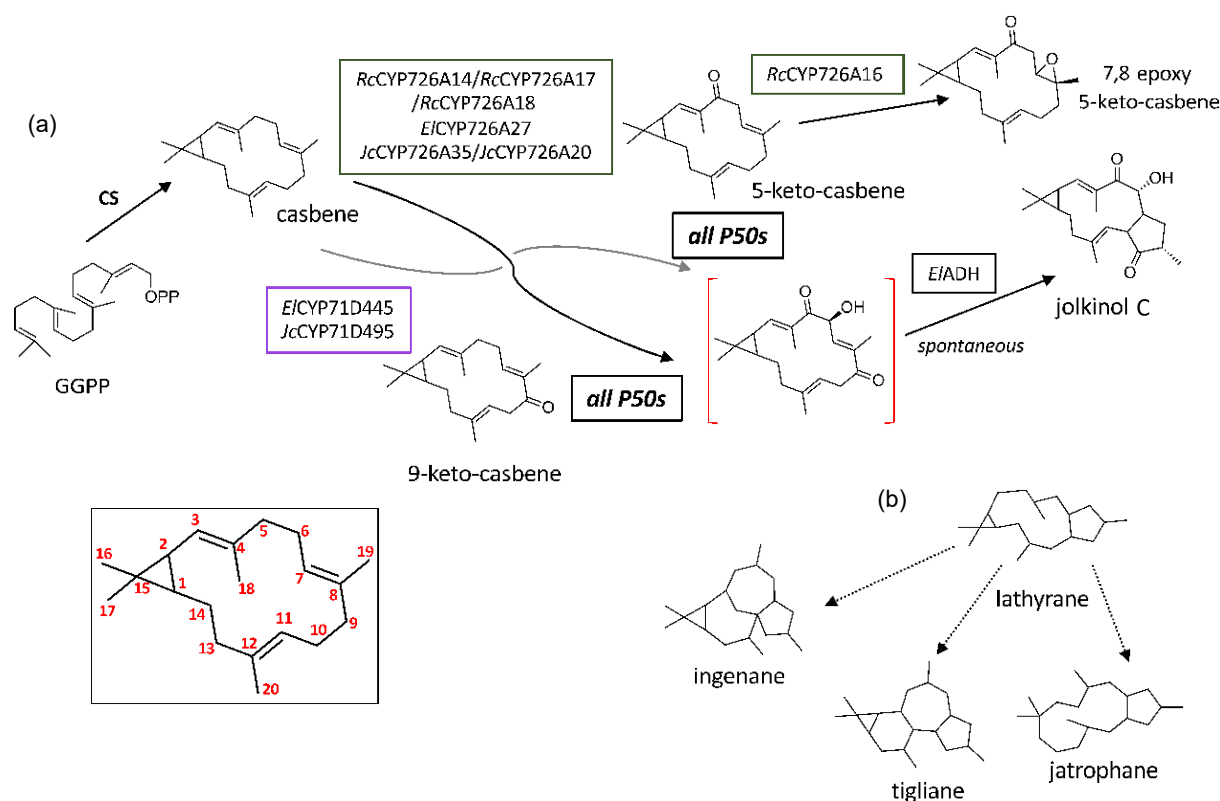


Figure 4. (a) Pathway to jolkinol C and (b) hypothesized route to additional macrocyclic diterpene backbones.

Sequential steps are represented by a single arrow, while curved double arrows indicate steps where multiple enzymes can interact with multiple substrates in a network fashion. In this pathway, the CYP71Ds (purple box) hydroxylate the 9-position while the CYP726s hydroxylate the 5-position (green box), but this can occur in any order. Both can hydroxylate the 6-position, together forming the unstable intermediate 6-hydroxy-5,9-diketocasbene (in red brackets), which can non-enzymatically rearrange to jolkinol C. The addition of EIADH was found to significantly increase jolkinol C production by improving conversion of hydroxy-moieties to keto-groups. For clarity, additional side-products of these enzymes are not shown.

(b) Conversion of jolkinol C to additional rearranged terpenes has not yet been elucidated and is indicated by the dotted arrows. CS, casbene synthase. Species names are abbreviated as Rc, *Ricinus communis*; El, *Euphorbia lathyris*; Jc, *Jatropha curcas*. Inset shows standard casbene numbering.

Beyond TPSs and P450s

Up to this point, the various pathways discussed have relied on TPS or P450 networks to create an array of diterpenoids. Yet some plants may not have expanded P450 and diTPS gene families, and rely on other enzyme types to create chemical diversity. One example of this is the steviol glycosides, which accumulate in *planta* to an astounding 25% of leaf dry weight. While over 30 different steviol glycosides have been reported, stevioside and rebaudioside A (Reb A) make up the bulk while the rest accumulate in low to trace amounts (Ceunen & Geuns, 2013). The diterpene core, steviol, is derived from a linear diTPS-CYP pathway that mirrors biosynthesis of the hormone gibberellin until the last step. An ent-CPP synthase cyclizes GGPP, and the class I diTPS kaurene synthase catalyzes transformation of ent-CPP to ent-kaurene (Richman et al., 1999). The first oxidation at C19 is catalyzed by kaurene oxidase (CYP701A5; Humphrey et al., 2006). A recent high-quality genome assembly of *Stevia rebaudiana* as well as early

pathway discovery work indicate that these first three enzymes are likely present as multiple copies in the genome, allowing for separate regulation for gibberellin versus steviol biosynthesis (Xu et al., 2021). From there the two pathways diverge, and the final oxidation at C13 is catalyzed by kaurenoic acid 13-hydroxylase (KAH) resulting in steviol. While the definitive identity of this enzyme in *S. rebaudiana* has not yet been verified, at least two P450s of the CYP716 and CYP714 subfamilies have been reported to have low to moderate KAH activity (Ceunen & Geuns, 2013; Wang et al., 2016). Additionally, four tandem duplicates in the CYP716 subfamily were found to be highly expressed in *S. rebaudiana* leaves, but have not been functionally verified (Xu et al., 2021). In attempts to engineer high-titer steviol glycoside production in microbial hosts, native enzymes were replaced in favor of a higher performing KAH (CYP714A2) from *A. thaliana* (Wang et al., 2016; Xu et al., 2022). The pathway then diverges into production of at least 14 steviol glycosides via a metabolic grid of four functionally characterized UDP-dependent glycosyl-

transferases (UGTs; UGT76G1, UGT74G1, UGT95C2, UGT91D2; Olsson et al., 2016; Richman et al., 2005; Wang et al., 2016). Hundreds of additional UGT candidates were identified in the genome analysis, of which 86 are expressed in relevant leaf tissues for steviol glycoside biosynthesis (Xu et al., 2021). UGTs are known to be quite promiscuous. For example, crude extracts of *A. thaliana* and tobacco have been shown to glycosylate steviol and other pathway intermediates (Humphrey et al., 2006). This raises difficulty in pathway engineering for a specific glycosyl variant, as is the case for steviol glycosides. Reb M and Reb D are two minor products of the steviol glycoside pathway that are preferred for their superior organoleptic properties to the more naturally abundant stevioside and Reb A (Olsson et al., 2016; Prakash et al., 2014). However, co-expression of the UGTs needed to produce these compounds results in numerous unwanted side-products. Intensive mutational analysis of the relevant UGT (UGT76G1) led to a variant with moderate improvements in product profile, but still multiple products (Olsson et al., 2016). As with promiscuity of the UGTs exponentially increases the diversity of the product profile, creating maximum output for the plant but inhibiting facile biotechnological use of the pathway.

MONOTERPENIDS/SESQUITERPENIDS

Monoterpenoids and sesquiterpenoids share many characteristics and functions with diterpenoids, but also have quite different and distinct roles in planta. Monoterpenoids are biosynthesized in the plastids from primarily MEP precursors as seen for diterpenoids, while sesquiterpenoids are generated in the cytosol from the MVA precursor pathway. Despite the two distinct pathways, together monoterpenoids and sesquiterpenoids constitute a large portion of the volatiles that plants emit (Muhlemann et al., 2014). Both classes often contribute to floral scents used to attract pollinators or to deter florivores. Volatile terpenoids also play an important part in defense against pathogens and general stress tolerance both above and below ground (Gershenzon & Dudareva, 2007). One example is caryophyllene, which is emitted from maize roots and attracts beneficial nematodes that feed on the root pest corn root-worm (*Diabrotica virgifera*; Rasman et al., 2005; Robert et al., 2012). α -Farnesene, which is emitted from apples, is an example of a sesquiterpene that functions as an insecticide, while also attracting birds that feed on the apples and aid in seed dispersal (Theis et al., 2014). Additionally, some of these terpenoids can also modulate the microbiome of the flower or vice versa (Hammer et al., 2003; Huang et al., 2012; Junker et al., 2011; Penuelas et al., 2014, reviewed by Dudareva et al., 2013). While both hydrocarbons and terpenes with a single hydroxylation are important volatiles, it is interesting to note that 92% of the known monoterpenoids are indeed hydroxylated according

to the Dictionary of Natural Products (Pateraki et al., 2015). Despite their generally more volatile nature, mono- and sesquiterpenoids are often also retained in plant tissue, and many plants have specialized storage cells, such as trichomes, laticifers and secretory ducts (Fahn, 1988; Tissier, 2018; Tissier et al., 2017). Storage compartments, whether internal or external, are excellent ways to store toxic terpenoids for potential usage against herbivores during tissue rupturing.

Sesquiterpenoids

The majority of sesquiTPSs are soluble proteins located in the cytosol that use (E,E)-FPP as a substrate. Nonetheless, a few sesquiTPSs have been found to be exceptions to this rule. In *Nicotiana tabacum*, a sesquiTPS (tobacco 5-epi-aristolochene synthase, TEAS) was characterized and found to accept (Z,E)-FPP in addition to (E,E)-FPP as a substrate (O'Maille et al., 2006). In addition, a sesquiTPS from sandalwood (*Santalum album*) produces sesquiterpenes with similar types of structures using both (E,E)-FPP and (Z,Z)-FPP as substrates (Jones et al., 2011). An example of a Z,Z-FPP prenyl transferase was discovered in *Solanum habrochaites*. This correlated with a sesquiTPS also identified in *S. habrochaites* that uses (Z,Z)-FPP but not (E,E)-FPP. The localization of both these enzymes was interestingly found to be in the chloroplasts (Sallaud et al., 2009). SesquiTPS enzymes can produce an astonishing variety of core terpene structures despite being limited in the variation of substrates. In fact, much of the diversity of sesquiterpenoids can be accounted for by the array of sesquiTPS products rather than extensive modifications, as seen for diterpenoids. These products may be cyclized, such as the guaienes (a C5- and C7-membered ring), eudesmanes (two C6-membered rings), germacrene (a C10-membered ring) and humulenes (a C11-membered ring); however, many sesquiterpenoids are also linear or monocyclic such as farnesene and bisabolene (Degenhardt et al., 2009). Some backbones already contain a hydroxy group due to water quenching of the carbocation cascade, such as kunzeal produced by TgTPS2 in *Thapsia garganica* (Pickel et al., 2012). In *Zea mays* (maize) a TPS, ZmEDS, was even found that produced eudesmanediol, which as the name indicates contains two alcohol groups (Liang et al., 2020). The multitude of backbones generated by the sesquiTPSs are due to various reactions that can occur during product formation, such as hydride shifts, methyl shifts, rearrangements, re- and de-protonations, in addition to the presence of three double-bonds in FPP. Extensive reviews of sesquiTPS product formation can be found in Degenhardt et al. (2009), Durairaj et al. (2019, 2021) and Xu & Dickschat (2020). One of the most astounding sesquiTPSs is from *A. grandis* (AgTPS), where 52 different sesquiterpenoid products were identified, though the product profile of the TPS was dominated by c-humulene while most

other products were found in negligible amounts (Steele et al., 1998). Unlike most TPSs, AgTPS has two rather than one divalent cation (Mg^{2+}) binding sites (the aspartate-rich DDxxD domains) that are located at the entrance of the active site. This may help the enzyme to achieve a much more complex product profile than seen for other multiproduct sesquiTPSs. Due to the sensitive energy balance of carbocation cascades, altering a single amino acid in the active site can significantly impact product profile. A study using a β -sesquiphellandrene synthase from *Persicaria minor* showed that a single mutation changed the product profile to include several hydroxylated sesquiterpenoids (Ker et al., 2020). Additional examples of multiproduct TPSs (both mono- and sesquiterpenoids) have been reviewed by Vattekkatte et al. (2018). Though multiproduct TPSs are common, many sesquiTPSs produce either one sole product or one major product with only very minor side-products. In kiwi fruit (*Actinidia deliciosa*), two examples of such single-product sesquiTPSs were described with either α -farnesene or (+)-germacrene D as the sole product (Nieuwenhuizen et al., 2009). Three sesquiTPSs from *Hyoscyamus muticus* exemplified this further, each producing a minimum of 93% of a single compound compared with the total amount of sesquiterpenoids detected.

Considering the multitude of sesquiTPS products, it is not surprising that sesquiTPSs that share high sequence similarity may in fact produce different compounds. This is seen for two *T. gargarica* TPSs, TgTPS1 and TgTPS2, which share 91% sequence identity but produce two different types of sesquiterpenoids, d-cadinene and kunzeaol, respectively. d-Cadinene is a eudesmane with the typical two six-membered rings, while kunzeaol is a hydroxylated germacrene with a 10-membered open ring structure. Additionally, TgTPS1 is a single-product TPS, while TgTPS2 produces several sesquiterpenoids in addition to kunzeaol (Pickel et al., 2012). Various mutation studies show that with just one or two amino acid changes in the active site of a sesquiTPS, the product profiles change substantially (Drew et al., 2016; Rising et al., 2000). In *Gossypium arboreum* (cotton), a (+)-d-cadinene synthase was subjected to a mutation study, and it was shown that changing a single amino acid can result in the production of a germacrene D-4-ol in addition to the original compound d-cadinene (Yoshikuni et al., 2006).

Many plants produce a variety of sesquiterpenoids, and it is common to see a portion of these based solely on one type of backbone structure. These backbones may have been modified by various hydroxylations as seen in the variety of caryophyllanes in *Eremophila spathulata* or acetylations as seen in *T. gargarica* (Bredahl et al., 2022; Drew et al., 2009). Most of the pathways for known sesquiterpenoids have not yet been elucidated, though in some species partial pathways have been found, though several further modifications are expected to occur (Mao et al.,

2016; Tian et al., 2018). In some of these instances the pathway is linear up until a certain point but branches out during the final modifications, as seen in the diterpenoid pathways.

As with diterpenoids, P450s are often the next class of enzymes to add further diversity to the sesquiterpenes. P450s often perform a single oxidation on the sesquiterpene backbone, though in several cases a cascade of oxidations occurs on the same carbon or same backbone. This is often seen in the formation of sesquiterpene lactones, described below. The promiscuity of P450s also renders them in some cases able to perform several consecutive hydroxylations on various positions on the same backbone. For example, in *S. habrochaites*, CYP71D184 can oxidize 7-epi-zingiberene into 9-hydroxy-zingiberene and 9-hydroxy-10,11-epoxyzingiberene (Zabel et al., 2021). One of the earliest modified sesquiterpenoid pathways to be studied was capsidiol biosynthesis in *N. tabacum*. The first step in the pathway is production of 5-epi-aristolochene by the TPS 5-epiaristolochene synthase (EAS). This is followed by hydroxylations at C1 and C3 by CYP71D20 to form capsidiol (Facchini & Chappell, 1992; Takahashi et al., 2005). Capsidiol biosynthesis is further reviewed in Banerjee and Hamberger (2018). Another interesting example of P450 promiscuity is found in *S. album*. Sandalwood is known for having an essential oil with fragrant sesquiterpenoids. These pathways have been reviewed previously and are therefore only mentioned in brief here (Banerjee & Hamberger, 2018). A network of nine P450s from the CYP76F subfamily was found to hydroxylate santalene and bergamotene backbones into corresponding santalols and bergamotols showing both a large and promiscuous P450 subfamily (Celedon et al., 2016; Diaz-Chavez et al., 2013).

Some of the most studied sesquiterpenoids belong to the sesquiterpene lactone subgroup due to their bioactivity and pharmaceutical activity (Chadwick et al., 2013). A few examples include sesquiterpenoids from *Tanacetum parthenium*, *T. gargarica* and *Artemisia annua* (Mathema et al., 2012; Thastrup et al., 1990; Tu, 2011). The enzymes responsible for lactone ring formation have been described in all three species, but the complexity and the type of enzymes involved in lactone ring formation depend on the pathway and the species in question, each having a unique approach (Andersen et al., 2017; Liu et al., 2014; Sy & Brown, 2002; Teoh et al., 2009). Kauniolide biosynthesis in *T. parthenium* and artemisinin biosynthesis in *A. annua* are briefly highlighted. While several of the biosynthetic steps for lactone ring formation have been described in various Asteraceae species, most progress has been made in *T. parthenium*, which is described here (Figure 5). Kauniolide biosynthesis is initiated by converting FPP to germacrene A, catalyzed by the sesquiTPS germacrene A synthase (GAS; Majdi et al., 2011). Germacrene A oxidase

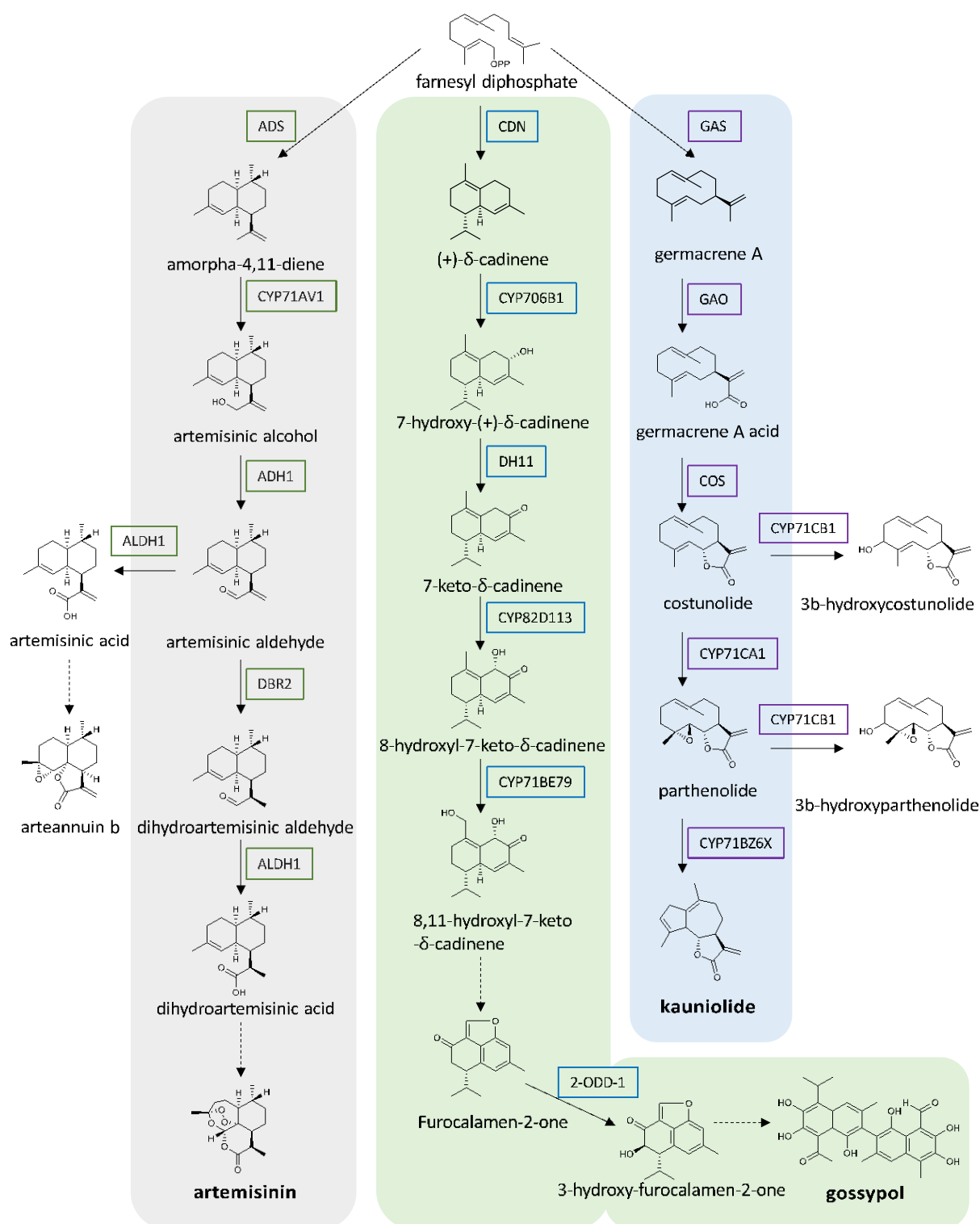


Figure 5. Three examples of distinct sesquiterpenoid pathways.

The gray box shows the artemisinin biosynthetic pathway in *Artemisia annua*. The green box shows the gossypol pathway in *Gossypium hirsutum*. The blue box shows the kauniolide pathway in *Tanacetum parthenium*. Dashed arrows denoted missing steps in the pathways or non-enzymatic steps. For clarity, additional side-products of DH1 in the gossypol pathway are not shown. 2-ODD-1, 2-oxoglutarate/Fe(II)-dependent dioxygenase; ADS, amorpha-4,11-diene synthase; ADH1, alcohol dehydrogenase 1; ALDH1, aldehyde dehydrogenase 1; COS, costunolide synthase; CDN, (+)- δ -cadinene synthase; DH1, alcohol dehydrogenase; DBR2, artemisinic aldehyde D11(13) reductase; GAO, germacrene A oxidase; GAS, germacrene A synthase.

(CYP71; GAO) performs a three-step oxidation of germacrene A to yield germacrene A acid [germacra-1(10),4,11(13)-trien-12-oic acid]. A second P450, costunolide synthase (CYP71BL2/COS) catalyzes a hydroxylation of germacrene A acid on position C6. The interesting outcome of these hydroxylations was seen regarding the formation of the lactone ring. The 6-*a*-germacrene A acid can perform a spontaneous lactonization to yield costunolide, a reoccurring theme. Costunolide is one of the possible precursors of many germacranolide, eudesmanolide and guaianolide sesquiterpenoids. GAS, GAO and COS are found in several Asteraceae including *Cichorium intybus*, *T. parthenium* and *Helianthus annuus*, but were all described in *T. parthenium* by Liu et al. (2014). Further steps modifying costunolide were elucidated in *T. parthenium*. In 2014, Liu et al. found a CYP71 clan P450, TpPTS/CYP71CA1, forming an epoxide and converting costunolide into parthenolide. This was followed in 2018 by the first discovery of a sesquiterpenoid P450 (TpKLS, CYP71BZ6X) able to perform the ring closure of a germacrene (parthenolide) thereby yielding kauniolide, a member of the diverse guaianolides (Liu et al., 2018). Though the pathway as described up until kauniolide appears to be linear, a P450 responsible for two potential side-branches was also discovered. Using yeast microsomes expressing CYP71CB1, feeding studies with costunolide and parthenolide found these converted to 3b-hydroxycostunolide and 3b-hydroxyparthenolide, respectively. Both metabolites have indeed been detected in *T. parthenium*, alluding to a role in planta. The genus *Tanacetum* in general produces a variety of different sesquiterpene lactones, though for *T. parthenium* at least costunolide and parthenolide are found in quantities sufficient for detection, parthenolide is furthermore one of the most abundant sesquiterpenoid lactones found in the plant (Abad et al., 1995). *T. parthenium* also produces a variety of further decorated guaianolides, and it is likely that these are based on modifications of kauniolide sesquiterpenoids and are awaiting discovery.

Artemisinin biosynthesis is an instructive example of a different set of enzymes generating the precursor steps needed for lactone ring formation. Depending on the expression system used for characterizing the individual enzymes involved in artemisinin biosynthesis, different activities have been reported (Teoh et al., 2006, 2009; Zhang et al., 2008). Here we only describe the pathway overview given by Judd et al. (2019) and Xie et al. (2016) (Figure 5). The first step in artemisinin biosynthesis is catalyzed by the sesquiTPS, ADS, which converts FPP to amorpho-4,11-diene (Bouwmeester et al., 1999; Mercke et al., 2000). CYP71AV1 then performs a hydroxylation of amorpho-4,11-diene to artemisinic alcohol. Alcohol dehydrogenase 1 (ADH1) converts artemisinic alcohol to artemisinic aldehyde. From here a reduction of artemisinic aldehyde to dihydroartemisinic aldehyde is catalyzed by

artemisinic aldehyde D11(13) reductase (DRB2). Aldehyde dehydrogenase 1 (ALDH1) is the final enzyme involved and forms dihydroartemisinic acid. The lactone ring is then expected to form spontaneously by photo-oxidation-based reactions. Further characterization of enzymes including in planta studies conversely showed additional complexity. Several side-branches are found with intermediates being converted away from the artemisinin path. These side-branches include the intermediate dihydroartemisinic aldehyde being converted to dihydroartemisinic alcohol by dihydroartemisinic aldehyde reductase (RED1; Ryden et al., 2010). Another branch is the conversion of artemisinic aldehyde to artemisinic acid by aldehyde dehydrogenase 1 (ALDH1) from which arteannuin A is formed.

Gossypol, hemigossypolone and heliocides are cadinene-type sesquiterpene aldehydes found in *G. hirsutum*. Significant progress has been made in the discovery of the enzymes responsible for the central backbone, though the final steps to gossypol are still awaiting discovery (Figure 5). The initial biosynthetic step is performed by a (+)-d-cadinene TPS (CDN) followed by oxidation by CYP706B1 to 7-hydroxy-(+)-d-cadinene. An alcohol dehydrogenase, DH1, converts 7-hydroxy-(+)-d-cadinene to 7-keto-d-cadinene. Two P450s, CYP82D113 and CYP71BE7, yield single oxidations generating 8-hydroxy-7-keto-d-cadinene and 8,11-dihydroxy-7-keto-d-cadinene, respectively. While there are several steps missing mid-pathway, virus-induced gene silencing led to identification of an additional step further downstream, namely a 2-oxoglutarate/Fe(II)-dependent dioxygenase (2-OGD-1), which converted furocalamen-2-one to a new compound, 3-hydroxy-furocalamen-2-one (Tian et al., 2018). Additionally, DH1 was found to be promiscuous and uses 8-hydroxy-7-keto-d-cadinene and 8,11-dihydroxy-7-keto-d-cadinene as substrates.

As seen for other classes of terpenoids, various sesquiterpenoids have also been found as glycosides. One example is *Dendrobium nobile* where eight guaiane and eudesmane type sesquiterpene glycosides have been isolated to date (Tan et al., 2023; Zhao et al., 2001). The sesquiterpenoid field has not progressed as far as the diterpenoid field when it comes to elucidating long and complex pathways, especially when it comes to acetylated and glycosylated sesquiterpenoids. Recently, however, a breakthrough was made in tea plant (*Camellia sinensis* (L.) O. Kuntze). During cold stress in tea plants, the linear and hydroxylated sesquiterpenoid nerolidol accumulated in a glycosylated form and is thought to play a role in cold adaptation. A glucosyltransferase, UGT91Q2, found to be induced by cold stress was indeed shown to produce nerolidol glucoside (Zhao, Zhang, et al., 2020).

Monoterpenoids

The majority of known monoterpenoids are derived from geranyl diphosphate (GPP), produced from one IPP and one DMAPP by geranyl diphosphate synthase (GPPS) in

the plastids. In tomato (*Solanum lycopersicum*), a neryl diphosphate synthase (NDPS1) was found to produce neryl diphosphate (NPP; Akhtar et al., 2013). Monoterpenoids derived from two DMAPP units have also been described, and constitute a small subclass known as irregular monoterpenoids (Epstein & Poulter, 1973). As seen for other classes of terpenoids, monoterpenoids are also present with different types of backbones such as acyclic, monocyclic and bicyclic structures (Degenhardt et al., 2009). For regular monoterpenoids, the first committed step is performed by a monoterpene synthase (monoTPS). As seen for many sesquiTPSs, it is not uncommon for monoTPSs to produce multiple products. Vattekkatte et al. (2018) lists several examples of multi-product monoTPSs, but a few well-known examples include a 1,8-cineole synthase from *Nicotiana suaveolens* that produces six out of the eight flower volatiles found in the plant (Roeder et al., 2007) and a limonene synthase from lavender (*Lavandula angustifolia*) that produces six monoterpenes also found in flowers (Landmann et al., 2007).

The oxidative metabolic networks giving rise to a vast diversity of monoterpenes of biotechnological, commercial and agricultural relevance have been reviewed for menthol (*Mentha piperita*), linalool derivatives (*A. thaliana*) and iridoid precursor geraniol (*Catharanthus roseus*, *A. thaliana*; Banerjee & Hamberger, 2018; Ilc et al., 2016). For an update and elaboration on the vast network of monoterpene functionalization in glandular trichomes of members of the Lamiaceae, we refer the reader to Lange & Srividy (2019). In the following, we will focus on advances in network discovery in this family, which is exceptionally rich and chemically diverse in monoterpene metabolites

(Lange, 2015). A few examples of remarkable monoterpene biosynthesis in other families are also highlighted. Increasingly available high-quality transcriptomes have in recent years fueled the discovery and functional annotation of genes that span networks in monoterpene metabolism in the mints (Mint Evolutionary Genomics Consortium, 2018).

Specifically in thyme, three distinct chemotypes were explored to identify the routes to thymol, carvacrol and the further oxidized thymoquinone (Krause et al., 2021). Like the pathway to the diterpene resin acids and the macrocyclic jolkinol C described above, thyme provides an example of a route involving unstable intermediates. In thyme, the activity of seven members of the CYP71 subfamily were shown to yield specifically two diene intermediates from gamma-terpinene, which can spontaneously dehydrate to the aromatic p-cymene. However, when combined with a short-chain dehydrogenase, two allylic ketone intermediates were detected. These can isomerize via keto-enol tautomerism into the aromatic alcohols thymol and carvacrol. Two P450s of the CYP76S and CYP736A families then can intercept and oxidize these products to the shared product thymohydroquinone. It is currently unclear whether the final oxidation to thymoquinone occurs enzymatically, or is spontaneous (Figure 6; adapted from Krause et al., 2021). In-depth analysis of the recently published genome of Chinese native thyme *Thymus quinque-costatus* genome (Sun et al., 2022) may provide further insights into the evolution of these pathways. Furthermore, the reported presence of 10 and structural elucidation of seven monoterpene glycosides indicates that the networks are more expansive than just the routes to the oxidized

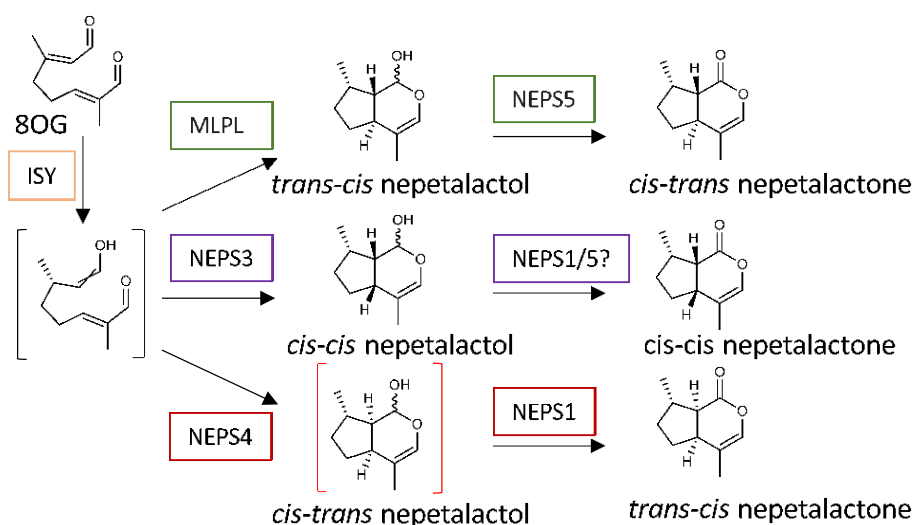


Figure 6. Nepetalactone biosynthesis in *Nepeta* spp.

Undefined stereochemistry is depicted by a crossed double-bond. 8OG, 8-oxogeraniol; ISY, iridoid synthase; NEP, nepetalactol-related short-chain reductase/dehydrogenase.

scaffolds, and with yet to be discovered enzymes plausibly of the UGT family (Kitajima et al., 2004).

Two illustrative examples of pathway discovery using chromosome-scale genome assemblies are found in nepetalactone catnip (*Nepeta* spp., Lichman et al., 2020) and *Mentha longifolia* L. (Vining et al., 2022). To investigate the repeated evolution of the unusual volatile nepetalactone iridoid-type monoterpenes, Lichman et al. combined a comparative genomic approach, with phylogenetic analysis and enzyme characterization. This specific example highlights that complex networks can arise through enzymatic activities in the pathways beyond TPSs and P450s. Specifically, the biosynthesis of nepetalactones involves dephosphorylation of geranyl diphosphate by geraniol synthase, hydroxylation by geraniol-8-hydroxylase and oxidation to the ketone 8-oxogeraniol. This linear route was then suggested to be expanded by action of iridoid synthase yielding nepetalactol a decade ago (Geu-Flores et al., 2012). The pathway was refined by the demonstration that short-chain reductases/dehydrogenases are required for stereoselective formation of nepetalactone in either cis-trans or cis-cis configuration by nepetalactone synthase 1 (NEPS1) and cis-cis-nepetalactol (NEPS3) in *Nepeta* spp. (catmint; Lichman et al., 2019). Intriguingly, the genomes of two *Nepeta* species revealed the presence of BGCs, which together with co-expression analysis finally clarified the routes to three of the four possible stereoisomers of nepetalactone. The authors reported specific sequences including four NEPS and a major latex protein-like (MLPL) enzyme spanning the metabolic network (Figure 7; adapted from Lichman et al., 2020).

Like thyme, *M. longifolia* accessions were reported to have highly diverse chemotypes, which can open the door to the discovery of enzymes contributing to characteristic

profiles. Even though an earlier draft genome of *M. longifolia* existed (Vining et al., 2017), the chromosome scale reference genome published in 2022 impressively set the stage to identify and characterize the genetic underpinning for the monoterpene composition (Vining et al., 2022). Integration of transcriptomic data, specifically from the glandular trichomes, and putative orthology to the large number of functionally characterized homologs in other mint species (i.e. peppermint) involved in the pathway to (-)-mentone was used to mine the genome.

This approach identified some, but not all, menthone biosynthetic candidate genes including those encoding the key TPS for limonene (LS), the P450 catalyzing 3-hydroxylation of limonene (L3H), isopiperitenone dehydrogenase (ISPD), isopiperitenone reductase (ISPR) and pulegone reductase (PulR). The latter three were experimentally validated as recombinant enzymes. It is unclear, however, why the chemotype of the sequenced accession accumulates predominantly pulegone and only traces of menthone (79% and 0.06%, respectively), despite high expression and apparent functionality of the PulR. Yet, with detection of metabolites of the non-menthone, piperitenone-type, which originate from limonene as well, mint monoterpenes follow suit to the examples above and are formed through complex metabolic networks. It is noteworthy that the genomic mapping of the candidate genes indicated the presence of at least one cluster of non-homologous genes of monoterpene metabolism (LS, L3H; Vining et al., 2022).

In Brassicaceae, *A. thaliana* is a good example of a species with an active monoterpenoid network, specifically linalool metabolism. This has been extensively reviewed previously and therefore only a few enzymes are highlighted here. Two monoTPSs, TPS10 and TPS14, were

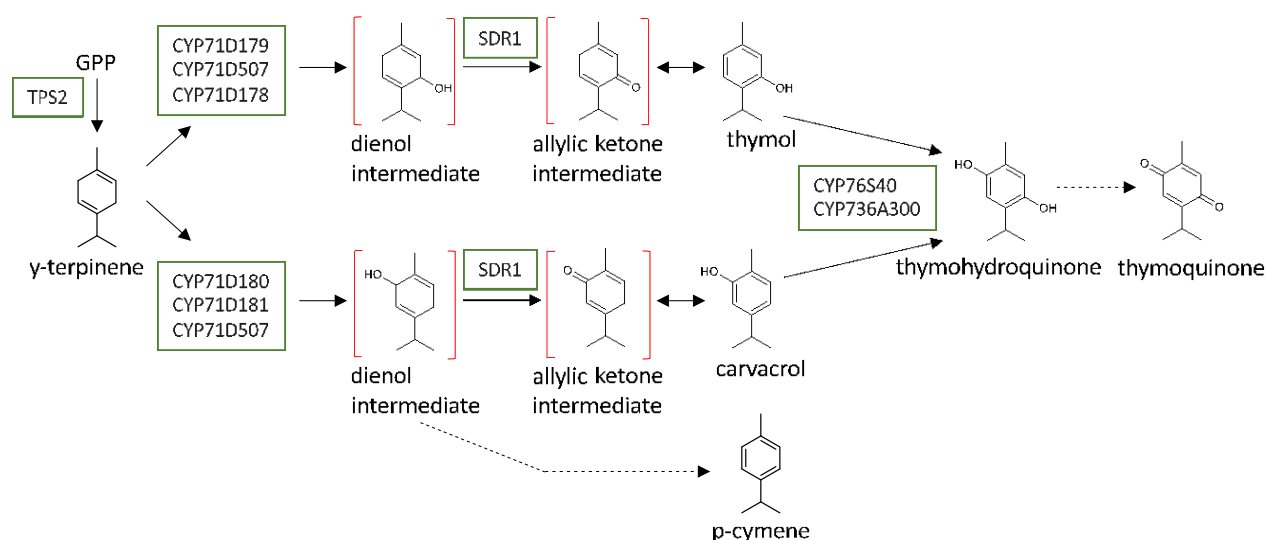


Figure 7. Biosynthetic pathways of thymol, carvacrol, p-cymene and thymohydroquinone in thyme and oregano. SDR, short-chain dehydrogenase/reductase.

characterized as (R)- and (S)-linalool synthases, respectively (Ginglinger et al., 2013). *A. thaliana* harbors several P450s from both the CYP71 and CYP76 families that can hydroxylate linalool. These include CYP76C3 and CYP71B31 that produce multiple linalool derivatives from (3S) and (3R) linalool, however not in equivalent quantities (Ginglinger et al., 2013). Additional CYP76s were found to also participate in linalool oxidation, with CYP76C1 recognized to be the enzyme mainly responsible for linalool oxidation in flowers (Boachon et al., 2015; Höfer et al., 2014).

Grapevine (*Vitis vinifera*) from the Vitaceae family is another producer of linalool with several TPSs responsible for this (Martin et al., 2010). Recently CYP76F14 was found to oxidize linalool in several steps to (E)-8-carboxylinalool (Ilc et al., 2017). (E)-8-Carboxylinalool is present as glucose ester in grape berries, with expected involvement of a UGT in the biosynthesis. Of further importance to the wine industry is that linalool is a precursor of the wine odorant wine lactone.

Pyrethrins are an important and unique type of metabolites that are valued for their anti-insecticidal activity, and are found in several species in Asteraceae including *Tanacetum cinerariifolium*. The various pyrethrin structures consist of two parts derived from two different pathways, an acid moiety from an irregular monoterpene pathway and an alcohol moiety derived from jasmonic acid. The monoterpene acid moiety is either pyrethric acid or chrysanthemic acid, and several different metabolites of the class of pyrethrin have been described (Matsuda, 2012). The pathway to pyrethric acid was recently discovered and includes four enzymes. The initial enzyme, TcCDS, involved in the monoterpene branch was discovered in 2001 and found to utilize two DMAPP to produce chrysanthemyl diphosphate (Rivera et al., 2001). Later additional studies showed that TcCDS could also form chrysanthemol, which is the actual precursor of pyrethrins (Yang et al., 2014). The following steps up until pyrethric acid were only discovered recently by Xu et al. (2019). Chrysanthemol is converted to chrysanthemic acid by alcohol dehydrogenase (2 TcADH2), and aldehyde dehydrogenase (1 TcALDH1; Xu et al., 2018). CYP71BZ is a chrysanthemol 10-hydroxylase (TcCHH) that oxidizes C10 of chrysanthemol to form a carboxylic group thereby producing 10-carboxychrysanthemic acid. The final step to pyrethric acid is performed by 10-carboxychrysanthemic acid 10-methyltransferase (TcCCMT). A GDGLIP (TcGLIP) is responsible for linking the acid moiety to the alcohol moiety (Kikuta et al., 2012).

Mono- and sesquiterpenoids – blurred lines

One of the most promiscuous plant P450s discovered so far, CYP706A3, has been found in *A. thaliana* (Boachon et al., 2019). CYP706A3 is a key player in the sesquiterpene metabolic network but can also oxidize

monoterpenoids. CYP706A3 was found to cluster with TPS11 on the *A. thaliana* genome. TPS11 is a sesquiterpene multiproduct enzyme, with (+)- α -barbatene and (+)-thujopsene among the major products (Tholl et al., 2005). The substrates of CYP706A3 are hydrocarbons and the hydroxylation performed on these is a key factor in switching the affected classes from volatiles released to retained and stored in flowers plausibly due to increased polarity. Interestingly, insect larvae were observed to avoid feeding on flowers expressing CYP706A3. As seen before with promiscuous P450s, CYP706A3 did not oxidize all TPS11 products with the same efficiency, but seemed to favor the ‘bulky multi-cyclic sesquiterpenoids’. The promiscuity of CYP706A3 was further supported by not only single oxidations on multiple substrates, but also successive oxidations of the same substrates. Despite the clustering with a sesquiterpene producing TPS, additional screening in microsome assays with pure standards of several monoterpenoids, α -pinene, (+)-sabinene, β -pinene and α -phellandrene, corresponding to the product of *A. thaliana* TPS24, also showed oxidation of these.

CONCLUSION

The evidence for complex networks in the pathways presented here demonstrates how plants often rely on a small set of enzymes to generate distinct, yet variegated, chemical profiles. Similar strategies are applicable beyond terpenoids and are often observed in other specialized metabolite families. In particular, the reliance on promiscuous P450s and other modifying and conjugating enzymes is common to the biosynthesis of alkaloids, flavonoids and glucosinolates. In our survey of terpenoid biosynthesis, it is apparent that within a particular plant lineage, a single or few terpene backbones can give rise to the majority of terpenoid diversity. Within the diterpenoids, most of this diversity is generated through the activity of modifying enzymes such as P450s and other oxidoreductases as well as glycosyl and acyl transferases. In particular, the tendency of these enzymes to act on multiple substrates and thus create multiple network edges exponentially diversifies the product profile. Some multiproduct dTPSs exist, but they tend to be the exception, whereas volatile mono- and sesquiterpene pathways appear to rely largely on diversification through TPSs (both by multi-product single enzymes and large gene families), consistent with biological functions based on volatility. In the cases where mono- and sesquiterpenes are heavily modified, they reflect diterpenoid pathways in that one backbone is used to generate a class of related compounds.

The human bias in approaching specialized metabolism is to seek a linear pathway leading to single compounds of interest. Though this is the case in limited plant species where a single or few compounds predominate, there are typically many related compounds present in

various quantities. Moreover, 'intermediate' compounds of some pathways can accumulate to detectable levels and may also be bioactive. The terpenoid pathways presented here support the concept that plants benefit from a network of specialized metabolism that generates a diverse array of bioactive compounds, enabling defense against a variety of environmental threats. Those studied now are simply a snapshot of a dynamic evolutionary process that changes along with constantly shifting selective pressures. Moreover, although plants rely on a repertoire of both redundant and promiscuous enzymes for these networks, they have also developed extensive regulatory mechanisms to limit interactions and control product outcomes. Integrating study of the native host metabolism through precise transcriptomics, proteomics and metabolomics approaches along with biochemical enzyme characterization will improve our understanding of how plants control such complex network interactions. As we continue to learn how plants accomplish sophisticated biosynthesis of specialized metabolites, this knowledge will also serve as a lesson for biotechnological control of plant enzymes when engineering production of a single desired compound.

AUTHOR CONTRIBUTIONS

ERL, TBA and BH conceived and wrote the manuscript. All authors contributed to revisions.

ACKNOWLEDGEMENTS

This work was supported by the Michigan State University Strategic Partnership Grant program ('Evolutionary-Driven Genome Mining of Plant Biosynthetic Pathways') to BH through Georgia Research Alliance funds to C. Robin Buell. BH gratefully acknowledges the US Department of Energy Great Lakes Bioenergy Research Center Cooperative Agreement DE-SC0018409, startup funding from the Department of Biochemistry and Molecular Biology, and support from AgBioResearch (MCL02454). ERL is supported by the NSF Graduate Research Fellowship Program (DGE-1848739). BH is in part supported by the National Science Foundation under Grant Number 1737898. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation. Michigan State University occupies the ancestral, traditional and contemporary Lands of the Anishinaabeg—Three Fires Confederacy of Ojibwe, Odawa and Potawatomi peoples. The University resides on Land ceded in the 1819 Treaty of Saginaw.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

- Abad, M.J., Bermejo, P. & Villar, A. (1995) An approach to the genus *Tanacetum* L. (Compositae): phytochemical and pharmacological review. *Phytotherapy Research*, 9, 79–92. Available from: <https://doi.org/10.1002/ptr.2650090202>
- Aharoni, A., Giri, A.P., Deuerlein, S., Griepink, F., de Kogel, W.-J., Verstappen, F.W.A. et al. (2003) Terpenoid metabolism in wild-type and transgenic *Arabidopsis* plants. *Plant Cell*, 15, 2866–2884. Available from: <https://doi.org/10.1105/tpc.016253>
- Akhtar, T.A., Matsuba, Y., Schauvinhold, I., Yu, G., Lees, H.A., Klein, S.E. et al. (2013) The tomato cis-prenyltransferase gene family. *The Plant Journal*, 73, 640–652. Available from: <https://doi.org/10.1111/tpj.12063>
- Andersen, T.B., Martinez-Swatson, K.A., Rasmussen, S.A., Boughton, B.A., Jørgensen, K., Andersen-Ranberg, J. et al. (2017) Localization and in-vivo characterization of *Thapsia garganica* CYP76AE2 indicates a role in thapsigargin biosynthesis. *Plant Physiology*, 174, 56–72. Available from: <https://doi.org/10.1104/pp.16.00055>
- Andersen-Ranberg, J., Kongstad, K.T., Nielsen, M.T., Jensen, N.B., Pateraki, I., Bach, S.S. et al. (2016) Expanding the landscape of diterpene structural diversity through stereochemically controlled combinatorial biosynthesis. *Angewandte Chemie, International Edition*, 55, 2142–2146. Available from: <https://doi.org/10.1002/anie.201510650>
- Aubourg, S., Lecharny, A. & Bohlmann, J. (2002) Genomic analysis of the terpenoid synthase (AtTPS) gene family of *Arabidopsis thaliana*. *Molecular Genetics and Genomics*, 267, 730–745. Available from: <https://doi.org/10.1007/s00438-002-0709-y>
- Aubourg, S., Takvorian, A., Cheron, A., Kreis, M. & Lecharny, A. (1997) Structure, organization and putative function of the genes identified within a 23.9-kb fragment from *Arabidopsis thaliana* chromosome IV. *Gene*, 199, 241–253. Available from: [https://doi.org/10.1016/S0378-1119\(97\)00374-0](https://doi.org/10.1016/S0378-1119(97)00374-0)
- Banerjee, A. & Hamberger, B. (2018) P450s controlling metabolic bifurcations in plant terpene specialized metabolism. *Phytochemistry Reviews*, 17, 81–111. Available from: <https://doi.org/10.1007/s11101-017-9530-4>
- Bathe, U. & Tissier, A. (2019) Cytochrome P450 enzymes: a driving force of plant diterpene diversity. *Phytochemistry*, 161, 149–162. Available from: <https://doi.org/10.1016/j.phytochem.2018.12.003>
- Battaile, J., Dunning, R.L. & Loomis, W.D. (1961) Biosynthesis of terpenes. I. Chromatography of peppermint oil terpenes. *Biochimica et Biophysica Acta*, 51, 538–544. Available from: [https://doi.org/10.1016/0006-3002\(61\)90611-4](https://doi.org/10.1016/0006-3002(61)90611-4)
- Birtic, S., Dussort, P., Pierre, F.-X., Bily, A.C. & Roller, M. (2015) Carnosic acid. *Phytochemistry*, 115, 9–19. Available from: <https://doi.org/10.1016/j.phytochem.2014.12.026>
- Boachon, B., Burdloff, Y., Ruan, J.-X., Rojo, R., Junker, R.R., Vincent, B. et al. (2019) A promiscuous CYP706A3 reduces terpene volatile emission from *Arabidopsis* flowers, affecting florivores and the floral microbiome. *Plant Cell*, 31, 2947–2972. Available from: <https://doi.org/10.1105/tpc.19.00320>
- Boachon, B., Junker, R.R., Miesch, L., Bassard, J.-E., Höfer, R., Caillieau-deaux, R. et al. (2015) CYP76C1 (cytochrome P450)-mediated linalool metabolism and the formation of volatile and soluble linalool oxides in *Arabidopsis* flowers: a strategy for defense against floral antagonists. *Plant Cell*, 27, 2972–2990. Available from: <https://doi.org/10.1105/tpc.15.00399>
- Bohlmann, J., Martin, D., Oldham, N.J. & Gershenzon, J. (2000) Terpenoid secondary metabolism in *Arabidopsis thaliana*: cDNA cloning, characterization, and functional expression of a myrcene/(E)-beta-ocimene synthase. *Archives of Biochemistry and Biophysics*, 375, 261–269. Available from: <https://doi.org/10.1006/abbi.1999.1669>
- Bohlmann, J., Meyer-Gauen, G. & Croteau, R. (1998) Plant terpenoid synthases: molecular biology and phylogenetic analysis. *Proceedings of the National Academy of Sciences of the United States of America*, 95, 4126–4133. Available from: <https://doi.org/10.1073/pnas.95.8.4126>
- Boutanaev, A.M., Moses, T., Zi, J., Nelson, D.R., Mugford, S.T., Peters, R.J. et al. (2015) Investigation of terpene diversification across multiple sequenced plant genomes. *Proceedings of the National Academy of Sciences of the United States of America*, 112, E81–E88. Available from: <https://doi.org/10.1073/pnas.1419547112>
- Bouwmeester, H.J., Wallaart, T.E., Janssen, M.H.A., van Loo, B., Jansen, B.J.M., Posthumus, M.A. et al. (1999) Amorpha-4,11-diene synthase catalyses the first probable step in artemisinin biosynthesis. *Phytochemistry*, 52, 843–854. Available from: [https://doi.org/10.1016/S0031-9422\(99\)00206-X](https://doi.org/10.1016/S0031-9422(99)00206-X)
- Bredahl, E.K., Kjaerulff, L., Ndi, C., Semple, S., Buirchell, B., Møller, B.L. et al. (2022) Isolation and structure elucidation of caryophyllane sesquiterpenoids from leaves of *Eremophila spatulata*. *Phytochemistry Letters*, 47, 156–163. Available from: <https://doi.org/10.1016/j.phytol.2021.12.010>
- Celedon, J.M., Chiang, A., Yuen, M.M.S., Diaz-Chavez, M.L., Madilao, L.L., Finnegan, P.M. et al. (2016) Heartwood-specific transcriptome and

© 2023 The Authors.

The Plant Journal published by Society for Experimental Biology and John Wiley & Sons Ltd.,

The Plant Journal, (2023), 114, 1178–1201

- metabolite signatures of tropical sandalwood (*Santalum album*) reveal the final step of (Z)-santalol fragrance biosynthesis. *The Plant Journal: For Cell and Molecular Biology*, 86, 289–299. Available from: <https://doi.org/10.1111/tpj.13162>
- Ceunen, S. & Geuns, J.M.C. (2013) Steviol glycosides: chemical diversity, metabolism, and function. *Journal of Natural Products*, 76, 1201–1228. Available from: <https://doi.org/10.1021/np400203b>
- Chadwick, M., Trewin, H., Gawthrop, F. & Wagstaff, C. (2013) Sesquiterpenoids lactones: benefits to plants and people. *International Journal of Molecular Sciences*, 14, 12780–12805. Available from: <https://doi.org/10.3390/ijms140612780>
- Chen, F., Tholl, D., Bohlmann, J. & Pichersky, E. (2011) The family of terpene synthases in plants: a mid-size family of genes for specialized metabolism that is highly diversified throughout the kingdom. *The Plant Journal*, 66, 212–229. Available from: <https://doi.org/10.1111/j.1365-3113.2011.04520.x>
- Chen, F., Tholl, D., D'Auria, J.C., Farooq, A., Pichersky, E. & Gershenzon, J. (2003) Biosynthesis and emission of terpenoid volatiles from *Arabidopsis* flowers. *Plant Cell*, 15, 481–494. Available from: <https://doi.org/10.1105/tpc.007989>
- Croteau, R., Burbott, A.J. & Loomis, W.D. (1973) Enzymatic cyclization of neryl pyrophosphate to -terpineol by cell-free extracts from peppermint. *Biochemical and Biophysical Research Communications*, 50, 1006–1012. Available from: [https://doi.org/10.1016/0006-291x\(73\)91506-4](https://doi.org/10.1016/0006-291x(73)91506-4)
- Croteau, R. & Karp, F. (1977) Biosynthesis of monoterpenes: partial purification and characterization of 1,8-cineole synthetase from *Salvia officinalis*. *Archives of Biochemistry and Biophysics*, 179, 257–265. Available from: [https://doi.org/10.1016/0003-9861\(77\)90110-2](https://doi.org/10.1016/0003-9861(77)90110-2)
- Crowley, M.P., Godin, P.J., Inglis, H.S., Snarey, M. & Thain, E.M. (1962) The biosynthesis of the "pyrethrins". I. The incorporation of ^{14}C -labelled compounds into the flowers of *Chrysanthemum cinerariaefolium* and the biosynthesis of chrysanthemum monocarboxylic acid. *Biochimica et Biophysica Acta*, 60, 312–319. Available from: [https://doi.org/10.1016/0006-3002\(62\)90406-7](https://doi.org/10.1016/0006-3002(62)90406-7)
- Degenhardt, J., Köllner, T.G. & Gershenzon, J. (2009) Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. *Phytochemistry, Evolution of Metabolic Diversity*, 70, 1621–1637. Available from: <https://doi.org/10.1016/j.phytochem.2009.07.030>
- Demissie, Z.A., Erland, L.A.E., Rheault, M.R. & Mahmoud, S.S. (2013) The biosynthetic origin of irregular monoterpenes in *Lavandula*. *The Journal of Biological Chemistry*, 288, 6333–6341. Available from: <https://doi.org/10.1074/jbc.M112.431171>
- Dennis, D.T. & West, C.A. (1967) Biosynthesis of gibberellins. 3. The conversion of (J)-kaurene to (J)-kauren-19-oic acid in endosperm of *Echinocystis macrocarpa* Greene. *The Journal of Biological Chemistry*, 242, 3293–3300. Available from: [https://doi.org/10.1016/S0021-9258\(18\)95909-0](https://doi.org/10.1016/S0021-9258(18)95909-0)
- Diaz-Chavez, M.L., Moniodis, J., Madilao, L.L., Jancsik, S., Keeling, C.I., Barbours, E.L. et al. (2013) Biosynthesis of sandalwood oil: *Santalum album* CYP76F cytochromes P450 produce santalols and bergamotol. *PLoS One*, 8, e75053. Available from: <https://doi.org/10.1371/journal.pone.0075053>
- Drew, D.P., Andersen, T.B., Sweetman, C., Möller, B.L., Ford, C. & Simonsen, H.T. (2016) Two key polymorphisms in a newly discovered allele of the *Vitis vinifera* TPS24 gene are responsible for the production of the rotundone precursor α -guaiane. *Journal of Experimental Botany*, 67, 799–808. Available from: <https://doi.org/10.1093/jxb/erv491>
- Drew, D.P., Krichau, N., Reichwald, K. & Simonsen, H.T. (2009) Guaianolides in apiaceae: perspectives on pharmacology and biosynthesis. *Phytochemistry Reviews*, 8, 581–599. Available from: <https://doi.org/10.1007/s11101-009-9130-z>
- Dudareva, N., Klempien, A., Muhlemann, J.K. & Kaplan, I. (2013) Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *The New Phytologist*, 198, 16–32. Available from: <https://doi.org/10.1111/nph.12145>
- Durairaj, J., Di Girolamo, A., Bouwmeester, H.J., de Ridder, D., Beekwilder, J. & van Dijk, A.D.J. (2019) An analysis of characterized plant sesquiterpene synthases. *Phytochemistry*, 158, 157–165. Available from: <https://doi.org/10.1016/j.phytochem.2018.10.020>
- Durairaj, J., Melillo, E., Bouwmeester, H.J., Beekwilder, J., de Ridder, D. & van Dijk, A.D.J. (2021) Integrating structure-based machine learning and co-evolution to investigate specificity in plant sesquiterpene synthases. *PLoS Computational Biology*, 17, e1008197. Available from: <https://doi.org/10.1371/journal.pcbi.1008197>
- Ehlting, J., Sauveplane, V., Olry, A., Ginglinger, J.-F., Provart, N.J. & Werck-Reichhart, D. (2008) An extensive (co-)expression analysis tool for the cytochrome P450 superfamily in *Arabidopsis thaliana*. *BMC Plant Biology*, 8, 47. Available from: <https://doi.org/10.1186/1471-2229-8-47>
- Epstein, W.W. & Poulter, C.D. (1973) A survey of some irregular monoterpenes and their biogenetic analogies to presqualene alcohol. *Phytochemistry*, 12, 737–747. Available from: [https://doi.org/10.1016/0031-9422\(73\)80670-3](https://doi.org/10.1016/0031-9422(73)80670-3)
- Erb, M. & Kliebenstein, D.J. (2020) Plant secondary metabolites as defenses, regulators, and primary metabolites: the blurred functional trichotomy. *Plant Physiology*, 184, 39–52. Available from: <https://doi.org/10.1104/pp.20.00433>
- Esposito, M., Nothias, L.-F., Nedev, H., Gallard, J.-F., Leyssen, P., Retailleau, P. et al. (2016) *Euphorbia dendroidea* latex as a source of jatrophone esters: isolation, structural analysis, conformational study, and anti-CHIKV activity. *Journal of Natural Products*, 79, 2873–2882. Available from: <https://doi.org/10.1021/acs.jnatprod.6b00644>
- Facchini, P.J. & Chappell, J. (1992) Gene family for an elicitor-induced sesquiterpene cyclase in tobacco. *Proceedings of the National Academy of Sciences of the United States of America*, 89, 11088–11092. Available from: <https://doi.org/10.1073/pnas.89.22.11088>
- Fahn, A. (1988) Secretory tissues in vascular plants. *The New Phytologist*, 108, 229–257. Available from: <https://doi.org/10.1111/j.1469-8137.1988.tb04159.x>
- Fattahian, M., Ghanadian, M., Ali, Z. & Khan, I.A. (2020) Jatrophone and rearranged jatrophone-type diterpenes: biogenesis, structure, isolation, biological activity and SARs (1984–2019). *Phytochemistry Reviews*, 19, 265–336. Available from: <https://doi.org/10.1007/s11101-020-09667-8>
- Field, B. & Osbourn, A.E. (2008) Metabolic diversification-independent assembly of operon-like gene clusters in different plants. *Science*, 320, 543–547. Available from: <https://doi.org/10.1126/science.1154990>
- Forman, V., Callari, R., Folly, C., Heider, H. & Hamberger, B. (2017) Production of putative diterpene carboxylic acid intermediates of triptolide in yeast. *Molecules (Basel, Switzerland)*, 22, 981. Available from: <https://doi.org/10.3390/molecules22060981>
- Gao, W., Hillwig, M.L., Huang, L., Cui, G., Wang, X., Kong, J., Yang, B. & Peters, R.J. (2009) A Functional Genomics Approach to Tanshinone Biosynthesis Provides Stereochemical Insights. *Organic Letters*, 11(22), 5170–5173. <https://doi.org/10.1021/ol902051v>
- Geisler, K., Jensen, N.B., Yuen, M.M.S., Madilao, L. & Bohlmann, J. (2016) Modularity of conifer diterpene resin acid intermediates: P450 enzymes of different CYP720B clades use alternative substrates and converge on the same products. *Plant Physiology*, 171, 152–164. Available from: <https://doi.org/10.1104/pp.16.00180>
- Gericke, O., Hansen, N.L., Pedersen, G.B., Kjaerulf, L., Luo, D., Staerk, D. et al. (2020) Neryl neryl diphosphate is the precursor of serrulatane, viscidane and cembrane-type diterpenoids in *Eremophila* species. *BMC Plant Biology*, 20, 91. Available from: <https://doi.org/10.1186/s12870-020-2293-x>
- Gershenzon, J. & Dudareva, N. (2007) The function of terpene natural products in the natural world. *Nature Chemical Biology*, 3, 408–414. Available from: <https://doi.org/10.1038/nchembio.2007.5>
- Geu-Flores, F., Sherden, N.H., Courdavault, V., Burlat, V., Glenn, W.S., Wu, C. et al. (2012) An alternative route to cyclic terpenes by reductive cyclization in iridoid biosynthesis. *Nature*, 492, 138–142. Available from: <https://doi.org/10.1038/nature11692>
- Ginglinger, J.-F., Boachon, B., Höfer, R., Paetz, C., Köllner, T.G., Miesch, L. et al. (2013) Gene Coexpression analysis reveals complex metabolism of the monoterpene alcohol linalool in *Arabidopsis* flowers. *Plant Cell*, 25, 4640–4657. Available from: <https://doi.org/10.1105/tpc.113.117382>
- Gonzalez, M.A. (2015) Aromatic abietane diterpenoids: their biological activity and synthesis. *Natural Product Reports*, 32, 684–704. Available from: <https://doi.org/10.1039/C4NP00110A>
- Guo, J., Ma, X., Cai, Y., Ma, Y., Zhan, Z., Zhou, Y.J. et al. (2016) Cytochrome P450 promiscuity leads to a bifurcating biosynthetic pathway for tanshinones. *The New Phytologist*, 210, 525–534. Available from: <https://doi.org/10.1111/nph.13790>
- Hamberger, B. & Bak, S. (2013) Plant P450s as versatile drivers for evolution of species-specific chemical diversity. *Philosophical Transactions of the*

- Royal Society B: Biological Sciences, 368, 20120426. Available from: <https://doi.org/10.1098/rstb.2012.0426>
- Hamberger, B., Ehlting, J., Barbazuk, B. & Douglas, C.J. (2006) Chapter four - Comparative genomics of the shikimate pathway in Arabidopsis, Populus trichocarpa and Oryza sativa: shikimate pathway gene family structure and identification of candidates for missing links in phenylalanine biosynthesis. In: Romeo, J.T. (Ed.) Recent advances in phytochemistry, integrative plant biochemistry. Oxford, UK: Elsevier, pp. 85–113. Available from: [https://doi.org/10.1016/S0079-9920\(06\)80038-9](https://doi.org/10.1016/S0079-9920(06)80038-9)
- Hamberger, B. & Hahlbrock, K. (2004) The 4-coumarate:CoA ligase gene family in Arabidopsis thaliana comprises one rare, sinapate-activating and three commonly occurring isoenzymes. Proceedings of the National Academy of Sciences of the United States of America, 101, 2209–2214. Available from: <https://doi.org/10.1073/pnas.0307307101>
- Hamberger, B., Ohnishi, T., Hamberger, B., Seguin, A. & Bohlmann, J. (2011) Evolution of diterpene metabolism: sitka spruce CYP720B4 catalyzes multiple oxidations in resin acid biosynthesis of conifer defense against insects. Plant Physiology, 157, 1677–1695. Available from: <https://doi.org/10.1104/pp.111.185843>
- Hammer, K.A., Carson, C.F. & Riley, T.V. (2003) Antifungal activity of the components of Melaleuca alternifolia (tea tree) oil. Journal of Applied Microbiology, 95, 853–860. Available from: <https://doi.org/10.1046/j.1365-2672.2003.02059.x>
- Hansen, N.L., Heskes, A.M., Hamberger, B., Olsen, C.E., Hallström, B.M., Andersen-Ranberg, J. et al. (2017) The terpene synthase gene family in Tripterygium wilfordii harbors a labdane-type diterpene synthase among the monoterpane synthase TPS-b subfamily. The Plant Journal: For Cell and Molecular Biology, 89, 429–441. Available from: <https://doi.org/10.1111/tpj.13410>
- Hansen, N.L., Kjaerulf, L., Heck, Q.K., Forman, V., Staerk, D., Möller, B.L. et al. (2022) Tripterygium wilfordii cytochrome P450s catalyze the methyl shift and epoxidations in the biosynthesis of triptonide. Nature Communications, 13, 5011. Available from: <https://doi.org/10.1038/s41467-022-32667-5>
- Hartmann, T. (2008) The lost origin of chemical ecology in the late 19th century. Proceedings of the National Academy of Sciences of the United States of America, 105, 4541–4546. Available from: <https://doi.org/10.1073/pnas.0709231105>
- Hefner, J., Rubenstein, S.M., Ketchum, R.E., Gibson, D.M., Williams, R.M. & Croteau, R. (1996) Cytochrome P450-catalyzed hydroxylation of taxa-4(5),11(12)-diene to taxa-4(20),11(12)-dien-5-ol: the first oxygenation step in taxol biosynthesis. Chemistry & Biology, 3, 479–489. Available from: [https://doi.org/10.1016/S1074-5521\(96\)90096-4](https://doi.org/10.1016/S1074-5521(96)90096-4)
- Höfer, R., Boachon, B., Renault, H., Gavira, C., Miesch, L., Iglesias, J. et al. (2014) Dual function of the cytochrome P450 CYP76 family from Arabidopsis thaliana in the metabolism of monoterpenols and phenylurea herbicides. Plant Physiology, 166, 1149–1161. Available from: <https://doi.org/10.1104/pp.114.244814>
- Huang, M., Sanchez-Moreiras, A.M., Abel, C., Sohrabi, R., Lee, S., Gershenzon, J. et al. (2012) The major volatile organic compound emitted from Arabidopsis thaliana flowers, the sesquiterpene (E)- β -caryophyllene, is a defense against a bacterial pathogen. The New Phytologist, 193, 997–1008. Available from: <https://doi.org/10.1111/j.1469-8137.2011.04001.x>
- Humphrey, T.V., Richman, A.S., Menassa, R. & Brandle, J.E. (2006) Spatial organization of four enzymes from Stevia rebaudiana that are involved in steviol glycoside synthesis. Plant Molecular Biology, 61, 47–62. Available from: <https://doi.org/10.1007/s11103-005-5966-9>
- Ignea, C., Athanasakoglou, A., Ioannou, E., Georgantea, P., Trikkas, F.A., Loupassaki, S. et al. (2016) Carnosic acid biosynthesis elucidated by a synthetic biology platform. Proceedings of the National Academy of Sciences of the United States of America, 113, 3681–3686. Available from: <https://doi.org/10.1073/pnas.1523787113>
- Ilc, T., Halter, D., Miesch, L., Lauvoisard, F., Kriegshauser, L., Ilg, A. et al. (2017) A grapevine cytochrome P450 generates the precursor of wine lactone, a key odorant in wine. The New Phytologist, 213, 264–274. Available from: <https://doi.org/10.1111/nph.14139>
- Ilc, T., Parage, C., Boachon, B., Navrot, N. & Werck-Reichhart, D. (2016) Monoterpenol oxidative metabolism: role in plant adaptation and potential applications. Frontiers in Plant Science, 7, 509. Available from: <https://doi.org/10.3389/fpls.2016.00509>
- Inabuy, F.S., Fishedick, J.T., Lange, I., Hartmann, M., Srividya, N., Parrish, A.N. et al. (2017) Biosynthesis of diterpenoids in Tripterygium adventitious root cultures. Plant Physiology, 175, 92–103. Available from: <https://doi.org/10.1104/pp.17.00659>
- Irmisch, S., Möller, A.T., Schmidt, L., Günther, J., Gershenzon, J. & Köllner, T.G. (2015) One amino acid makes the difference: the formation of entkaurene and 16 α -hydroxy-entkaurene by diterpene synthases in poplar. BMC Plant Biology, 15, 262. Available from: <https://doi.org/10.1186/s12870-015-0647-6>
- James, W.H. & Hollinger, M.E. (1954) The utilization of carotene. II. From sweet potatoes by young human adults. The Journal of Nutrition, 54, 65–74. Available from: <https://doi.org/10.1093/jn/54.1.65>
- Jia, M., Mishra, S.K., Tufts, S., Jernigan, R.L. & Peters, R.J. (2019) Combinatorial biosynthesis and the basis for substrate promiscuity in class I diterpene synthases. Metabolic Engineering, 55, 44–58. Available from: <https://doi.org/10.1016/j.ymben.2019.06.008>
- Jia, M. & Peters, R.J. (2016) Extending a single residue switch for abbreviating catalysis in plant entkaurene synthases. Frontiers in Plant Science, 7, 1765. Available from: <https://doi.org/10.3389/fpls.2016.01765>
- Jia, Q., Brown, R., Köllner, T.G., Fu, J., Chen, X., Wong, G.K.-S. et al. (2022) Origin and early evolution of the plant terpene synthase family. Proceedings of the National Academy of Sciences of the United States of America, 119, e2100361119. Available from: <https://doi.org/10.1073/pnas.2100361119>
- Johnson, S.R., Bhat, W.W., Bibik, J., Turmo, A., Hamberger, B., Evolutionary Mint Genomics Consortium et al. (2019) A database-driven approach identifies additional diterpene synthase activities in the mint family (Lamiaceae). The Journal of Biological Chemistry, 294, 1349–1362. Available from: <https://doi.org/10.1074/jbc.RA118.006025>
- Johnson, S.R., Bhat, W.W., Sadre, R., Miller, G.P., Garcia, A.S. & Hamberger, B. (2019) Promiscuous terpene synthases from Prunella vulgaris highlight the importance of substrate and compartment switching in terpene synthase evolution. The New Phytologist, 223, 323–335. Available from: <https://doi.org/10.1111/nph.15778>
- Jones, C.G., Moniodis, J., Zulak, K.G., Scalfidi, A., Plummer, J.A., Ghisalberty, E.L. et al. (2011) Sandalwood fragrance biosynthesis involves sesquiterpene synthases of both the terpene synthase (TPS)-a and TPS-b subfamilies, including santalene synthases. The Journal of Biological Chemistry, 286, 17445–17454. Available from: <https://doi.org/10.1074/jbc.M111.231787>
- Judd, R., Bagley, M.C., Li, M., Zhu, Y., Lei, C., Yuzuak, S. et al. (2019) Artemisinin biosynthesis in non-glandular trichome cells of Artemisia annua. Molecular Plant, 12, 704–714. Available from: <https://doi.org/10.1016/j.molp.2019.02.011>
- Junker, R.R., Gershenzon, J. & Unsicker, S.B. (2011) Floral odor bouquet loses its ant repellent properties after inhibition of terpene biosynthesis. Journal of Chemical Ecology, 37, 1323–1331. Available from: <https://doi.org/10.1007/s10886-011-0043-0>
- Karp, F., Harris, J.L. & Croteau, R. (1987) Metabolism of monoterpenes: demonstration of the hydroxylation of (+)-sabinene to (+)-cis-sabinol by an enzyme preparation from sage (Salvia officinalis) leaves. Archives of Biochemistry and Biophysics, 256, 179–193. Available from: [https://doi.org/10.1016/0003-9861\(87\)90436-x](https://doi.org/10.1016/0003-9861(87)90436-x)
- Karunanithi, P.S. & Zerbe, P. (2019) Terpene synthases as metabolic gatekeepers in the evolution of plant terpenoid chemical diversity. Frontiers in Plant Science, 10, 1166. Available from: <https://doi.org/10.3389/fpls.2019.01166>
- Keeling, C.I., Madilao, L.L., Zerbe, P., Dullat, H.K. & Bohlmann, J. (2011) The primary diterpene synthase products of Picea abies levopimaradiene/abietadiene synthase (PaLAS) are epimers of a thermally unstable diterpenol. The Journal of Biological Chemistry, 286, 21145–21153. Available from: <https://doi.org/10.1074/jbc.M111.245951>
- Kemboi, D., Siwe-Noundou, X., Krause, R.W.M., Langat, M.K. & Tembu, V.J. (2021) Euphorbia diterpenes: an update of isolation, structure, pharmacological activities and structure–activity relationship. Molecules, 26, 5055. Available from: <https://doi.org/10.3390/molecules26165055>
- Ker, D.-S., Chan, K.G., Othman, R., Hassan, M. & Ng, C.L. (2020) Site-directed mutagenesis of β sesquiphellandrene synthase enhances enzyme promiscuity. Phytochemistry, 173, 112286. Available from: <https://doi.org/10.1016/j.phytochem.2020.112286>

- Kikuta, Y., Ueda, H., Takahashi, M., Mitsumori, T., Yamada, G., Sakamori, K. et al. (2012) Identification and characterization of a GDSL lipase-like protein that catalyzes the ester-forming reaction for pyrethrin biosynthesis in *Tanacetum cinerariifolium*—a new target for plant protection. *The Plant Journal*, 71, 183–193. Available from: <https://doi.org/10.1111/j.1365-3113.2012.04980.x>
- King, A.J., Brown, G.D., Gilday, A.D., Forestier, E., Larson, T.R. & Graham, I.A. (2016) A cytochrome P450-mediated intramolecular carbon–carbon ring closure in the biosynthesis of multidrug-resistance-reversing lathyrane diterpenoids. *Chembiochem*, 17, 1593–1597. Available from: <https://doi.org/10.1002/cbic.201600316>
- King, A.J., Brown, G.D., Gilday, A.D., Larson, T.R. & Graham, I.A. (2014) Production of bioactive diterpenoids in the Euphorbiaceae depends on evolutionarily conserved gene clusters. *Plant Cell*, 26, 3286–3298. Available from: <https://doi.org/10.1105/tpc.114.129668>
- Kirby, J., Nishimoto, M., Park, J.G., Withers, S.T., Nowroozi, F., Behrendt, D. et al. (2010) Cloning of casbene and neocembre synthases from Euphorbiaceae plants and expression in *Saccharomyces cerevisiae*. *Phytochemistry*, 71, 1466–1473. Available from: <https://doi.org/10.1016/j.phytochem.2010.06.001>
- Kitajima, J., Ishikawa, T., Urabe, A. & Satoh, M. (2004) Monoterpenoids and their glycosides from the leaf of thyme. *Phytochemistry*, 65, 3279–3287. Available from: <https://doi.org/10.1016/j.phytochem.2004.09.010>
- Kjonaas, R. & Croteau, R. (1983) Demonstration that limonene is the first cyclic intermediate in the biosynthesis of oxygenated p-menthane monoterpenes in *Mentha piperita* and other *Mentha* species. *Archives of Biochemistry and Biophysics*, 220, 79–89. Available from: [https://doi.org/10.1016/0003-9861\(83\)90389-2](https://doi.org/10.1016/0003-9861(83)90389-2)
- Kjonaas, R.B., Venkatchalam, K.V. & Croteau, R. (1985) Metabolism of monoterpenes: oxidation of isopiperitenol to isopiperitenone, and subsequent isomerization to piperitenone by soluble enzyme preparations from peppermint (*Mentha piperita*) leaves. *Archives of Biochemistry and Biophysics*, 238, 49–60. Available from: [https://doi.org/10.1016/0003-9861\(85\)90139-0](https://doi.org/10.1016/0003-9861(85)90139-0)
- Köllner, T.G., Degenhardt, J. & Gershenzon, J. (2020) The product specificities of maize terpene synthases TPS4 and TPS10 are determined both by active site amino acids and residues adjacent to the active site. *Plants*, 9, 552. Available from: <https://doi.org/10.3390/plants9050552>
- Kramer, M. & Tarjan, R. (1959) Studies on carotene metabolism. IV. The biological value of carotene in various plants. *Internationale Zeitschrift für Vitaminforschung*, 30, 49–59.
- Krause, S.T., Liao, P., Crocoll, C., Boachon, B., Förster, C., Leidecker, F. et al. (2021) The biosynthesis of thymol, carvacrol, and thymohydroquinone in Lamiaceae proceeds via cytochrome P450s and a short-chain dehydrogenase. *Proceedings of the National Academy of Sciences of the United States of America*, 118, e2110092118. Available from: <https://doi.org/10.1073/pnas.2110092118>
- Kupchan, S.M., Court, W.A., Dailey, R.G., Gilmore, C.J. & Bryan, R.F. (1972) Triptolide and triptolidolide, novel antileukemic diterpenoid triepoxides from *Tripterygium wilfordii*. *Journal of the American Chemical Society*, 94, 7194–7195. Available from: <https://doi.org/10.1021/ja00775a078>
- Kutney, J.P., Hewitt, G.M., Kurihara, T., Salisbury, P.J., Sindelar, R.D., Stuart, K.L. et al. (1981) Cytotoxic diterpenes triptolide, triptolidolide, and cytotoxic triterpenes from tissue cultures of *Tripterygium wilfordii*. *Canadian Journal of Chemistry*, 59, 2677–2683. Available from: <https://doi.org/10.1139/v81-385>
- Landmann, C., Fink, B., Festner, M., Dregus, M., Engel, K.-H. & Schwab, W. (2007) Cloning and functional characterization of three terpene synthases from lavender (*Lavandula angustifolia*). *Archives of Biochemistry and Biophysics*, 465, 417–429. Available from: <https://doi.org/10.1016/j.abb.2007.06.011>
- Lange, B.M. (2015) The evolution of plant secretory structures and emergence of terpenoid chemical diversity. *Annual Review of Plant Biology*, 66, 139–159. Available from: <https://doi.org/10.1146/annurev-arplant-043014-114639>
- Lange, B.M. & Croteau, R. (1999) Genetic engineering of essential oil production in mint. *Current Opinion in Plant Biology*, 2, 139–144. Available from: [https://doi.org/10.1016/s1369-5266\(99\)80028-4](https://doi.org/10.1016/s1369-5266(99)80028-4)
- Lange, B.M. & Srividya, N. (2019) Enzymology of monoterpene functionalization in glandular trichomes. *Journal of Experimental Botany*, 70, 1095–1108. Available from: <https://doi.org/10.1093/jxb/ery436>
- Leong, B.J. & Last, R.L. (2017) Promiscuity, impersonation and accommodation: evolution of plant specialized metabolism. *Current Opinion in Structural Biology*, 47, 105–112. Available from: <https://doi.org/10.1016/j.sbi.2017.07.005>
- Leroi-Gourhan, A. (1975) The flowers found with Shanidar IV, a Neanderthal burial in Iraq. *Science*, 190, 562–564. Available from: <https://doi.org/10.1126/science.190.4214.562>
- Li, F.-S. & Weng, J.-K. (2017) Demystifying traditional herbal medicine with modern approach. *Nature Plants*, 3, 1–7. Available from: <https://doi.org/10.1038/nplants.2017.109>
- Liang, J., Wang, L., Liu, J., Shen, Q., Fu, J., Peters, R.J. et al. (2020) Probing enzymatic structure and function in the dihydroxylating sesquiterpene synthase ZmEDS. *Biochemistry*, 59, 2660–2666. Available from: <https://doi.org/10.1021/acs.biochem.0c00395>
- Lichman, B.R., Godden, G.T., Hamilton, J.P., Palmer, L., Kamileen, M.O., Zhao, D. et al. (2020) The evolutionary origins of the cat attractant nepetalactone in catnip. *Science Advances*, 6, eaba0721. Available from: <https://doi.org/10.1126/sciadv.aba0721>
- Lichman, B.R., Kamileen, M.O., Titchener, G.R., Saalbach, G., Stevenson, C.E.M., Lawson, D.M. et al. (2019) Uncoupled activation and cyclization in catmint reductive terpenoid biosynthesis. *Nature Chemical Biology*, 15, 71–79. Available from: <https://doi.org/10.1038/s41589-018-0185-2>
- Liu, Q., Beyraghdar Kashkooli, A., Manzano, D., Pateraki, I., Richard, L., Kolkman, P. et al. (2018) Kaunilide synthase is a P450 with unusual hydroxylation and cyclization-elimination activity. *Nature Communications*, 9, 4657. Available from: <https://doi.org/10.1038/s41467-018-06565-8>
- Liu, Q., Manzano, D., Tanic, N., Pesic, M., Bankovic, J., Pateraki, I. et al. (2014) Elucidation and in planta reconstitution of the parthenolide biosynthetic pathway. *Metabolic Engineering*, 23, 145–153. Available from: <https://doi.org/10.1016/j.ymben.2014.03.005>
- Loussouarn, M., Krieger-Liszak, A., Svilar, L., Bily, A., Birtic, S. & Havaux, M. (2017) Carnosic acid and carnosol, two major antioxidants of rosemary, act through different mechanisms. *Plant Physiology*, 175, 1381–1394. Available from: <https://doi.org/10.1104/pp.17.01183>
- Luo, D., Callari, R., Hamburger, B., Wubshet, S.G., Nielsen, M.T., Andersen-Ranberg, J. et al. (2016) Oxidation and cyclization of casbene in the biosynthesis of euphorbia factors from mature seeds of *Euphorbia lathyris* L. *Proceedings of the National Academy of Sciences of the United States of America*, 113, E5082–E5089. Available from: <https://doi.org/10.1073/pnas.1607504113>
- Ma, Y., Cui, G., Chen, T., Ma, X., Wang, R., Jin, B. et al. (2021) Expansion within the CYP71D subfamily drives the heterocyclization of tanshinones synthesis in *Salvia miltiorrhiza*. *Nature Communications*, 12, 685. Available from: <https://doi.org/10.1038/s41467-021-20959-1>
- Mahmoud, S.S. & Croteau, R.B. (2001) Metabolic engineering of essential oil yield and composition in mint by altering expression of deoxyxylulose phosphate reductoisomerase and menthofuran synthase. *Proceedings of the National Academy of Sciences of the United States of America*, 98, 8915–8920. Available from: <https://doi.org/10.1073/pnas.141237298>
- Majidi, M., Liu, Q., Karimzadeh, G., Malboobi, M.A., Beekwilder, J., Cankar, K. et al. (2011) Biosynthesis and localization of parthenolide in glandular trichomes of feverfew (*Tanacetum parthenium* L. Schulz Bip.). *Phytochemistry*, 72, 1739–1750. Available from: <https://doi.org/10.1016/j.phytochem.2011.04.021>
- Mao, H., Liu, J., Ren, F., Peters, R.J. & Wang, Q. (2016) Characterization of CYP71Z18 indicates a role in maize zealexin biosynthesis. *Phytochemistry*, 121, 4–10. Available from: <https://doi.org/10.1016/j.phytochem.2015.10.003>
- Martin, D.M., Aubourg, S., Schouwey, M.B., Daviet, L., Schalk, M., Toub, O. et al. (2010) Functional annotation, genome organization and phylogeny of the grapevine (*Vitis vinifera*) terpene synthase gene family based on genome assembly, FLcDNA cloning, and enzyme assays. *BMC Plant Biology*, 10, 226. Available from: <https://doi.org/10.1186/1471-2229-10-226>
- Mathema, V.B., Koh, Y.-S., Thakuri, B.C. & Sillanpää, M. (2012) Parthenolide, a sesquiterpene lactone, expresses multiple anti-cancer and anti-inflammatory activities. *Inflammation*, 35, 560–565. Available from: <https://doi.org/10.1007/s10753-011-9346-0>
- Matsuba, Y., Zi, J., Jones, A.D., Peters, R.J. & Pichersky, E. (2015) Biosynthesis of the diterpenoid lycosantalonal via neryleryl diphosphate in

- Solanum lycopersicum*. PLoS One, 10, e0119302. Available from: <https://doi.org/10.1371/journal.pone.0119302>
- Matsuda, K. (2012) Pyrethrin biosynthesis and its regulation in *Chrysanthemum cinerariaefolium*. In: Matsuo, N. & Mori, T. (Eds.) *Pyrethroids: from chrysanthemum to modern industrial insecticide, topics in current chemistry*. Berlin, Heidelberg: Springer, pp. 73–81. Available from: https://doi.org/10.1007/128_2011_271
- Mau, C.J. & West, C.A. (1994) Cloning of casbene synthase cDNA: evidence for conserved structural features among terpenoid cyclases in plants. *Proceedings of the National Academy of Sciences of the United States of America*, 91, 8497–8501.
- McCaskill, D. & Croteau, R. (1997) Prospects for the bioengineering of isoprenoid biosynthesis. *Advances in Biochemical Engineering/Biotechnology*, 55, 107–146. Available from: <https://doi.org/10.1007/BF0102064>
- Mercke, P., Bengtsson, M., Bouwmeester, H.J., Posthumus, M.A. & Brodeur, P.E. (2000) Molecular cloning, expression, and characterization of amorpho-4,11-diene synthase, a key enzyme of artemisinin biosynthesis in *Artemisia annua* L. *Archives of Biochemistry and Biophysics*, 381, 173–180. Available from: <https://doi.org/10.1006/abbi.2000.1962>
- Miller, G.P., Bhat, W.W., Lanier, E.R., Johnson, S.R., Mathieu, D.T. & Hamberger, B. (2020) The biosynthesis of the anti-microbial diterpenoid leubethanol in *Leucophyllum frutescens* proceeds via an all-cis prenyl intermediate. *The Plant Journal*, 104, 693–705. Available from: <https://doi.org/10.1111/tpj.14957>
- Mint Evolutionary Genomics Consortium. (2018) Phylogenomic mining of the mints reveals multiple mechanisms contributing to the evolution of chemical diversity in Lamiaceae. *Molecular Plant*, 11, 1084–1096. Available from: <https://doi.org/10.1016/j.molp.2018.06.002>
- Modi, V.V. & Patwa, D.K. (1959) Biosynthesis of carotenes in carrot extracts. *Nature*, 184, 983–984. Available from: <https://doi.org/10.1038/184983b0>
- Muhlemann, J.K., Klempien, A. & Dudareva, N. (2014) Floral volatiles: from biosynthesis to function. *Plant, Cell & Environment*, 37, 1936–1949. Available from: <https://doi.org/10.1111/pce.12314>
- Nguyen, T.-D., MacNevin, G. & Ro, D.-K. (2012) De novo synthesis of high-value plant sesquiterpenoids in yeast. *Methods in Enzymology*, 517, 261–278. Available from: <https://doi.org/10.1016/B978-0-12-404634-4.00013-9>
- Nieuwenhuizen, N.J., Wang, M.Y., Matich, A.J., Green, S.A., Chen, X., Yauk, Y.-K. et al. (2009) Two terpene synthases are responsible for the major sesquiterpenes emitted from the flowers of kiwifruit (*Actinidia deliciosa*). *Journal of Experimental Botany*, 60, 3203–3219. Available from: <https://doi.org/10.1093/jxb/erp162>
- Olsson, K., Carlsen, S., Semmler, A., Simon, E., Mikkelsen, M.D. & Møller, B.L. (2016) Microbial production of next-generation stevia sweeteners. *Microbial Cell Factories*, 15, 207. Available from: <https://doi.org/10.1186/s12934-016-0609-1>
- O'Maille, P.E., Chappell, J. & Noel, J.P. (2006) Biosynthetic potential of sesquiterpene synthases: alternative products of tobacco 5-epi-aristolochene synthase. *Archives of Biochemistry and Biophysics*, 448, 73–82. Available from: <https://doi.org/10.1016/j.abb.2005.10.028>
- Pang, H., Wu, L., Tang, Y., Zhou, G., Qu, C. & Duan, J. (2016) Chemical analysis of the herbal medicine *Salviae miltiorrhizae radix et rhizoma* (Danshen). *Molecules*, 21, 51. Available from: <https://doi.org/10.3390/molecules21010051>
- Pateraki, I., Andersen-Ranberg, J., Hamberger, B., Heskes, A.M., Martens, H.J., Zerbe, P. et al. (2014) Manoyl oxide (13R), the biosynthetic precursor of forskolin, is synthesized in specialized root cork cells in *Coleus forskohlii*. *Plant Physiology*, 164, 1222–1236. Available from: <https://doi.org/10.1104/pp.113.228429>
- Pateraki, I., Andersen-Ranberg, J., Jensen, N.B., Wubshet, S.G., Heskes, A.M., Forman, V. et al. (2017) Total biosynthesis of the cyclic AMP booster forskolin from *Coleus forskohlii*. *eLife*, 6, e23001. Available from: <https://doi.org/10.7554/eLife.23001>
- Pateraki, I., Heskes, A.M. & Hamberger, B. (2015) Cytochromes P450 for terpene functionalisation and metabolic engineering. *Advances in Biochemical Engineering/Biotechnology*, 148, 107–139. Available from: https://doi.org/10.1007/10_2014_301
- Patridge, E., Gareiss, P., Kinch, M.S. & Hoyer, D. (2016) An analysis of FDA-approved drugs: natural products and their derivatives. *Drug Discovery Today*, 21, 204–207. Available from: <https://doi.org/10.1016/j.drudis.2015.01.009>
- Pefuelas, J., Farre-Armengol, G., Llusia, J., Gargallo-Garriga, A., Rico, L., Sardans, J. et al. (2014) Removal of floral microbiota reduces floral terpene emissions. *Scientific Reports*, 4, 6727. Available from: <https://doi.org/10.1038/srep06727>
- Peters, R.J. (2010) Two rings in them all: the labdane-related diterpenoids. *Natural Product Reports*, 27, 1521–1530. Available from: <https://doi.org/10.1039/c0np00019a>
- Pickel, B., Drew, D.P., Manczak, T., Weitzel, C., Simonsen, H.T. & Ro, D.-K. (2012) Identification and characterization of a kunzeal synthase from *Thapsia garganica*: implications for the biosynthesis of the pharmaceutical thapsigargin. *The Biochemical Journal*, 448, 261–271. Available from: <https://doi.org/10.1042/BJ20120654>
- Poulose, A.J. & Croteau, R. (1978) Biosynthesis of aromatic monoterpenes: conversion of gamma-terpinene to p-cymene and thymol in *Thymus vulgaris* L. *Archives of Biochemistry and Biophysics*, 187, 307–314. Available from: [https://doi.org/10.1016/0003-9861\(78\)90039-5](https://doi.org/10.1016/0003-9861(78)90039-5)
- Prakash, I., Markosyan, A. & Bunders, C. (2014) Development of next generation stevia sweetener: Rebaudioside M. *Food*, 3, 162–175. Available from: <https://doi.org/10.3390/foods3010162>
- Rasmann, S., Köllner, T.G., Degenhardt, J., Hiltbold, I., Toepfer, S., Kuhlmann, U. et al. (2005) Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature*, 434, 732–737. Available from: <https://doi.org/10.1038/nature03451>
- Richman, A., Swanson, A., Humphrey, T., Chapman, R., McGarvey, B., Pocs, R. et al. (2005) Functional genomics uncovers three glucosyltransferases involved in the synthesis of the major sweet glucosides of *Stevia rebaudiana*. *The Plant Journal*, 41, 56–67. Available from: <https://doi.org/10.1111/j.1365-3113.2004.02275.x>
- Richman, A.S., Gijzen, M., Starratt, A.N., Yang, Z. & Brandle, J.E. (1999) Diterpene synthesis in *Stevia rebaudiana*: recruitment and up-regulation of key enzymes from the gibberellin biosynthetic pathway. *The Plant Journal*, 19, 411–421. Available from: <https://doi.org/10.1046/j.1365-3113.1999.00531.x>
- Rising, K.A., Starks, C.M., Noel, J.P. & Chappell, J. (2000) Demonstration of Germacrene A as an intermediate in 5-epi-aristolochene synthase catalysis. *Journal of the American Chemical Society*, 122, 1861–1866. Available from: <https://doi.org/10.1021/ja993584h>
- Rivera, S.B., Swedlund, B.D., King, G.J., Bell, R.N., Hussey, C.E., Shattuck-Eidens, D.M. et al. (2001) Chrysanthemyl diphosphate synthase: isolation of the gene and characterization of the recombinant non-head-to-tail monoterpene synthase from *Chrysanthemum cinerariaefolium*. *Proceedings of the National Academy of Sciences of the United States of America*, 98, 4373–4378. Available from: <https://doi.org/10.1073/pnas.071543598>
- Ro, D.-K., Arimura, G.-I., Lau, S.Y.W., Piers, E. & Bohlmann, J. (2005) Loblolly pine abietadienol/abietadienal oxidase PtAO (CYP720B1) is a multifunctional, multisubstrate cytochrome P450 monooxygenase. *Proceedings of the National Academy of Sciences of the United States of America*, 102, 8060–8065. Available from: <https://doi.org/10.1073/pnas.0500825102>
- Robert, C.A.M., Erb, M., Duployer, M., Zwahlen, C., Doyen, G.R. & Turlings, T.C.J. (2012) Herbivore-induced plant volatiles mediate host selection by a root herbivore. *The New Phytologist*, 194, 1061–1069. Available from: <https://doi.org/10.1111/j.1469-8137.2012.04127.x>
- Roeder, S., Hartmann, A.-M., Effmert, U. & Piechulla, B. (2007) Regulation of simultaneous synthesis of floral scent terpenoids by the 1,8-cineole synthase of *Nicotiana suaveolens*. *Plant Molecular Biology*, 65, 107–124. Available from: <https://doi.org/10.1007/s11103-007-9202-7>
- Ryden, A.-M., Ruyter-Spira, C., Quax, W.J., Osada, H., Muranaka, T., Kayser, O. et al. (2010) The molecular cloning of dihydroartemisinic aldehyde reductase and its implication in artemisinin biosynthesis in *Artemisia annua*. *Planta Medica*, 76, 1778–1783. Available from: <https://doi.org/10.1055/s-0030-1249930>
- Salazar, D., Lokvam, J., Mesones, I., Vasquez Pilco, M., Ayarza Zuniga, J.M., de Valpine, P. et al. (2018) Origin and maintenance of chemical diversity in a species-rich tropical tree lineage. *Nature Ecology & Evolution*, 2, 983–990. Available from: <https://doi.org/10.1038/s41559-018-0552-0>
- Sallaud, C., Rontein, D., Onillon, S., Jabes, F., Duffe, P., Giacalone, C. et al. (2009) A novel pathway for sesquiterpene biosynthesis from Z,Z-farnesyl

- pyrophosphate in the wild tomato *Solanum habrochaites*. *Plant Cell*, 21, 301–317. Available from: <https://doi.org/10.1105/tpc.107.057885>
- Scheler, U., Brandt, W., Porzel, A., Rothe, K., Manzano, D., Bozic, D. et al. (2016) Elucidation of the biosynthesis of carnolic acid and its reconstitution in yeast. *Nature Communications*, 7, 12942. Available from: <https://doi.org/10.1038/ncomms12942>
- Shimura, K., Okada, A., Okada, K., Jikumaru, Y., Ko, K.-W., Toyomasu, T. et al. (2007) Identification of a biosynthetic gene cluster in rice for momilactones. *The Journal of Biological Chemistry*, 282, 34013–34018. Available from: <https://doi.org/10.1074/jbc.M703344200>
- Song, J.-J., Fang, X., Li, C.-Y., Jiang, Y., Li, J.-X., Wu, S. et al. (2022) A 2-oxoglutarate-dependent dioxygenase converts dihydrofuran to furan in *Salvia* diterpenoids. *Plant Physiology*, 188, 1496–1506. Available from: <https://doi.org/10.1093/plphys/kiab567>
- Song, Z., Lin, C., Xing, P., Fen, Y., Jin, H., Zhou, C. et al. (2020) A high-quality reference genome sequence of *Salvia miltiorrhiza* provides insights into tanshinone synthesis in its red rhizomes. *Plant Genome*, 13, e20041. Available from: <https://doi.org/10.1002/tpg2.20041>
- Steele, C.L., Crock, J., Bohlmann, J. & Croteau, R. (1998) Sesquiterpene synthases from grand fir (*Abies grandis*). Comparison of constitutive and wound-induced activities, and cDNA isolation, characterization, and bacterial expression of delta-selinene synthase and gamma-humulene synthase. *The Journal of Biological Chemistry*, 273, 2078–2089. Available from: <https://doi.org/10.1074/jbc.273.4.2078>
- Sun, M., Zhang, Y., Zhu, L., Liu, N., Bai, H., Sun, G. et al. (2022) Chromosome-level assembly and analysis of the thymus genome provide insights into glandular secretory trichome formation and monoterpenoid biosynthesis in thyme. *Plant Communications*, 3, 100413. Available from: <https://doi.org/10.1016/j.xplc.2022.100413>
- Swaminathan, S., Morrone, D., Wang, Q., Fulton, D.B. & Peters, R.J. (2009) CYP76M7 is an ent-cassadiene C11alpha-hydroxylase defining a second multifunctional diterpenoid biosynthetic gene cluster in rice. *Plant Cell*, 21, 3315–3325. Available from: <https://doi.org/10.1105/tpc.108.063677>
- Sy, L.-K. & Brown, G.D. (2002) The mechanism of the spontaneous autoxidation of dihydroartemisinin acid. *Tetrahedron*, 58, 897–908. Available from: [https://doi.org/10.1016/S0040-4020\(01\)01193-0](https://doi.org/10.1016/S0040-4020(01)01193-0)
- Takahashi, S., Zhao, Y., O'Maille, P.E., Greenhagen, B.T., Noel, J.P., Coates, R.M. et al. (2005) Kinetic and molecular analysis of 5-epiaristolochene 1,3-dihydroxylase, a cytochrome P450 enzyme catalyzing successive hydroxylations of sesquiterpenes. *The Journal of Biological Chemistry*, 280, 3686–3696. Available from: <https://doi.org/10.1074/jbc.M411870200>
- Tan, D., Song, Y., Wang, J., Gao, C., Qin, L., Zhao, Y. et al. (2023) Identification of sesquiterpene glycosides from *Dendrobium nobile* and their aglycosidase and α -amylase inhibitory activities. *Food Science and Technology*, 43, 99722. Available from: <https://doi.org/10.1590/fst.99722>
- Tantillo, J. (2011) Biosynthesis via carbocations: theoretical studies on terpene formation. *Natural Product Reports*, 28, 1035–1053. Available from: <https://doi.org/10.1039/C1NP00006C>
- Teoh, K.H., Polichuk, D.R., Reed, D.W. & Covello, P.S. (2009) Molecular cloning of an aldehyde dehydrogenase implicated in artemisinin biosynthesis in *Artemisia annua*. *Botany*, 87, 635–642. Available from: <https://doi.org/10.1139/B09-032>
- Teoh, K.H., Polichuk, D.R., Reed, D.W., Nowak, G. & Covello, P.S. (2006) *Artemisia annua* L. (Asteraceae) trichome-specific cDNAs reveal CYP71AV1, a cytochrome P450 with a key role in the biosynthesis of the antimalarial sesquiterpene lactone artemisinin. *FEBS Letters*, 580, 1411–1416. Available from: <https://doi.org/10.1016/j.febslet.2006.01.065>
- Thastrup, O., Cullen, P.J., Dröbak, B.K., Hanley, M.R. & Dawson, A.P. (1990) Thapsigargin, a tumor promoter, discharges intracellular Ca^{2+} stores by specific inhibition of the endoplasmic reticulum Ca^{2+} -ATPase. *Proceedings of the National Academy of Sciences of the United States of America*, 87, 2466–2470. Available from: <https://doi.org/10.1073/pnas.87.7.2466>
- The Arabidopsis Genome Initiative. (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature*, 408, 796–815. Available from: <https://doi.org/10.1038/35048692>
- Theis, N., Barber, N.A., Gillespie, S.D., Hazzard, R.V. & Adler, L.S. (2014) Attracting mutualists and antagonists: plant trait variation explains the distribution of specialist floral herbivores and pollinators on crops and wild gourds. *American Journal of Botany*, 101, 1314–1322. Available from: <https://doi.org/10.3732/ajb.1400171>
- Tholl, D., Chen, F., Petri, J., Gershenzon, J. & Pichersky, E. (2005) Two sesquiterpene synthases are responsible for the complex mixture of sesquiterpenes emitted from *Arabidopsis* flowers. *The Plant Journal*, 42, 757–771. Available from: <https://doi.org/10.1111/j.1365-3113.2005.02417.x>
- Tian, X., Ruan, J.-X., Huang, J.-Q., Yang, C.-Q., Fang, X., Chen, Z.-W. et al. (2018) Characterization of gossypol biosynthetic pathway. *Proceedings of the National Academy of Sciences of the United States of America*, 115, E5410–E5418. Available from: <https://doi.org/10.1073/pnas.1805085115>
- Tissier, A. (2018) Plant secretory structures: more than just reaction bags. *Current Opinion in Biotechnology*, 49, 73–79. Available from: <https://doi.org/10.1016/j.copbio.2017.08.003>
- Tissier, A., Morgan, J.A. & Dudareva, N. (2017) Plant volatiles: going 'in' but not 'out' of trichome cavities. *Trends in Plant Science*, 22, 930–938. Available from: <https://doi.org/10.1016/j.tplants.2017.09.001>
- Trapp, S.C. & Croteau, R.B. (2001) Genomic organization of plant terpene synthases and molecular evolutionary implications. *Genetics*, 158, 811–832. Available from: <https://doi.org/10.1093/genetics/158.2.811>
- Tu, L., Su, P., Zhang, Z., Gao, L., Wang, J., Hu, T. et al. (2020) Genome of *Tripterygium wilfordii* and identification of cytochrome P450 involved in triptolide biosynthesis. *Nature Communications*, 11, 971. Available from: <https://doi.org/10.1038/s41467-020-14776-1>
- Tu, Y. (2011) The discovery of artemisinin (qinghaosu) and gifts from Chinese medicine. *Nature Medicine*, 17, 1217–1230. Available from: <https://doi.org/10.1038/nm.2471>
- Upper, C.D. & West, C.A. (1967) Biosynthesis of gibberellins. II. Enzymic cyclization of geranylgeranyl pyrophosphate to kaurene. *The Journal of Biological Chemistry*, 242, 3285–3292.
- Vattekatte, A., Garms, S., Brandt, W. & Boland, W. (2018) Enhanced structural diversity in terpenoid biosynthesis: enzymes, substrates and cofactors. *Organic & Biomolecular Chemistry*, 16, 348–362. Available from: <https://doi.org/10.1039/c7ob02040f>
- Vining, K.J., Johnson, S.R., Ahkami, A., Lange, I., Parrish, A.N., Trapp, S.C. et al. (2017) Draft genome sequence of *Mentha longifolia* and development of resources for mint cultivar improvement. *Molecular Plant*, 10, 323–339. Available from: <https://doi.org/10.1016/j.molp.2016.10.018>
- Vining, K.J., Pandelova, I., Lange, I., Parrish, A.N., Lefors, A., Kronmiller, B. et al. (2022) Chromosome-level genome assembly of *Mentha longifolia* L. reveals gene organization underlying disease resistance and essential oil traits. *G3: Genes, Genomes, Genetics*, 12, jkac112. Available from: <https://doi.org/10.1093/g3journal/jkac112>
- Vogel, B.S., Wildung, M.R., Vogel, G. & Croteau, R. (1996) Abietadiene synthase from grand fir (*Abies grandis*). cDNA isolation, characterization, and bacterial expression of a bifunctional diterpene cyclase involved in resin acid biosynthesis. *The Journal of Biological Chemistry*, 271, 23262–23268. Available from: <https://doi.org/10.1074/jbc.271.38.23262>
- Volf, M., Segar, S.T., Miller, S.E., Isua, B., Sisol, M., Aubona, G. et al. (2018) Community structure of insect herbivores is driven by conservatism, escalation and divergence of defensive traits in *Ficus*. *Ecology Letters*, 21, 83–92. Available from: <https://doi.org/10.1111/ele.12875>
- Wang, J., Li, S., Xiong, Z. & Wang, Y. (2016) Pathway mining-based integration of critical enzyme parts for de novo biosynthesis of steviolglycosides sweetener in *Escherichia coli*. *Cell Research*, 26, 258–261. Available from: <https://doi.org/10.1038/cr.2015.111>
- Wang, S., Alseekh, S., Fernie, A.R. & Luo, J. (2019) The structure and function of major plant metabolite modifications. *Molecular Plant*, 12, 899–919. Available from: <https://doi.org/10.1016/j.molp.2019.06.001>
- Wang, Z., Nelson, D.R., Zhang, J., Wan, X. & Peters, R.J. (2022) Plant (di)terpenoid evolution: from pigments to hormones and beyond. *Natural Product Reports*, 40, 452–469. Available from: <https://doi.org/10.1039/D2NP00054G>
- Wang, Z. & Peters, R.J. (2022) Tanshinones: leading the way into Lamiaceae labdane-related diterpenoid biosynthesis. *Current Opinion in Plant Biology*, 66, 102189. Available from: <https://doi.org/10.1016/j.pbi.2022.102189>
- Weng, J.-K., Lynch, J.H., Matos, J.O. & Dudareva, N. (2021) Adaptive mechanisms of plant specialized metabolism connecting chemistry to function. *Nature Chemical Biology*, 17, 1037–1045. Available from: <https://doi.org/10.1038/s41589-021-00822-6>
- Weng, J.-K., Philippe, R.N. & Noel, J.P. (2012) The rise of chemodiversity in plants. *Science*, 336, 1667–1670. Available from: <https://doi.org/10.1126/science.1217411>

- Whitehead, S.R., Bass, E., Corrigan, A., Kessler, A. & Poveda, K. (2021) Interaction diversity explains the maintenance of phytochemical diversity. *Ecology Letters*, 24, 1205–1214. Available from: <https://doi.org/10.1111/ele.13736>
- Wilderman, P.R. & Peters, R.J. (2007) A single residue switch converts abietadiene synthase into a pimaradiene specific cyclase. *Journal of the American Chemical Society*, 129, 15736–15737. Available from: <https://doi.org/10.1021/ja074977g>
- Wildung, M.R. & Croteau, R. (1996) A cDNA clone for taxadiene synthase, the diterpene cyclase that catalyzes the committed step of taxol biosynthesis. *The Journal of Biological Chemistry*, 271, 9201–9204. Available from: <https://doi.org/10.1074/jbc.271.16.9201>
- Wisecaver, J.H., Borowsky, A.T., Tzin, V., Jander, G., Kliebenstein, D.J. & Rokas, A. (2017) A global coexpression network approach for connecting genes to specialized metabolic pathways in plants. *Plant Cell*, 29, 944–959. Available from: <https://doi.org/10.1105/tpc.17.00009>
- Wong, J., de Rond, T., d'Espaux, L., van der Horst, C., Dev, I., Rios-Solis, L. et al. (2018) High-titer production of lathyrane diterpenoids from sugar by engineered *Saccharomyces cerevisiae*. *Metabolic Engineering*, 45, 142–148. Available from: <https://doi.org/10.1016/j.ymben.2017.12.007>
- Xie, D.-Y., Ma, D.-M., Judd, R. & Jones, A.L. (2016) Artemisinin biosynthesis in *Artemisia annua* and metabolic engineering: questions, challenges, and perspectives. *Phytochemistry Reviews*, 15, 1093–1114. Available from: <https://doi.org/10.1007/s11101-016-9480-2>
- Xu, H. & Dickschat, J.S. (2020) Germacrene A-A Central Intermediate in Sesquiterpene Biosynthesis. *Chemistry*, 26, 17318–17341. Available from: <https://doi.org/10.1002/chem.202002163>
- Xu, H., Li, W., Schillmiller, A.L., van Eekelen, H., de Vos, R.C.H., Jongsma, M.A. et al. (2019) Pyrethric acid of natural pyrethrin insecticide: complete pathway elucidation and reconstitution in *Nicotiana benthamiana*. *The New Phytologist*, 223, 751–765. Available from: <https://doi.org/10.1111/nph.15821>
- Xu, H., Moghe, G.D., Wiegert-Rininger, K., Schillmiller, A.L., Barry, C.S., Last, R.L. et al. (2018) Coexpression analysis identifies two oxidoreductases involved in the biosynthesis of the monoterpene acid moiety of natural pyrethrin insecticides in *Tanacetum cinerariifolium*. *Plant Physiology*, 176, 524–537. Available from: <https://doi.org/10.1104/pp.17.01330>
- Xu, J., Ai, Y., Wang, J., Xu, J., Zhang, Y. & Yang, D. (2017) Converting S-limonene synthase to pinene or phellandrene synthases reveals the plasticity of the active site. *Phytochemistry*, 137, 34–41. Available from: <https://doi.org/10.1016/j.phytochem.2017.02.017>
- Xu, M., Wilderman, P.R. & Peters, R. (2007) Following evolution's lead to a single residue switch for diterpene synthase product outcome. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 7397–7401. Available from: <https://doi.org/10.1073/pnas.0611454104>
- Xu, X., Yuan, H., Yu, X., Huang, S., Sun, Y., Zhang, T. et al. (2021) The chromosome-level stevia genome provides insights into steviol glycoside biosynthesis. *Horticulture Research*, 8, 129. Available from: <https://doi.org/10.1038/s41438-021-00565-4>
- Xu, Y., Wang, X., Zhang, C., Zhou, X., Xu, X., Han, L. et al. (2022) De novo biosynthesis of rubusoside and rebaudiosides in engineered yeasts. *Nature Communications*, 13, 3040. Available from: <https://doi.org/10.1038/s41467-022-30826-2>
- Yang, T., Gao, L., Hu, H., Stoopen, G., Wang, C. & Jongsma, M.A. (2014) Chrysanthemyl diphosphate synthase operates in planta as a bifunctional enzyme with chrysanthemol synthase activity. *The Journal of Biological Chemistry*, 289, 36325–36335. Available from: <https://doi.org/10.1074/jbc.M114.623348>
- Yoshida, R., Yoshimura, T. & Hemmi, H. (2020) Reconstruction of the “archaeal” mevalonate pathway from the methanogenic archaeon *Methanosarcina mazei* in *Escherichia coli* cells. *Applied and Environmental Microbiology*, 86, e02889-19. Available from: <https://doi.org/10.1128/AEM.02889-19>
- Yoshikuni, Y., Martin, V.J.J., Ferrin, T.E. & Keasling, J.D. (2006) Engineering cotton (+)-delta-cadinene synthase to an altered function: germacrene D-4-ol synthase. *Chemistry & Biology*, 13, 91–98. Available from: <https://doi.org/10.1016/j.chembiol.2005.10.016>
- Zabel, S., Brandt, W., Porzel, A., Athmer, B., Bennewitz, S., Schäfer, P. et al. (2021) A single cytochrome P450 oxidase from *Solanum habrochaites* sequentially oxidizes 7-epi-zingiberene to derivatives toxic to whiteflies and various microorganisms. *The Plant Journal*, 105, 1309–1325. Available from: <https://doi.org/10.1111/tpl.15113>
- Zerbe, P., Hamberger, B., Yuen, M.M.S., Chiang, A., Sandhu, H.K., Madilao, L.L. et al. (2013) Gene discovery of modular diterpene metabolism in nonmodel systems. *Plant Physiology*, 162, 1073–1091. Available from: <https://doi.org/10.1104/pp.113.218347>
- Zhang, Y., Teoh, K.H., Reed, D.W., Maes, L., Goossens, A., Olson, D.J.H. et al. (2008) The molecular cloning of artemisinic aldehyde delta11(13) reductase and its role in glandular trichome-dependent biosynthesis of artemisinin in *Artemisia annua*. *The Journal of Biological Chemistry*, 283, 21501–21508. Available from: <https://doi.org/10.1074/jbc.M803090200>
- Zhao, M., Zhang, N., Gao, T., Jin, J., Jing, T., Wang, J. et al. (2020) Sesquiterpene glucosylation mediated by glucosyltransferase UGT91Q2 is involved in the modulation of cold stress tolerance in tea plants. *The New Phytologist*, 226, 362–372. Available from: <https://doi.org/10.1111/nph.16364>
- Zhao, Q., Luan, X., Zheng, M., Tian, X.-H., Zhao, J., Zhang, W.-D. et al. (2020) Synergistic mechanisms of constituents in herbal extracts during intestinal absorption: focus on natural occurring nanoparticles. *Pharmaceutics*, 12, 128. Available from: <https://doi.org/10.3390/pharmaceutics12020128>
- Zhao, W., Ye, Q., Tan, X., Jiang, H., Li, X., Chen, K. et al. (2001) Three new sesquiterpene glycosides from *Dendrobium nobile* with immunomodulatory activity. *Journal of Natural Products*, 64, 1196–1200. Available from: <https://doi.org/10.1021/np0102612>
- Zhou, F. & Pichersky, E. (2020) More is better: the diversity of terpene metabolism in plants. *Current Opinion in Plant Biology*, 55, 1–10. Available from: <https://doi.org/10.1016/j.pbi.2020.01.005>
- Zhou, K., Gao, Y., Hoy, J.A., Mann, F.M., Honzatko, R.B. & Peters, R.J. (2012) Insights into diterpene cyclization from structure of bifunctional abietadiene synthase from *Abies grandis*. *The Journal of Biological Chemistry*, 287, 6840–6850. Available from: <https://doi.org/10.1074/jbc.M111.337592>
- Zi, J., Mafu, S. & Peters, R.J. (2014) To gibberellins and beyond! Surveying the evolution of (Di)Terpenoid metabolism. *Annual Review of Plant Biology*, 65, 259–286. Available from: <https://doi.org/10.1146/annurev-arplant-050213-035705>
- Zi, J. & Peters, R.J. (2013) Characterization of CYP76AH4 clarifies phenolic diterpenoid biosynthesis in the Lamiaceae. *Organic & Biomolecular Chemistry*, 11, 7650–7652. Available from: <https://doi.org/10.1039/c3ob41885e>