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Non-volatile natural products in plant glandular trichomes: chemistry, biological activities and biosynthesis†

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Plant glandular trichomes (GTs) are adaptive structures that are well known as "phytochemical factories" due to their impressive capacity to biosynthesize and store large quantities of specialized natural products. The natural products in GTs are chemically diverse and mostly function as defense chemicals, therefore GTs are frequently regarded as "the first defense line" of plants against biotic and abiotic stresses. More importantly, many GT natural products are commercially desirable, thanks to their significant biological activities, thus attracting extensive interest in their biosynthesis. Consequently, it is well known that plant GTs are not only important reservoirs of biologically active natural products but are also a valuable bank of novel biosynthetic genes and enzymes. The non-volatile or oxygenated natural products in plant GTs, which need longer biosynthetic pathways and more energy from the plants, are of particular interest due to their more extensive biological activities and high commercial value. This review mainly focuses on these non-volatile natural products in plant GTs, including their chemistry, biological activities and biosynthesis. The methods employed for investigating natural products and their biosynthesis in plant GTs are also comprehensively discussed.

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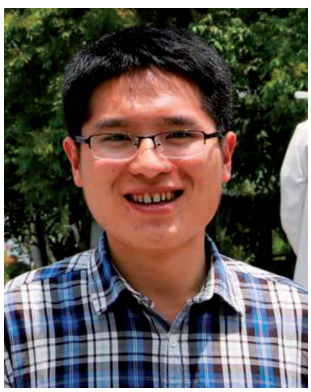

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1. Introduction

Plant glandular trichomes (GTs) are specified epidermal structures distributed on the surfaces of the aerial organs of approximately 30% of vascular plants, including angiosperms, gymnosperms and bryophytes, and play important roles in plant resistance against herbivores and pathogens and in plant–environment interactions.[1] GTs exhibit great morphological diversity in different plant species and even within one species but typically consist of a glandular head at the apex of a stalk. Capitate and peltate are two usual types of GTs that are frequently present in plants, especially the Asteraceae, Lamiaceae, Cannabaceae and Solanaceae families (Fig. 1). Capitate GTs are composed of one or a few glandular cells atop a single- or multi-cellular stalk (Fig. 1A–C, E and F). Peltate GTs usually consist of a large multicellular glandular head at the tip of a (short) stalk cell, and the glandular head is often surmounted by a thick cuticle to form a large sub-cuticular storage cavity in which the natural products are accumulated (Fig. 1D–F). Different GT types have distinct properties and may have evolved due to different selection pressures confronted by the plants during their adaption to the environment.

Sheng-Hong Li received his Ph.D. degree in Phytochemistry under the guidance of Prof. Han-Dong Sun from Kunming Institute of Botany, Chinese Academy of Sciences in 2001. He subsequently worked as an Assistant Professor and then Associate Professor at the same institute. In 2004, he moved to the Max Planck Institute for Chemical Ecology in Germany for his post-doctoral research with Prof. Jonathan Gershenzon and Dr. Bernd Schneider. Since 2007, he has been employed as a full professor and group leader by Kunming Institute of Botany, Chinese Academy of Sciences. Currently, he is a deputy director of the State Key Laboratory of Phytochemistry and Plant Resources in West China. He received the Alexander von Humboldt Research Fellowship in 2005 and National Science Fund for Distinguished Young Scholars in 2015, and was selected as a member of the National Ten thousand Talent Program (2018), the Leading Talent Program of Ministry of Science and Technology (2017), and the Hundred Talent Program of the Chinese Academy of Sciences (2010). He has published over 100 research papers. His major research interests focus on the chemistry, biological functions and biosynthesis of plant natural products, especially various terpenoids in plant specialized structures such as glandular trichomes.

A common feature of plant GTs is their extraordinary capacity to produce, store and sometimes secrete chemodiversified natural products including volatile, semi-volatile and non-volatile compounds. Remarkably, the amount of GT products may reach 30% of the leaf dry weight,[2] and the chemodiversity and amount are influenced by environmental conditions and seasonal variation. Tremendous efforts have been made to reveal the chemical structures of GT products, covering several major types of natural products including various types of terpenoids, phenolics, glycerides, and so on. These special chemicals frequently play crucial roles in the interactions of plants with other organisms and the surrounding environment, and particularly in plant defense against herbivores and pathogens due to their toxic, antifeedant, anti-fungal and antibacterial activities. Meanwhile, a large number of GT products possess significant pharmacological activities including psychoactive, anti-parasitic, antitumor, antimicrobial, antiviral, antioxidative and antithrombotic properties. Accordingly, plant GTs have been considered to be important reservoirs of bioactive natural products for new drug discovery.

The natural products accumulated in GTs have been generally considered to be biosynthesized *in situ*, which makes GTs attractive as "biofactories" for producing high-value natural products. GTs can be separated from the epidermis and then the intermediate compounds, mRNA and proteins are readily captured from the isolated GTs. Through the combination of expressed sequence tag (EST)/unigene sequencing of GTs-specific cDNA libraries and proteome analysis, more and more biosynthetic genes and enzymes with unusual functions, which are critical for the synthetic biology of useful natural products, have been discovered from various plant GTs, as has been well exemplified by the elucidation of biosynthetic pathways of terpenoids in Lamiaceae and Asteraceae plant GTs. Therefore, plant GTs have also been regarded as a valuable bank of novel biosynthetic genes and enzymes as well as excellent experimental systems for investigating the biosynthetic pathways of GTs-specific natural products.

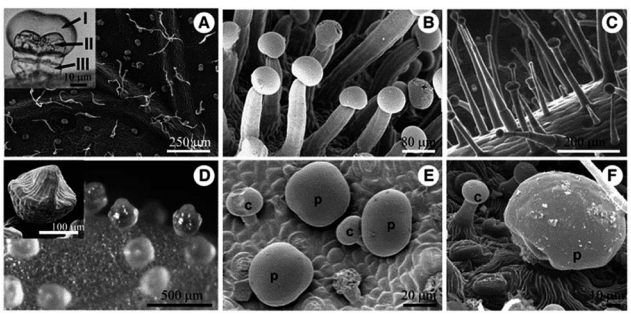


Fig. 1 Examples of plant glandular trichomes (GTs). (A) Capitate GTs of *Artemisia annua* (Asteraceae). I, II, III indicate storage cavity, secretory cells, and stalk cells, respectively. (B) Capitate GTs of *Cannabis sativa* (Cannabaceae). (C) Capitate GTs of *Nicotiana benthamiana* (Solanaceae). (D) Peltate GTs of *Humulus lupulus* (Cannabaceae). (E) Peltate and capitate GTs of *Salvia divinorum* (Lamiaceae). (F) Peltate and capitate GTs of *Rosmarinus officinalis* (Lamiaceae). c and p indicate capitate and peltate GTs, respectively. Photos were reproduced from the literature with permission.[3–9]

Recognizing the importance of plant GTs, many excellent reviews have appeared. For example, the mechanical surface abrasion procedures for the isolation and preparation of plant trichomes were reviewed by Gershenzon *et al.*,[10] Wagner *et al.*,[11] and Tissier.[12] Besides, Dai *et al.*,[13] Tissier,[12] and Champagne and Boutry[14] summarized the methods for the localization of natural products and the analysis of transcriptomics and proteomics of plant GTs. To provide better guidance for the follow-up studies related to GTs, it is necessary to summarize the approaches, especially the recently developed techniques, for the systematic investigation of the chemistry and biosynthesis of GTs-specific compounds.

In 1984, the chemistry, biological activities and biosynthesis of natural products produced by plant GTs were first summarized in a book edited by Rodriguez *et al.*[15] Since then, a large number of GTs-specific natural products have been identified, but the subsequent reviews only listed some representative examples.[12,16–19] Therefore, a comprehensive and updated report on the chemistry and biological activities of plant GTs natural products is necessary.

In 2000, Hallahan reviewed monoterpenoid (mostly volatile) biosynthesis in the GTs of Lamiaceae plants.[20] In 2013, Lange and Turner reviewed the terpenoid biosynthesis in plant GTs but focused specifically on the essential oils and oleoresins.[5] The volatile compounds in plant GTs were considered to play important ecological roles, such as attracting pollinators, and protection from herbivores and pathogens.[1] The evolution, differentiation and development of GTs,[21] the strategies for bioengineering development and metabolism of glandular tissues in plants[16] and the applications of plant GTs in metabolic engineering[22] have also been well reviewed, but will not be covered herein.

In this review, we aim to emphasize the non-volatile or oxygenated natural products in plant GTs that need longer biosynthetic pathways and require more plant energy, and are of particular interest due to their extensive biological activities and high commercial value, *e.g.*, anti-malarial artemisinin (**119**), the psychoactive cannabinoids (**223–231**) and salvinorin A (**182**), and the antioxidative carnosic acid (**186**). Since the 1960s, 489 non-volatile natural products have been identified from the GTs of 81 plant species and the biosynthetic pathways of a number of bioactive compounds have been investigated. In particular, the progress on the precise collection of GTs, highly sensitive chemical analysis and high-throughput "omics" techniques appropriate for micro-samples have made significant contributions in this field. The methods for investigating the chemistry and biosynthesis of GTs-specific compounds are comprehensively discussed, and the advantages and shortcomings of each procedure are also presented. Through this review, we seek to highlight the great potential of GTs as "phytochemical factories" for the production and accumulation of bioactive compounds, and to underscore the importance of using plant GTs as an efficient timesaving and cost-saving strategy for the discovery of bioactive natural products and their biosynthetic pathways in current natural product research (Fig. 2).

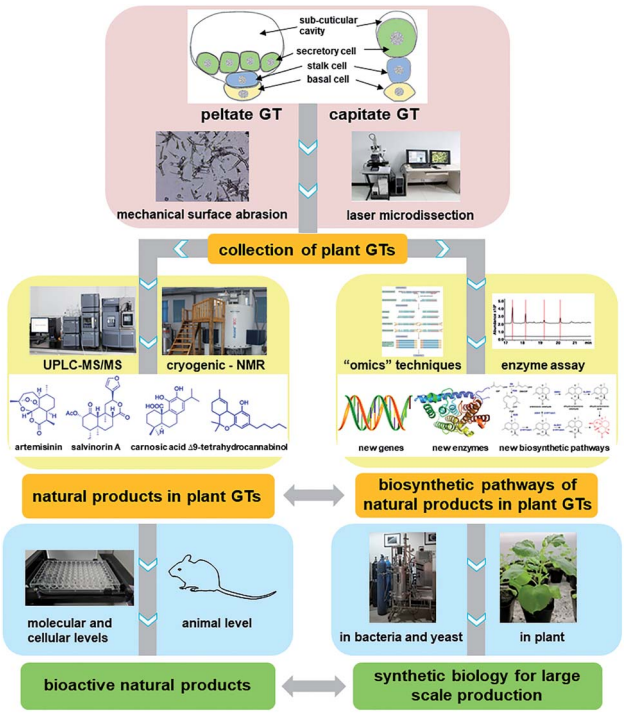


Fig. 2 An outline of plant glandular trichomes as an excellent experimental system and shortcut for the discovery of bioactive natural products and new genes and biosynthetic pathways.

2. Methods for investigating the chemistry and biosynthesis of GTs-specific compounds

Plant GTs are tiny structures with glandular heads of approximately 15–115 μm in diameter, which are often scattered in plant aerial surfaces and interspersed with other plant protuberances, making it a big challenge to investigate the chemistry and biosynthesis of GT-specific natural products. Since the 1960s, considerable progress, including the separation and collection of GTs, localization and discovery of natural products in plant GTs, and elucidation of biosynthetic pathways of GTs-specific compounds, has been made to achieve the goals of a deeper understanding and the rational utilization of plant GTs.

2.1 Separation and collection of plant GTs

Investigation of the chemistry and biosynthesis of plant GTs-specific compounds requires the availability of rapid, efficient and accurate methods for obtaining adequate quantities of GTs. The initial method for collecting GTs relied on the excision of epidermal strips from young leaves.[23] Later, many mechanical surface abrasion procedures were developed, including gently brushing, touching, abrading or scraping leaf surfaces with a soft bristle toothbrush, a microscope slide, glass beads, or needles and tweezers under a stereomicroscope.[24–27] However, with these methods, it was hard to prepare GTs with sufficient quality or quantity for further studies and accordingly, more efficient techniques were developed. Slone and Kelsey described the mechanical homogenization of aerial tissue followed by isolation and partial purification of the isolated GTs heads by Percoll density gradient centrifugation.[28] Gershenzon *et al.* modified the mechanical procedures by abrasion of the leaf surfaces with glass beads in a commercial cell homogenizer followed by filtration.[29] This method can be adapted to a broad range of plant species with leaves of varying size, toughness and surface topography. Yerger *et al.* then developed a physical method that sheared GTs from frozen plant tissues with powdered dry ice.[30] To collect the GTs tightly attached to leaf veins that were densely covered with non-GTs, quartz sand was used to chafe the leaflets.[31] Although the above-mentioned methods are simple and viable for most laboratories, the collected GTs samples inevitably contain significant quantities of non-glandular tissues and other contaminants. Moreover, these methods cannot realize the separation of a single type of GT and seem unsuitable for the isolation of those GTs embedded in the epidermal cells.

In order to isolate specific cell types of multicellular GTs, a method based on the laser microdissection pressure catapulting (LMPC) technique was developed.[32] Using this method, one outer pair of apical cells and two pairs of sub-apical, chloroplast-containing cells, were isolated from *Artemisia annua* GTs (Fig. 3).[32] The RNA extracted from the microdissected cells was then used for real-time quantitative PCR to localize biosynthetic genes of artemisinin (**119**).[32,33] Laser microdissection (LMD) was also used to isolate and collect specific types of GTs such as peltate GTs from *Colquhounia coccinea* var. *mollis* and capitate GTs from *Cannabis sativa*.[34,35] Although a professional instrument is needed, the introduction of LMD to GTs isolation attained a whole new level of precision.

2.2 Localization and discovery of natural products in plant GTs

Since the GT sample amounts gained are usually very limited and natural products cannot be amplified as DNA, the localization and discovery of natural products in plant GTs are enormous challenges and need highly sensitive and accurate analytical techniques. Early studies for the localization of GT-specific compounds mainly relied on histochemical staining. For example, Fast Blue B was used as a potential staining

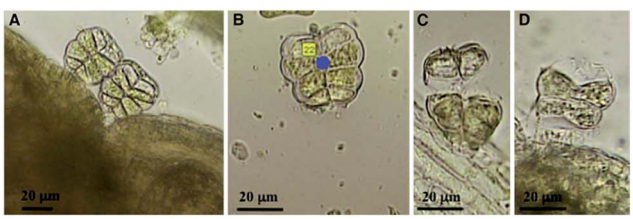


Fig. 3 Demonstration of laser microdissection pressure catapulting (LMPC) in isolating individual cells of plant GTs. (A) Two *Artemisia annua* GTs before microdissection. (B) Single *A. annua* GTs after microdissection. (C) Two apical cells after microdissection. (D) Four sub-apical cells after microdissection. Photos were reproduced from the literature with permission.[33]

reagent for the localization of cannabinoids in GTs of *C. sativa*.[36] Approaches for collecting trichome exudates were also developed, such as gently touching the plant surface with a microscope slide followed by rinsing the slide with organic solvent, wiping the plant surface with a solvent-soaked cotton swab, and washing the plant surface rapidly with organic solvent.[37,38] The isolated GTs or trichome exudates were then analyzed with a combination of modern analytical techniques such as microspectrofluorometry coupled with multispectral fluorescence microimaging, high performance liquid chromatography (HPLC), ultraperformance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), liquid chromatography tandem time-of-flight mass spectrometry (LC-TOF-MS), Raman and ^{1}H NMR spectroscopies.[39–42]

Recently, mass spectrometry imaging (MSI) has been developed to directly profile and image natural products in plant GTs (Fig. 4). Based on the laser desorption/ionization (LDI)-MSI technique, acyl sugars, alkaloids, flavonoids, and terpenoid acids were detected in the GTs of wild tomato *Solanum habrochaite*,[43] and flavonoids and naphthoquinones were localized to the GTs of *Hypericum perforatum*.[44] Using the matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) technique, sesquiterpene lactones were confirmed to be accumulated in the capitate GTs of *Smallanthus sonchifolius* and sunflowers (*Helianthus annuus*),[45,46] and bioactive diterpenoids were localized to the peltate GTs of *Salvia divinorum* and *Vitex agnus-castus*.[47,48] A time-of-flight secondary ion mass spectrometry (TOF-SIMS) dual beam depth profiling was applied to precisely detect and localize bioactive vedelianin (**232**) in the GTs lying at the surface of leaves and fruits of *Macaranga vedeliana*.[49] In addition, the negative ion 'chip-based' nanospray tandem mass spectrometry apparatus was utilized to identify flavonoids **301–305** in the GTs of *Lychnophora ericoides*.[50] A leaf spray-MS technique was used to directly detect natural products in the GTs of *Glycyrrhiza lepidota*.[51] Moreover, pressure probe electrospray ionization mass spectrometry with internal electrode capillary (IEC-PPESI-MS) was applied to analyze and compare the single-cell metabolic profiles of stalk and GT cells of tomato (*S. lycopersicum*).[52] These detection methods, no doubt, gave highly intuitive, precise and sensitive ways to localize bioactive metabolites in plant GTs. However, all these methods have the risk of missing new compounds.

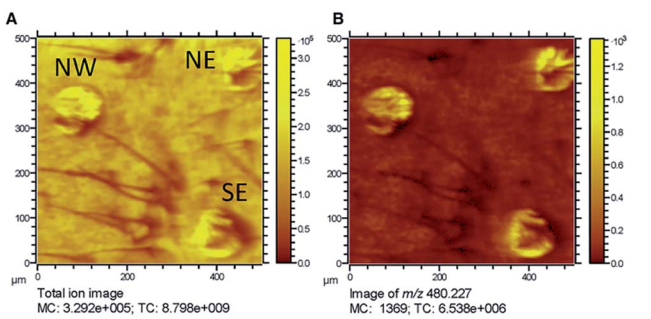


Fig. 4 Demonstration of the mass spectrometry imaging (MSI) technique in detecting natural products in plant GTs. (A) Total ion image showing three GTs of *Macaranga vedeliana* detected in the ion image (labeled NW, NE, and SE). (B) Image of the *m*/*z* 480.227 $[M]^+$ ion of vedelianin (**232**). Image size 500 × 500 μm, 256 × 256 pixels, pixel size 2 × 2 μm. Photos were reproduced from the literature with permission.[49]

The chromatographic analysis of GT exudates of *Leucosceptrum canum* followed by target compound isolation and structure elucidation with classical phytochemical techniques led to the identification of leucosceptrane sesterterpenoids (or leucosceptroids, **193–197**) with an unprecedented skeleton.[38] Recent development of LMD coupled with UPLC-MS/MS and/or cryogenic ^{1}H NMR spectroscopy has facilitated the identification of natural products in plant GTs. Thus, colquhounane sesterterpenoids (or colquhounoids, **198–200**) with another novel skeleton were identified from the peltate GTs of *C. coccinea* var. *mollis* using LMD coupled with UPLC-MS/MS (Fig. 5).[35] In addition, four new labdane diterpenoids **169–172**, together with the known **173**, were localized to the capitate GTs of *Paragutzlaffia henryi* using LMD coupled with cryogenic ^{1}H NMR.[53]

2.3 Elucidation of biosynthetic pathways of natural products in plant GTs

Exploring the GTs instead of the whole plant has been considered as an efficient approach to access the genes and enzymes involved in the biosynthesis and regulation of GTs-specific bioactive compounds. In the past, the incubation of labelled precursors with GTs or cell-free extracts has been extensively adopted in biosynthesis studies of many natural products such as cembrenoids, acylsugars and *N*-acyl nornicotines in tobacco GTs.[25,37,54–56] Techniques for the separation and purification of enzymes from the isolated GTs cells were then developed as demonstrated in an investigation of acylsugar biosynthesis in tomato.[57]

Coupled with the mechanical collection of GTs at low temperatures, methods for the isolation of high-quality GTs-specific RNAs were established.[58] On that basis, the technology for the EST/unigene sequencing of GTs-specific cDNA libraries was rapidly developed and widely employed for the systematic investigation of the biosynthesis and regulation of GTs-specific natural products. Based on GTs-specific EST libraries, the key genes responsible for the biosynthesis of well-known bioactive compounds, such as artemisinin (**119**), carnosic acid (**186**), salvinorin A (**182**) and cannabinoids (**223–231**), were identified, as subsequently discussed in more detail in Section 4.[59–61] Likewise, advances in proteome approaches to plant GTs provided another avenue for the identification of relevant biosynthetic enzymes. The proteomics of the GTs of *Nicotiana tabacum* and hops (*Humulus lupulus*) were reported.[62,63] However, identifying GTs-specific proteins from only proteomic mass spectra while lacking any DNA sequence or transcriptome sequence information remains a big challenge.

A number of techniques such as *in situ* hybridization, qRT-PCR, expression of reporter genes, and immunolocalization have been used to reveal the cell-specific distribution of individual genes and proteins. Using immunogold labelling with

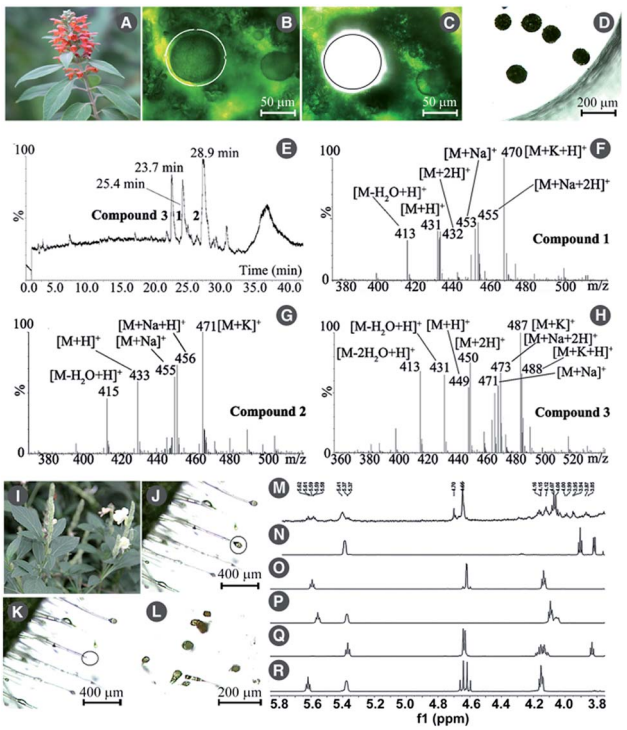


Fig. 5 Demonstration of laser microdissection (LMD) coupled with UPLC-MS/MS and/or cryogenic NMR spectroscopy in the identification of natural products in plant GTs. (A) *Colquhounia coccinea* var. *mollis* plant in bloom. (B) Intact peltate GTs of *C. coccinea* var. *mollis* before microdissection. (C) Remaining leaf tissue after microdissection. (D) Collected peltate GTs by LMD. (E–H) Microchemical analysis of natural products in the microdissected peltate GTs with UPLC/MS/MS. (I) *Paragutzlaffia henryi* plant in bloom. (J) Intact capitate GTs of *P. henryi* before microdissection. (K) Remaining tissue after microdissection. (L) Collected capitate GTs by LMD. (M–R) Cryogenic ^{1}H NMR analysis of *P. henryi* capitate GTs and comparison of natural products isolated from the GT extracts. Photos were reproduced from the literature with permission.[35,53]

silver enhancement, a key enzyme for the biosynthesis of **119** was localized to the GTs of *A. annua*.[32] Moreover, based on the qRT-PCR analysis of different GT cell types separated by the LMPC technique, 11 key genes for the biosynthesis of **119** were further localized to the sub-apical cells of the GTs of *A. annua*.[33]

The combination of GTs-specific transcriptome and metabolome information would be helpful in understanding the biosynthesis and regulation of GTs natural products. TrichOME is an integrated database of genes and metabolites in plant GTs, which has been developed in recent years and is freely available at http://www.planttrichome.org/.[13] Currently, the TrichOME database hosts 4,230,576 Sanger/454 ESTs sequenced from trichome and non-trichome control tissues of 16 species, as well as the UPLC-MS data for hops and *Medicago sativa*.

3. Non-volatile natural products in plant GTs and their biological activities

In this section, the chemical structures of specialized non-volatile compounds reported from plant GTs since the 1960s are summarized. Based on their biogenesis, the compounds were divided into five major groups and miscellaneous types. In addition, information about the compound name, source of isolation, and reference can be found in the ESI (Tables S1–S10†).

3.1 Terpenoids

Terpenoids are extremely rich in plant GTs, including sesquiterpenoids, diterpenoids, sesterterpenoids, triterpenoids, as well as a large number of monoterpenoids, which are mostly volatile and are not covered here.

3.1.1 Sesquiterpenoids. A total number of 158 non-volatile sesquiterpenoids (**1–158**) were discovered from the GTs of various plant species (Fig. 6–9, Table S1†). The bisabolene-type sesquiterpenoids **1–10** and chemically diversified sesquiterpene lactones (STLs, **11–120**, **129–153**) were all isolated from the GTs of the Asteraceae family. These STLs mainly include xanthane (**11–21**), germacrane (**23–117**), eudesmane (**22**, **121–134**), and guaiane (**118**, **135–153**) sesquiterpenoids. STLs were used as chemotaxonomic markers of the Asteraceae family, especially in the Heliantheae tribe, and it was suggested that different chemical profiles might be correlated with the plant speciation.[64–67] For example, the dominant chemotypes found in the GTs of *Heliunthus* and *Viguiera* species were 1,10-epoxidized (**56–72**) and 1-keto-2,3-unsaturated (**85–97**, **99–103**) heliangolides.[65] Besides, glycosides of bisabolene (**154**)[68] and campheranane (**155–158**)[69] were identified from the GTs of Acanthaceae and Solanaceae plants, respectively. Extensive biological activities including anti-parasitic, anti-cancer and anti-inflammatory effects and defensive functions were documented for these sesquiterpenoids.

3.1.1.1 Anti-parasitic activity of plant GTs sesquiterpenoids. Remarkable anti-parasitic activity has been reported for these STLs from plant GTs. The most famous example is the antimalarial drug artemisinin (**119**) (also called qinghaosu), an STL endoperoxide localized to the capitate GTs of *A. annua*.[70] Artemisinin combination therapies remain the first-line treatments for *Plasmodium falciparum* malaria.[71] It seems that the antimalarial activity of **119** against drug-resistant *P. falciparum* strains, with IC_{50} values of 10^{-8}–10^{-7} mol L^{-1} *in vitro*, is attributable to its peroxide.[72] Thus, the unusual structure and potency of **119** makes it a powerful alternative to the alkaloidal antimalarials, saving hundred thousands of lives each year.[71] Parthenin (**135**),

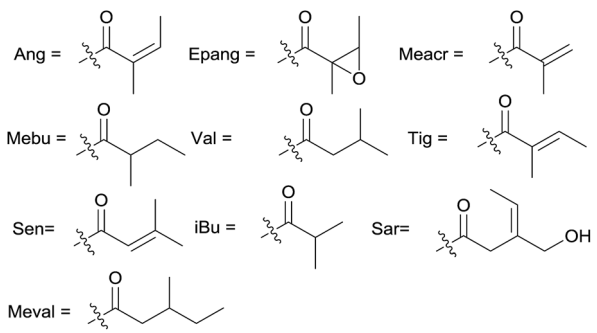


Fig. 6 Structure abbreviations used in this review.

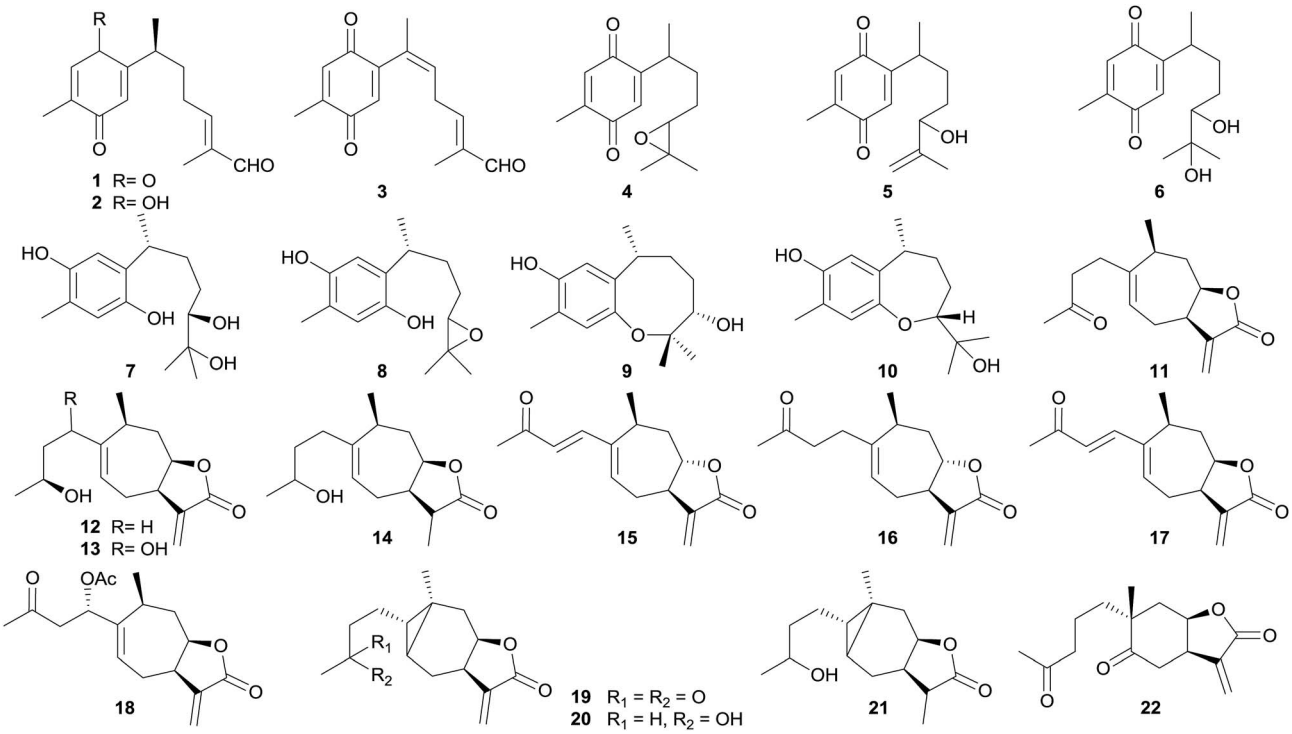


Fig. 7 The chemical structures of sesquiterpenoids **1–22** from plant GTs.

identified in the GTs of *Parthenium hysterophorus*,[73] also showed antimalarial activity against the sexual stage transmission of *P. falciparum*, suggesting an independent mechanism in comparison to **119**.[74] Transmission-blocking drugs might be a new tactic for preventing the spread of artemisinin-resistant parasites.[74,75]

Moreover, the anti-parasitic activity of STLs has been observed against many other parasites as well. For example, tagitinin C (**37**) a major sesquiterpenoid in the GTs of *Tithonia diversifolia*, possesses potent antitrypanosomal activity against *Trypanosoma brucei* (TC221) with IC_{50} of 0.0042 μg mL^{-1}.[76,77] Budlein A (**86**), which was mainly localized to the GTs of *H. tuberosus*, showed strong schistosomicidal activity against *Schistosoma mansoni*, accompanied with high cytotoxicity.[78]

3.1.1.2 Anti-cancer activity of plant GTs sesquiterpenoids. Due to their cytotoxicity, the STLs compartmentalized in plant GTs are thought to prevent autotoxicity.[79] The selective anti-cancer activity of STLs are functionalized through targeting specific signalling pathways, making them drug candidates in cancer clinical trials.[80] Besides the antimalarial activity, **119** and its derivatives also showed promising anti-cancer activity in *in vitro*, *in vivo* and clinical studies, and synergistic effects with regular chemotherapeutics and minimal toxicity.[81] **119** is currently in clinical trials for the treatment of metastatic breast cancer (ClinicalTrials.gov: NCT00764036) and colorectal cancer (Isrctn.org: ISRCTN05203252). The anti-oxidative and tumorigenesis suppression activities were recently found for artemisitene (**120**), an analogue of **119** also localized to the capitate GTs of *A. annua*.[82,83]

Tomentosin (**11**), detected in the GTs of the *Scalesia* species,[84] exhibited telomere shortening and apoptotic effects on human cervical cancer SiHa and HeLa cell lines, with IC_{50} values of 7.10 and 5.87 μM, respectively.[85] Xanthanolides **15–18** from the trichome secretions of *Xanthium strumarium*[86] were found to possess anti-tumor activity, and the activity of xanthatin (**15**) was mediated *via* glycogen synthases kinase-3β and β-catenin.[87] TNF-related apoptosis-inducing ligand (TRAIL) is a new target for cancer therapy due to its ability to selectively induce apoptosis in cancer cells, and significant TRAIL-resistance overcoming activity for xanthinosin (**16**) was observed.[88] Cnicin (**35**), a major component in the GTs of *Centaurea maculosa*,[89] showed cytotoxicity against a panel of human cancer cell lines, with IC_{50} values ranging from 0.77 to 12.5 μM.[90]

The germacranolides uvedalin (**48**) and enhydrin (**49**) were found to be accumulated in the GTs of *Smallanthus sonchifolius*[45] and both compounds showed potential cytotoxicity against HeLa, HL-60, and murine B16-F10 melanoma cell lines, with IC_{50} values ranging from 0.74 to 6.55 μM.[91] Parthenolide (**52**), localized in the GTs of feverfew (*Tanacetum parthenium*), is a potential agent for treating migraine prophylaxis and cancers.[92] Moderate cytotoxicity was observed for tagitinin C (**37**), leptocarpin (**71**), gerin (**128**), ambrosin (**136**), and zaluzanin C (**139**) against multiple cancer cell lines.[93–99] Besides, **37** also exhibited anti-tobacco mosaic virus (TMV) activity.[96]

3.1.1.3 Anti-inflammatory activity of plant GTs sesquiterpenoids. The plants containing STLs have often been used in traditional Chinese medicine against inflammation. Tomentosin (**11**) exhibited anti-inflammatory activity through the inhibition of multiple inflammatory mediators by the suppression of the transcription factor NF-κB and MAP (mitogen-activated

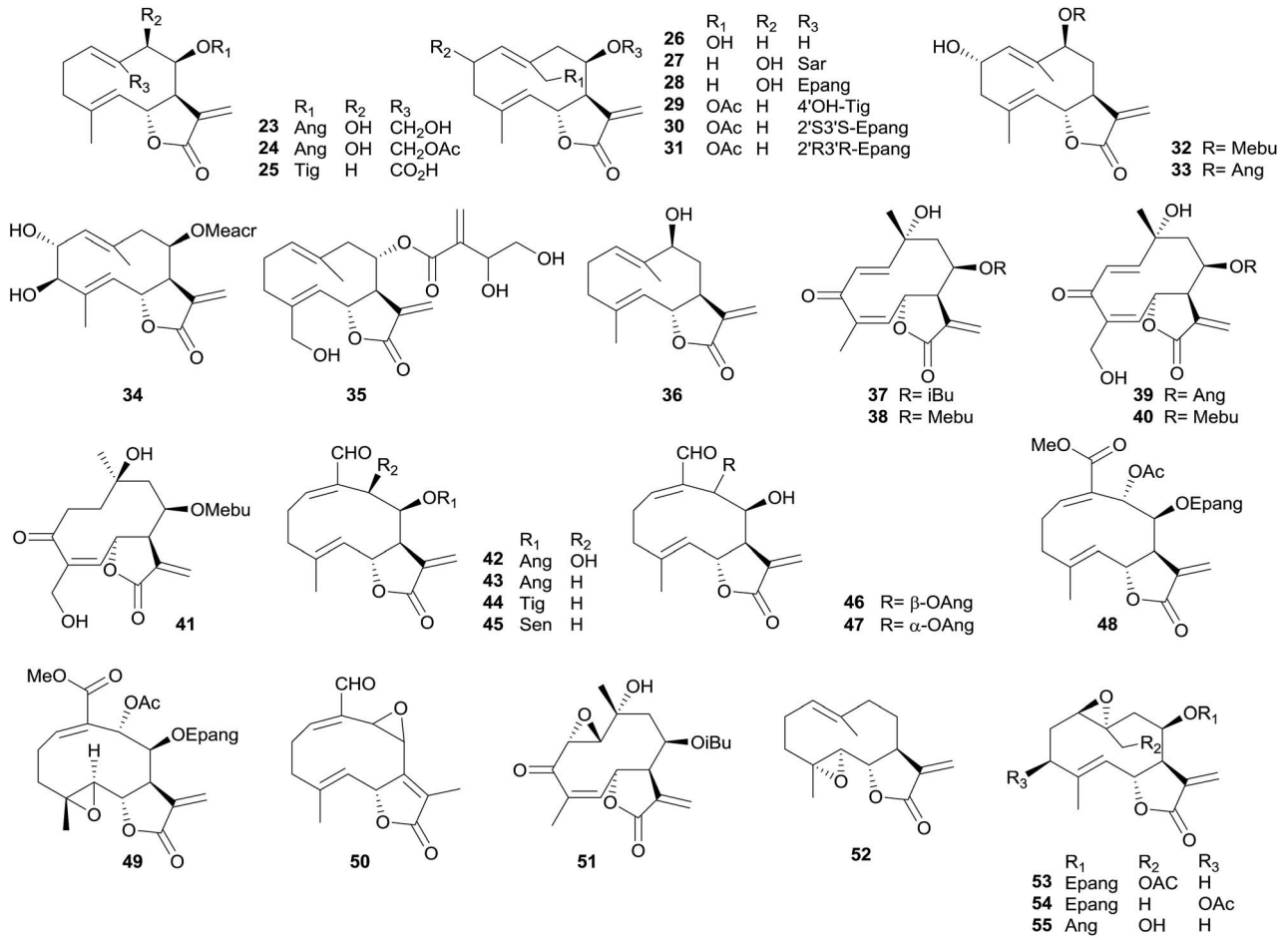


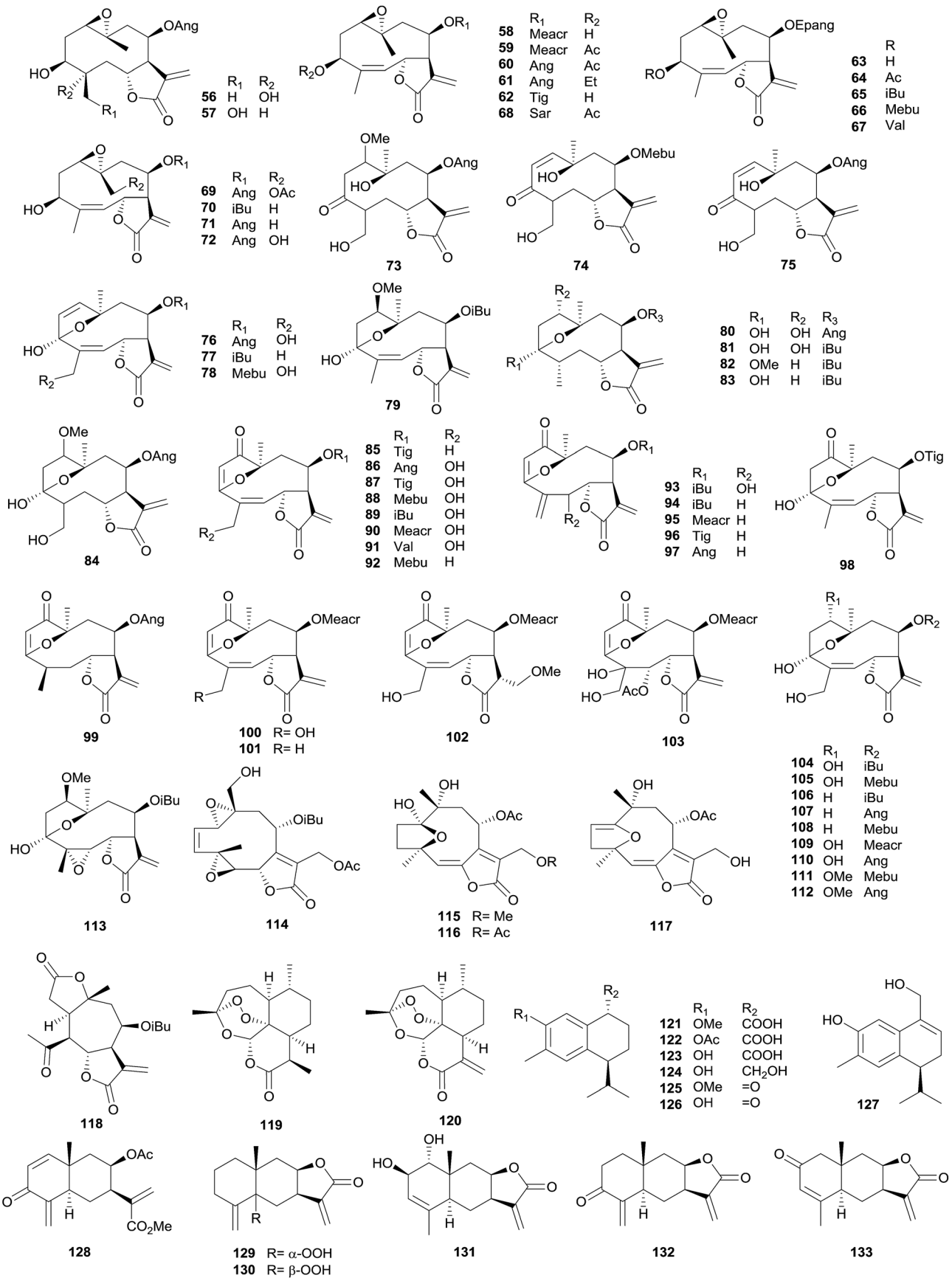
Fig. 8 *(Contd.)*

protein) kinase activation.[100] Carabrone (**19**) and 8β-angeloyloxy-14-oxo-acanthospermolide (**43**) showed inhibitory activity toward the LPS-induced nitric oxide production in RAW264.7 cells, with IC_{50} values of 5.3 μM [101] and 12.0 μM,[102] respectively. Cnicin (**35**) showed inhibitory effects on NF-κB and inducible nitric oxide synthase (iNOS) with IC_{50} values of 1.8 and 6.5 μM, respectively.[103] Moderate inhibitory activity of heliangine (**62**) and guaianolides **145–149** against NF-κB was also observed.[36,104] The structural relationships of STLs against cancer and inflammation were reviewed, which suggested the importance of alkylating center reactivity (mainly the α-methylene-γ-lactone), side chain lipophilicity, and molecular geometry.[80]

3.1.1.4 Defensive functions of plant GTs sesquiterpenoids. Increasing evidence has revealed the defensive functions of STLs stored in the GTs of Asteraceae species, such as their allergy effects, antifeedant and antifungal activities, which might contribute to the evolutionary success and wide distribution of Asteraceae plants.[79] The allergic contact dermatitis caused by sunflowers (*H. annuus*) has been generally thought to be related to the trichome exudates.[105] Chemical investigation indicated that the STLs **36**, **39**, **56**, **57**, **73–76**, and **84** could be localized in the GTs either from leaves or anther appendages of sunflowers.[27,105–107] The experimental sensitization in guinea pigs revealed that the hemiketal-containing 1-*O*-methyl-4,5-dihydroniveusin A (**84**) induced the strongest response among the tested compounds.[105]

Cnicin (**35**) also displayed termiticidal activity against the Formosan subterranean termite *Coptotermes formosanus*,[108] and antifeedant activity against the larvae of the generalist noctuid moth *Spodoptera littoralis*.[109] The STLs from sunflowers were antifeedants and neurotoxicants of the adult rootworm *Diabrotica virgifera*, and the most potent compound was epoxylactone angelate argophyllin A (**56**).[110,111] Argophyllin B (**57**) reduced the larval mass of the sunflower moth *Homeosoma electellum*.[112] The dichloromethane-rinsed GTs extract of *Tithonia diversifolia* showed antifeedant activity against *Chlosyne lacinia* larvae, which led to the identification of *seco*-guaianolide **118** and guaianolides **150**, **151**.[76]

Phytotoxicity was reported for calamenene-type sesquiterpenoids **121–127** found in the dichloromethane rinsate of camphorweed (*Heterotheca subaxillaris*) aerial organs. Among them, 2-methoxy-calamenene-14-carboxylic acid (**121**) showed the strongest inhibitory activity against the seedling growth of *Agrostis stolonifera* and *Lactuca sativa*, as well as duckweed (*Lemna pausicostata*).[113] Pseudoguaianolides parthenin (**135**)



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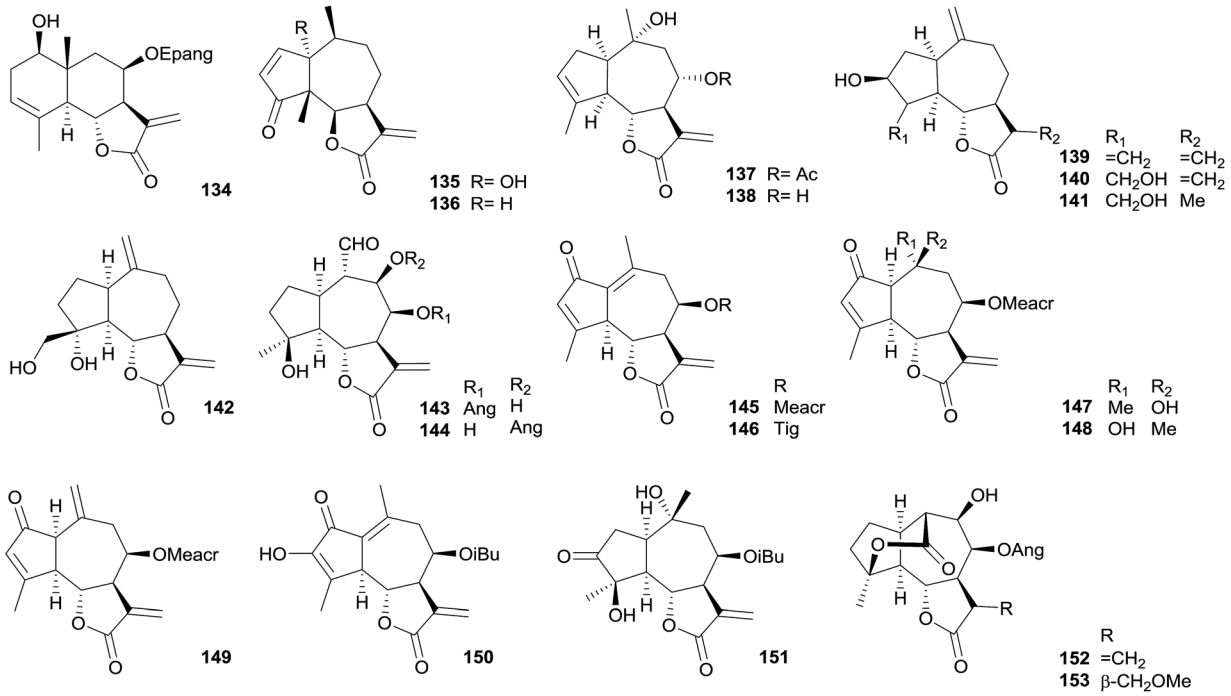


Fig. 8 The chemical structures of sesquiterpenoids 23–153 from plant GTs.

and ambrosin (**136**) were found in the GTs of *Parthenium hysterophorous*, an invasive flowering annual weed that causes allergic contact dermatitis.[73] Its invasive ability might be related to the phytotoxic and allelopathic properties of **135**.[114,115]

Cumambrins A (**137**) and B (**138**) were localized to the GTs of *Artemisia nova*, and antibacterial activity was observed for **137**, whereas **138** bearing a free hydroxyl group at C-8 was not active against any of the tested bacterial strains.[116,117]

3.1.2 Diterpenoids. Thirty-four non-volatile diterpenoids (**159–192**) have been found from the GTs of different plant species, including five skeletal types, cembrane (**159–164**), labdane (**166–176**), clerodane (**165**, **177–185**), abietane (**186–187**), and kaurene (**188–192**) diterpenoids (Fig. 10, Table S2†), mostly from Lamiaceae, Solanaceae, Asteraceae, and Acanthaceae families. Broad biological activities were reported for these diterpenoids, such as psychoactive, trypanocidal, anti-malarial, and anti-inflammatory activities, and some ecological functions were also described.

(−)-Kolavenol (**165**), (−)-hardwickiic acid (**180**) and salvinorins A–D (**182–185**) were found to be accumulated in the peltate GTs of the psychoactive sage *S. divinorum*.[3,48] Salvinorin A (**182**), a major bioactive component of this plant, was the first known diterpenoid with psychoactive effects as a non-nitrogenous hallucinogen.[118,119] A more intense hallucination effect was observed for **182** as compared to the common hallucinogens such as lysergic acid diethylamide (LSD) and mescaline, whose target is the 5-HT_{2A} receptor.[118,119] A pharmacological study indicated that **182** was a potent and selective κ opioid receptor agonist, indicating its therapeutic potential for perceptual distortions related diseases (*e.g.*, schizophrenia, dementia, and bipolar disorders), pain, drug addiction, and gastrointestinal disturbances.[120] Besides, **180** is a selective δ-opioid receptor (DOR) agonist.[121] Accumulated evidence has suggested the important role of the opioid system in the control of neurological disorders, for example, the DOR modulation has multiple neuroprotective functions.[122]

(−)-Kolavenol (**165**) a biosynthetic precursor of **182** was found to have trypanocidal activity (IC_{50} = 8.6 μM) against *Trypanosoma brucei rhodesiense*, which causes the acute form of human African trypanpsomiasis.[123] *ent*-Kaurenoic acid (**188**) and grandiflorenic acid (**189**) were detected in the GTs of *Montanoa tomentosa*,[124] both showing antimalarial activity against *P. falciparum* chloroquine-resistant clone W2, with IC_{50} values of 21.3 and 23.3 μM, respectively.[125,126] Tobacco (*N. tabacum*) is an important economic crop of Solanaceous plants, and cembranoids (**159–164**), *cis*-abienol (**166**), and 15-hydroxy abienol (**167**) were identified from the exudate of its GTs.[25,58,127] Cembratriene-diols (CBT-diols) **163** and **164** can block behavioral sensitization to nicotine, and function as noncompetitive antagonists of nicotine acetylcholine receptors (AChR).[128] Moreover, neuroprotective activity was observed for **163** in *in vitro* and *in vivo* models of brain ischemia, suggesting that **163** could be a lead compound in developing ischemic stroke medications.[129]

(−)-Sclareol (**168**), accumulated in the GTs of clary sage *S. sclarea*, has been used as a starting material for Ambrox production, which is an ambergris analogue used in the flavour and fragrance industries.[130,131] Immune-regulating, anti-tumour, and anti-osteoarthritic activities of **168** were also reported both *in vitro* and *in vivo*.[132]

Leoheterin (**174**) and galeopisin (**175**) were found in the GTs of peltate in *Leonurus japonicus*, a traditional Chinese medicine

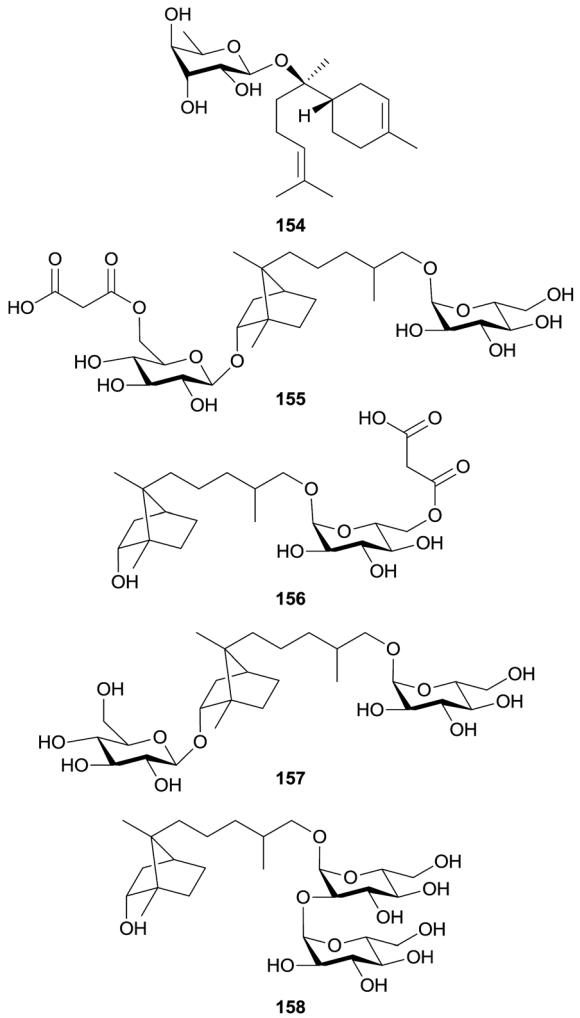


Fig. 9 The chemical structures of sesquiterpenoids **154–158** from plant GTs.

for the treatment of gynecological diseases.[133] **174** showed potential antithrombotic activity through the inhibition of arachidonic acid-induced rabbit platelet aggregation. Besides, moderate anti-inflammatory and anti-proliferative effects were observed for **174** and **175**.[133]

Carnosic acid (**186**) and carnosol (**187**) were found to be abundant in the GTs of rosemary (*Rosmarinus officinalis*).[134] Both compounds showed anti-oxidative, antimicrobial, and neuroprotective effects, and have been widely used in the food, nutritional health and cosmetics industries.[135,136] Meanwhile, **187** is a potent Nrf2/ARE pathway activator with low toxicity, exhibiting neuroprotective activity in the mouse model.[136]

The diterpenoids secreted by GTs and aggregated on plant surfaces usually serve as defense chemicals against insects and microbes. *Peronospora tabacina*, a fungal pathogen causing tobacco blue mold, has been a major economic threat. α-4,8,13-Duvatriene-1,3-diol (α-DVT) (**159**) and β-DVT (**160**) showed significant inhibitory activity against the sporangia germination of *P. tabacina*, with IC_{50} values of 3.0 and 2.9 μg cm^{-2}, respectively.[137] *cis*-Abienol (**166**) and (−)-sclareol (**168**) inhibited wilt disease of tobacco, tomato, and *Arabidopsis* plants caused by the soil-borne bacterial pathogen *Ralstonia solanacearum*.[138] Moreover, **168** can induce plant resistance against the root-knot nematode by ethylene-dependent enhancement of lignin accumulation.[139]

Paraguhenryisins A–D (**169–172**) and physacoztomatin (**173**) are localized to the capitate GTs of *Paragutzlaffia henryi* (Acanthaceae).[53] Phytotoxic activity of **171** and **172** on the inhibition of seed germination and seedling root elongation of *Arabidopsis thaliana* was observed, and **171** was the most abundant and active compound, indicating a role of these diterpenoids as alleochemicals of *P. henryi*.[53] Seguiniilactones A–C (**177–179**) were identified in the peltate GTs of *C. seguinii*, exhibiting significant antifeedant activity against a generalist insect *Spodoptera exigua*, and seguiniilactone A (**177**) (EC_{50} = 0.22 μg cm^{-2}) was approximately 17-fold more potent than the commercial neem oil (1% azadirachtin, EC_{50} = 3.71 μg cm^{-2}).[140] Besides, the moderate antifungal activity of seguiniilactone C (**179**) was observed against six predominant fungal strains isolated from the diseased leaves of *C. seguinii*. Salvianduline D (**181**) secreted from the GTs of *Salvia blepharophylla*[141] showed phytotoxic activity against the seed germination of *Papaver rhoeas* and *Avena sativa*.[142]

The kaurene glycoside stevioside is a natural sweetener found from *Stevia rebaudiana* (Asteraceae). It was reported that the stevioside content in the leaves is positively correlated with its GTs distribution, but no direct proof is yet available for the accumulation of stevioside in the GTs.[143]

3.1.3 Sesterterpenoids. So far, only eight sesterterpenoids (**193–200**) have been reported from plant GTs by our laboratory, and exclusively from the Lamiaceae family (Fig. 11, Table S3†). Investigations on the chemistry and biological functions of the GTs of two Himalayan-Chinese Lamiaceae plants have led to the discovery of two unique classes of defensive sesterterpenoids with novel skeletons, leucosceptrane and colquhounane. Leucosceptroids A–C (**193–197**) were isolated from the large woody *L. canum* with an unusual dark brown colored nectar and were found to be major components of the GTs.[38,144] These compounds are potential deterrents against the larvae of two generalist herbivores, namely, the beet armyworm (*S. exigua*) and cotton bollworm (*Helicoverpa armigera*). Their inhibitory effect on the growth of four agricultural pathogenic fungi was also observed.[38]

Through LMD-UPLC-MS/MS analysis followed by target compound isolation, colquhounoids A–C (**198–200**) were discovered from the peltate GTs of *C. coccinea* var. *mollis*. Similar antifeedant and antifungal activities were also observed for them.[35] It is noteworthy that the natural contents of these two classes of sesterterpenoids in leaves were shown to be comparable to or even higher than their antifeedant EC_{50} values, suggesting that the amounts of these compounds in the plants were sufficiently high to deter the feeding by generalist insects in nature.[35,38]

3.1.4 Triterpenoids. Twenty-two triterpenoids (**201–222**) have been reported from plant GTs (Fig. 12, Table S4†). The widely distributed pentacyclic triterpenoids ursolic acid (**201**) and α-amyrin (**202**) were isolated from the leaf surface extract of

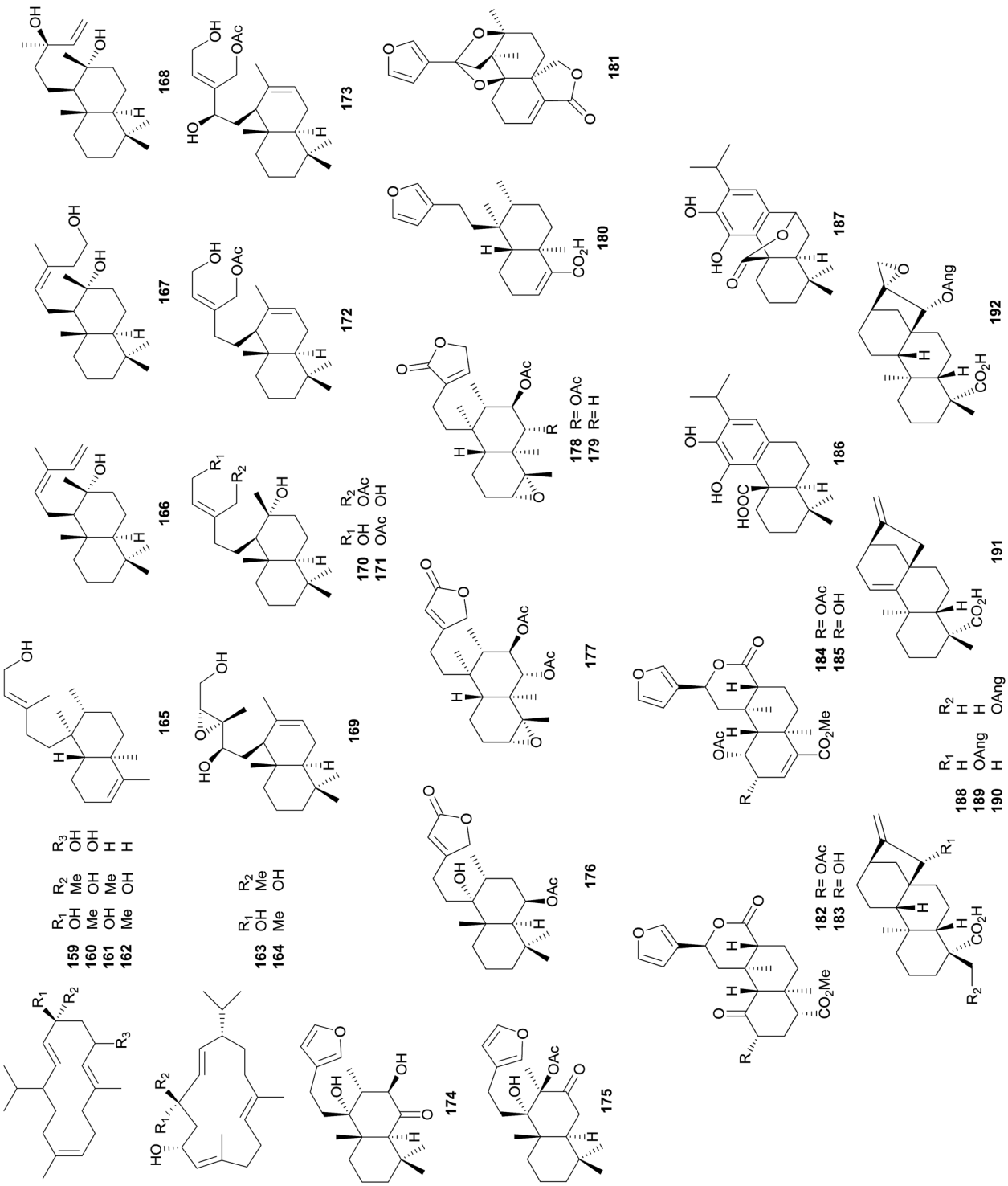


Fig. 10 The chemical structures of diterpenoids **159–192** from plant GTs.

S. blepharophylla, and were thought to be secreted from the GTs as waxy coatings of leaves for protecting the plant.[141] Broad pharmacological activities of **201** and **202** have been reported, including anti-cancer, anti-inflammatory, antioxidant, and hepatoprotective activities, and so on.[145] Two dammarane triterpenoids **203** and **204** were identified from the GTs on the stipules and leaves of *Cerasus yedoensis* (Rosaceae); their secretion was only observed during the spring time.[146]

Papyriferic acid (**222**) is a major product secreted by the GTs of the current-annual-growth twigs of the juvenile paper birch (*Betula neoalaskana*) and *B. pendula*, which was found to deter feeding by the snowshoe hare (*Lepus americanus*).[147] Pharmocological studies and molecular modelling revealed that **222** inhibited the succinate dehydrogenase activity of the snowshoe hare.[148,149]

3.1.5 Meroterpenoids. Meroterpenoids (not including routine prenylated compounds) were mainly reported from the GTs of *Cannabis sativa*, which has been well known for its production of the psychoactive terpenophenolic cannabinoids. Recently, cannabinoids **223–231** were precisely localized to the capitate-sessile and capitate-stalked GTs by a combination of LMD, LC-MS, and cryogenic NMR (Fig. 13, Table S5†).[34]

These cannabinoids were stored in the secretory cavities to protect the plant from insects and prevent autotoxicity, as the cytotoxic effect of Δ^9-tetrahydrocannabinolic acid (THCA, **223**) and cannabichromenic acid (CBCA, **229**) on plant cells was already observed.[150] Through years of endeavours, a number of molecular targets for phytocannabinoids, such as cannabinoid receptors (CB_1 and CB_2), glycine receptors, transient receptor potential (TRP) channels and G protein-coupled receptors have been identified.[151] Δ^9-Tetrahydrocannabinol (Δ^9-THC, **224**) is the most abundant and well-studied phytocannabinoid, accounting for the psychoactive property of marijuana. So far three medications based on cannabinoids, dronabinol (synthetic **224**), nabiximols [a 1 : 1 mixture of **224** and cannabidiol (CBD, **226**)] and Epidiolex (**226**) have been licensed. Dronabinol was approved by the U.S. Food and Drug Administration (FDA) in 1985 for treatment of chemotherapy-induced nausea and vomiting, and in 1992 for treatment of AIDS-associated anorexia and weight loss. Nabiximols was approved in the U.K. in 2010 for the treatment of multiple sclerosis. Moreover, **226** was demonstrated to be an anti-convulsant for pediatric epilepsy,[151,152] and was approved by the FDA in 2018 for the treatment of two rare and severe forms of epilepsy, Lennox-Gastaut and Dravet syndromes. In addition, it was reported that **226**, cannabigerol (CBG, **228**), cannabichromene (CBC, **230**) and cannabinol (CBN, **231**) stimulated and desensitized human TRP channels, which are relevant to the analgesic, anti-inflammatory and anti-cancer effects of cannabinoids.[153]

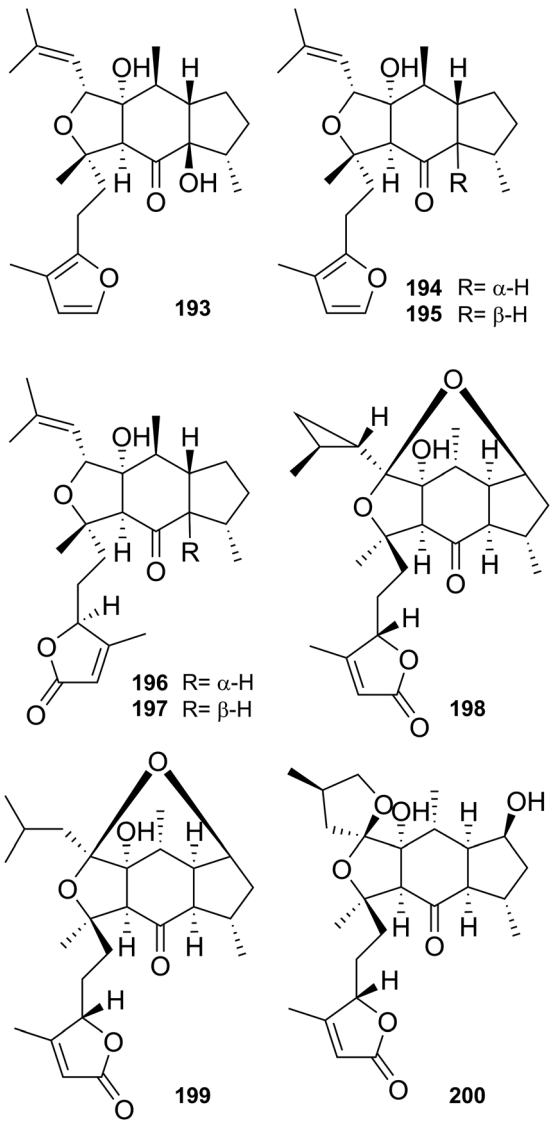


Fig. 11 The chemical structures of sesterterpenoids **193–200** from plant GTs.

3.2 Simple phenolics

Fifty-two simple phenolics (**233–284**) have been identified in plant GTs (Fig. 14, Table S6†), including a series of prenylated phenolics containing a C_5–C_{20} terpene side chain (**233–254**, **263–269**), phenolics with either an unsaturated or saturated C_5–C_{17} aliphatic side chain (**255–261**), dihydrophenanthrenes (**270**, **271**), and acylphloroglucinols (**272–284**) including two polyprenylated compounds (**272**, **273**). Although the polyprenylated acylphloroglucinols show broad pharmacological activities, these phenolics usually serve as defense chemicals for the plants, exhibiting contact allergic, anti-insect, and antimicrobial effects.

Some phenolic compounds secreted by plant GTs have been found to induce allergic effects caused by touching the plants. Reynolds and co-workers investigated the trichome exudates of some *Phacelia* species, identified prenylated phenolics **233–246** as the principal contact allergens of this genus. Geranylgeranylhydroquinone (**233**) and geranylbenzoquinone (**240**) elicited significant allergenic reactions on guinea pigs with 0.01 and 0.007 μM applications, respectively.[154–156] Reynolds studied another hydrophyllaceae plant, *Turricula parryi*, which caused severe human dermatitis, and farnesylhydroquinone derivatives **246–254** were discovered from its GTs exudate.[157] The garden geranium (*Pelargonium hortorum*) has inbred genotypes resistant

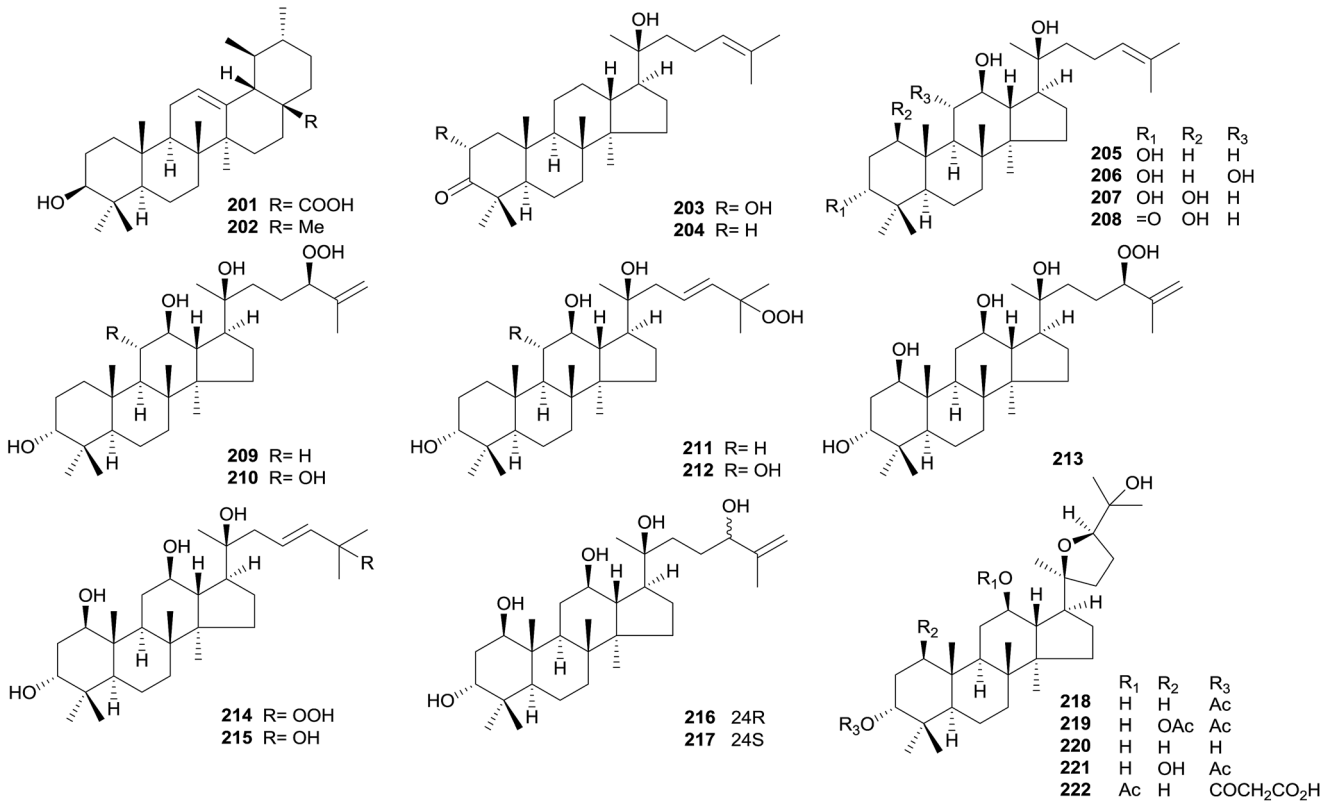


Fig. 12 The chemical structures of triterpenoids **201–222** from plant GTs.

or susceptible to pests. Unsaturated anacardic acids **255–258** were major constitutes of the trichome exudates of pest-resistant geranium. However, the pest-susceptible genotype lacked the ω^5 products but contained saturated 22:0 and 24:0 anacardic acids (**259**, **260**).[158,159] The unsaturated anacardic acids **255** and **256** could lower the growth rate and increase the mortality of the larvae of the Colorado potato beetle (*Leptinotarsa decemlineata*) by showing food deterrent effects.[160] In addition, **256** was reported to be a specific DNA polymerase β inhibitor ($IC_{50} = 1.4$ μM), which might potentiate the effect of DNA damage of cancer cells caused by clinical anti-cancer agents.[161] The benzoquinone primin (**261**) was isolated from the leaf surface extracts of *Primula obconica*,[162] and was found to have potent antiprotozoal and schistosomicidal activities against *T. brucei rhodesiense* ($IC_{50} = 0.14$ μM) and *Leishmania donovani* ($IC_{50} = 0.71$ μM), with low cytotoxicity to mammalian cells.[163] Compounds **261–263** were identified as major constituents excreted by the GTs of *Eriodictyon californicum*,

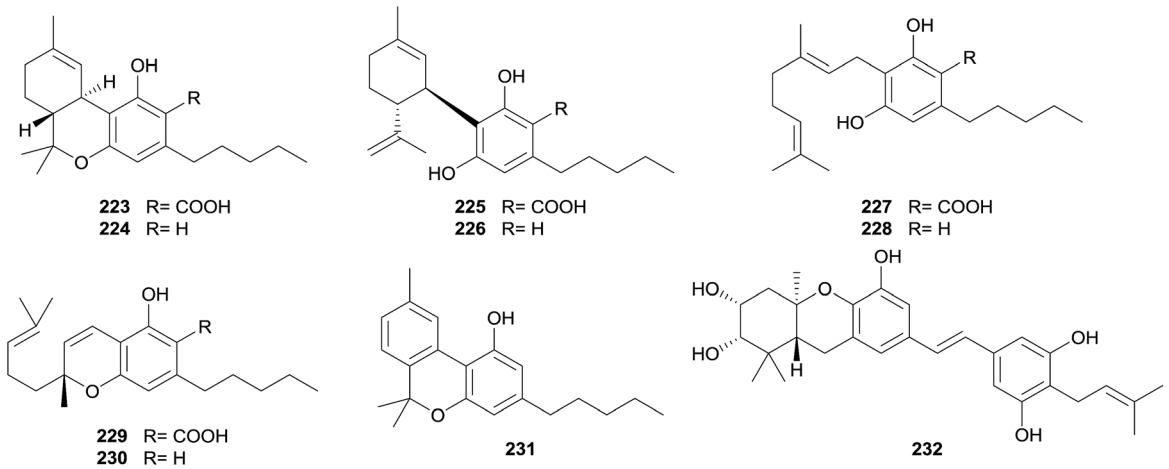


Fig. 13 The chemical structures of meroterpenoids **223–232** from plant GTs.

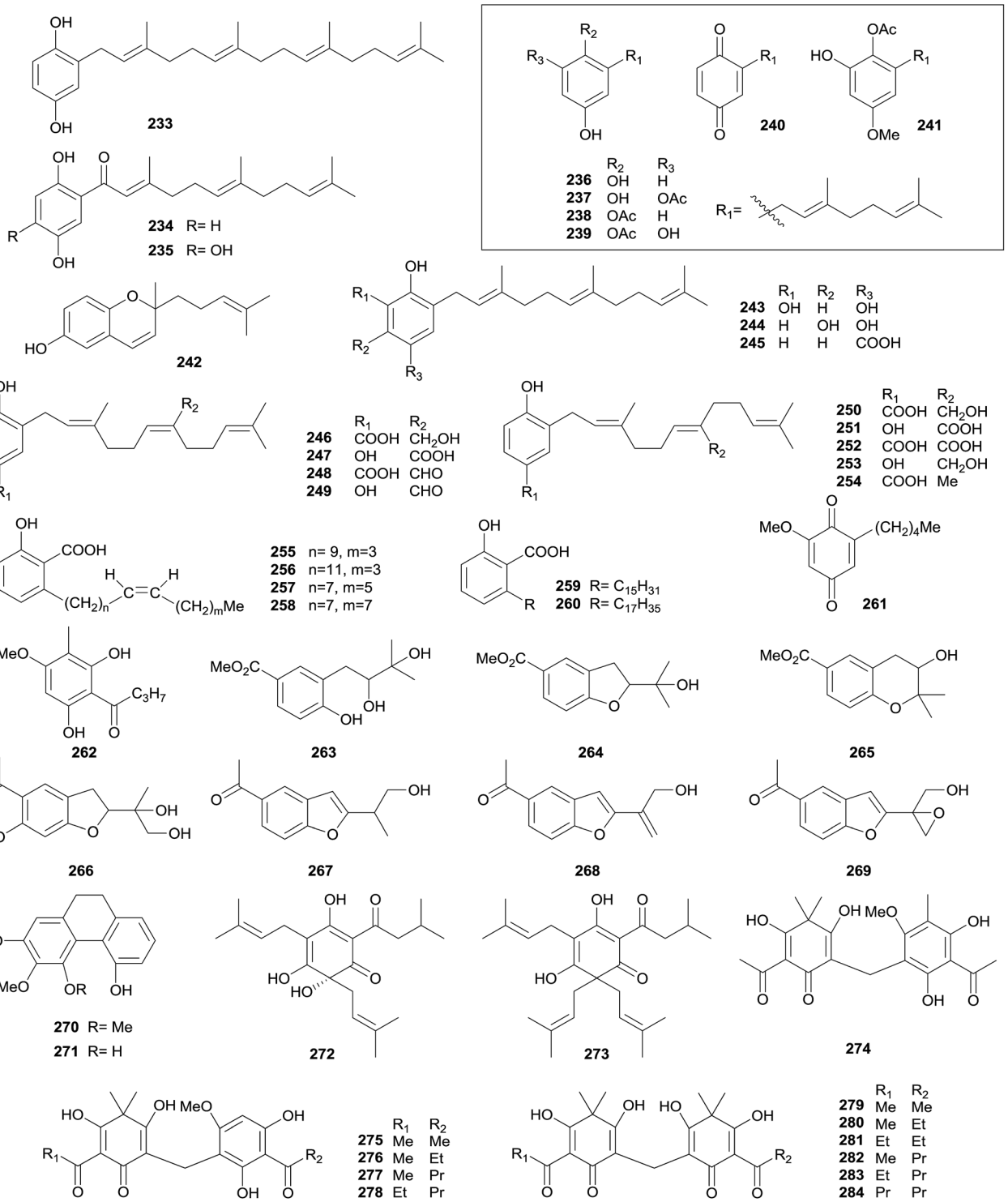


Fig. 14 The chemical structures of phenolics 233–284 from plant GTs.

being distributed on the leaves and twigs.[164] Antifungal activity was observed for compounds **263** and **265**.[165,166]

Hops (*H. lupulus*) is an important economic crop for the beer-brewing industry. The polyprenylated acylphloroglucinols humulone (**272**) and lupulone (**273**), also known as bitter acids and major compounds accounting for the flavour and biological activities of hops, were found to be accumulated with high concentrations in the GTs distributed on the adaxial surface of female cone bracts.[167,168] Compounds **272** and **273** exhibited various biological activities both *in vitro* and *in vivo*, such as anti-cancer, anti-inflammatory, and bactericidal activities, and had positive effects on metabolic disorders.[169]

Acylphloroglucinols **274–284** were found in the GTs exudates of the fronds of two *Dryopteris* ferns.[170] Among them, desaspidin PB

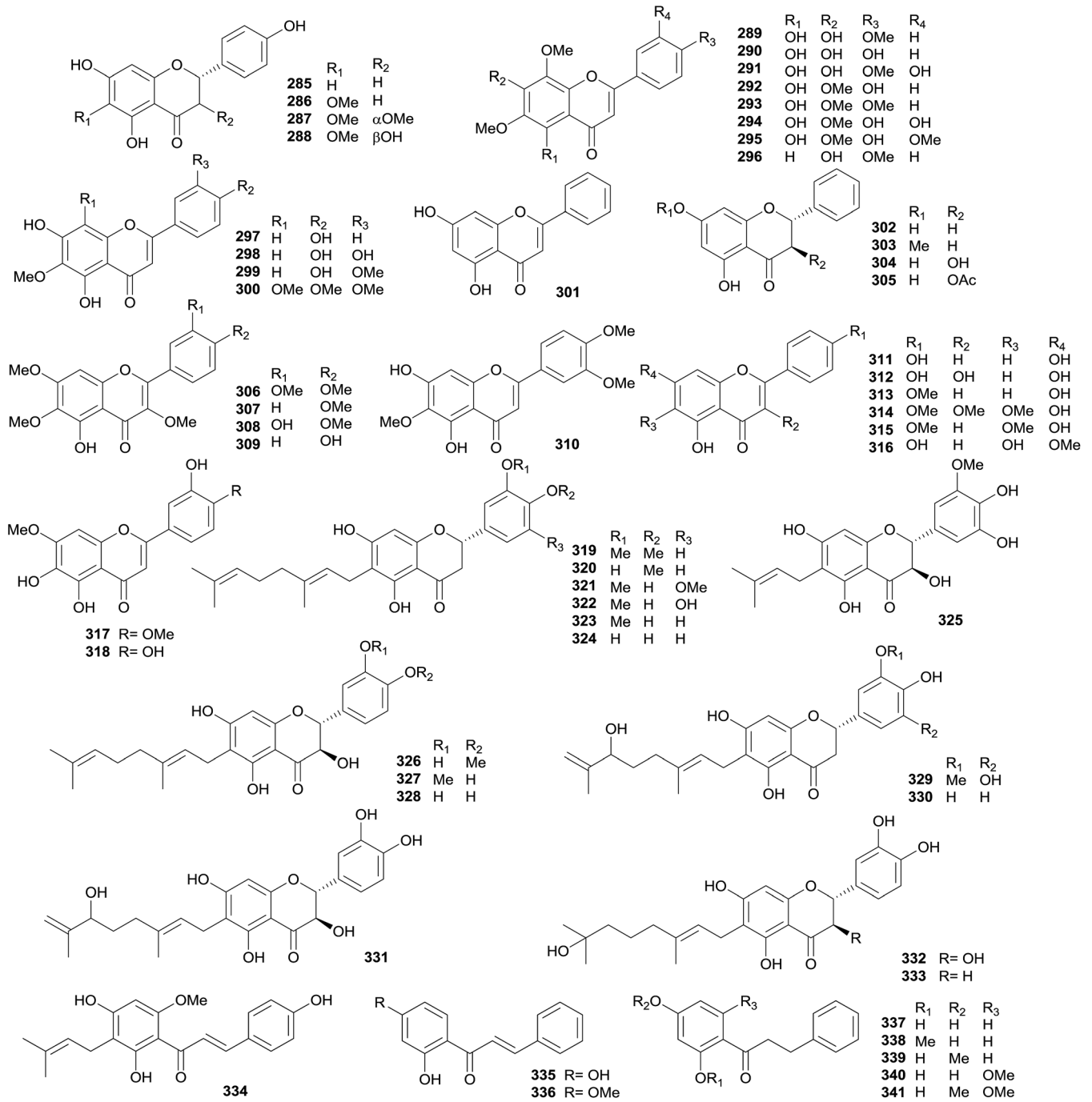


Fig. 15 The chemical structures of flavonoids **285–341** from plant GTs.

(**278**) exhibited antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, and *Dickeya zeae*, with minimal inhibition concentrations (MICs) ranging from 4 to 16 μg mL^{-1}.[171]

3.3 Flavonoids

Flavonoids are ubiquitous natural products in the plant kingdom, having extensive biological activities including anti-oxidative, anti-microbial, anti-cancer and anti-inflammatory activities. In total, 57 flavonoids (**285–341**) have been reported from plant GTs, including 16 prenylated compounds (**319–334**) with either a C_5 or C_{10} terpene side chain at their C6 position (Fig. 15, Table S7†).

Naringenin (**285**) was isolated as a major compound from the extracts of capitate GTs of two *Scalesia* species.[84] It displayed broad spectral bioactivities and therapeutic potential for the treatment of central nervous system diseases and cardiometabolic disorders.[172] Considering its efficacy and safety, this compound might be useful as a dietary supplement for the treatment of various human disorders.[172] Besides, **285**, **311–313** and some other derivatives were detected in the leaf GTs of five

Finnish birch species, *Betula pendula*, *B. pubescens* ssp. *pubescens*, *B. pubescens* ssp. *czerepanovii* and *B. nana*.[173]

Flavonoids found in the linear (**289–295**) and capitate (**296**) GTs of sunflowers (*H. annuus*) co-occurred with sesquiterpenoids.[174]

The co-occurrence of flavonoids with other natural products in plant GTs was thought to protect the plant from UV radiation or interact with pathogenic microorganisms.[175] Among them, nevadensin (**289**) showed anti-*Mycobacterium tuberculosis*, antitussive, anti-inflammatory, and anti-hypertensive effects,[176] and xanthomicrol (**292**) exhibited anti-spasmodic, anti-platelet, and anti-cancer effects.[177] Flavonoids **297–300** were identified in the GT exudates of three other *Helianthus* species, *H. angustifolius*, *H. floridanus*, *H. simulans*.[178] Hispidulin (**297**) is a potent benzodiazepine (BZD) receptor ligand that exhibited an anticonvulsive effect.[179] It also displayed anti-tumor activity by the suppression of pancreatic tumour growth and angiogenesis,[180] and the modulation of TRAIL.[181] Jaceosidin (**299**) was more potent than *cis*-platin in inhibiting the proliferation of human endometrial cancer cells.[182]

Using 'chip-based' nanospray tandem mass spectrometry, Leonardo and co-workers identified flavonoids **301–305** in the GTs of *L. ericoides*.[50] Chrysin (**301**) showed various bioactivities such as anti-inflammatory,[183] antiaging,[184] antiviral,[185] and anticancer activities,[186] but the low solubility has limited its pharmaceutical applications. Recently, chrysin nanoparticles encapsulated by copolymer were developed, which showed significantly improved bioavailability and efficacy as a chemotherapeutic agent in an A549-xenograft model *in vitro* and *in vivo*.[187] Pinocembrin (**302**) can inhibit neuroinflammation and protect against hemorrhagic brain, and its clinical trials for the treatment of ischemic stroke have been approved by the State Food and Drug Administration (SFDA) of China. More recently, **302** was reported as a promising drug candidate for the treatment of intracerebral hemorrhage and other acute brain injuries.[188]

The flavonoids **306–309** were identified from the leaf GTs exudate of *Laggera pterodonta*.[189] Anti-hypotensive and cytotoxic effects were observed for artemetin (**306**).[190,191] Casticin (**308**) was effective against multiple cancer cell lines *via* different molecular mechanisms.[192] Both **306** and **308** could potentiate the antimalarial effect of **119**.[193] Penduletin (**309**) could effectively inhibit the infection of enterovirus 71 (IC_{50} = 0.17 μM) and other human enteroviruses in cell culture.[194] The flavonoids **310** and **314–316** were identified in the GTs of *Artemisia umbelliformis* and *Heterotheca subaxillaris*, respectively.[113,195] The anti-tumour activities of eupatilin (**310**) and pectolinarigenin (**315**) have been reported; **310** inhibited the growth of human endometrial cancer cells[196] and **315** suppressed the tumour growth of nasopharyngeal cancer *in vivo*.[197] Nuchensin (**317**) and pedalitin (**318**) were isolated from the GTs of *S. blepharophylla*.[141] **318** is an inhibitor of the hepatitis C virus (HCV),[198] α-glucosidase,[199] and protein kinase CK2.[200] Prenylated flavonoids **319–333** were isolated from the secretion of GTs on the surface of immature fruits of *Paulownia tomentosa*. They showed potent scavenging effects on DPPH free radicals, with SC_{50} between 1.8 and 4.5 μg mL^{-1}.[201] The C-6 prenylated chalcone xanthohumol (**334**) is a potent and broad-spectrum cancer chemopreventive agent;[202] its antioxidative,[202] neuroprotective,[203] and α-glucosidase inhibitory[204] activities were also observed. The upper and lower epidermis of rolled hypostomatous leaves of *Empetrum nigrum* bear GTs, and investigation of the lipophilic exudates from the leaf surface has led to the identification of chalcones **335–341**.[205] Among them, 2′,4′-dihydrochalcone (**335**) showed selective anti-bacterial activity (IC_{50} = 23.8 μM) against *Mycobacterium tuberculosis* H37Ra.[206]

3.4 Acylsugars

Acylsugars appear to be rich in plant GTs. In total, 75 *O*-acyl sugars (**342–416**) consisting of either glucose or sucrose esterified by aliphatic acids of different chain lengths have been reported from the GTs of Solanaceae, Caryophyllaceae, Martyniaceae, and Geraniaceae species (Fig. 16, Table S8†). Acyl sugars can deter or repel aphids and beet armyworms, and adhere, immobilize or suffocate arthropods due to their sticky and surfactant properties.[11] The trichome exudate of *Datura wrightii* can reduce the larvae growth of *Manduca sexta*, and was identified as a complex mixture of sugar esters, with the major compound being 3′-*O*-hexanoyl glucose (**353**).[207] The GTs of *N. attenuata* stored a rich mixture of *O*-acyl sugars that can protect tobacco plants from fungal pathogens and a specialist herbivore, *Manduca sexta*. *O*-Acyl sugars **354–368** were characterized by UPLC/Q-TOF-MS analysis from the trichome exudate of *N. attenuata*.[208] Interestingly, these compounds play important roles not only in the direct defense but also in the indirect defense of wild tobacco. The *O*-acyl sugars in the GTs of *N. attenuata* can be digested by Lepidopteran herbivores (*e.g. M. sexta*) after feeding, and several volatile aliphatic acids are produced; the omnivorous predator *Pogonomyrmex rugosus* can then use these airborne chemical signals to locate their prey.[209]

3.5 Glycerides

Glycerides seem to be also rich in plant GTs. Altogether 67 glycerides (**417–483**) have been reported from the GTs of the Pedaliaceae, Martyniaceae, and Scrophulariaceae families (Fig. 17, Table S9†). Chemical investigation of the GTs exudate of *Ceratotheca triloba* revealed nine 1-*O*-acetyl-2-*O*-[(*R*)-3-acetyloxy-fatty acyl]-3-*O*-malonylglycerols (**417–425**), with **423** as the most abundant compound (41%).[210] The Martyniaceae plants *Lbicella lutea* and *Proboscidea louisiana* have rich GTs on their leaves and stems, and 2-*O*-[(3*R*,6*S*)-3,6-diacetyloxyfattyacyl] glycerols (**426–430**, **436–440**, and **446–448**) and their deacetoxy compounds 2-*O*-[(3*R*)-3-acetyloxyfattyacyl]glycerols (**431–435**, **441–445**, and **449–450**) were detected in the GTs exudates of both plants.[211] Glycerides **450–453** and **454–483** were isolated from the GTs exudates of two Scrophulariaceae species, *Verbascum blattaria* f. *erubescens* and *Paulownia tomentosa*, respectively.[212,213] However, the biological significance of these trichome glycerides are yet to be investigated.

3.6 Miscellaneous

Some fatty acids stored in the GTs were also found. 2-Undecanone from the type VI GTs of wild tomato *Lycopersicon hirsutum* f. *glabratum* caused the larval and pupal deformity and

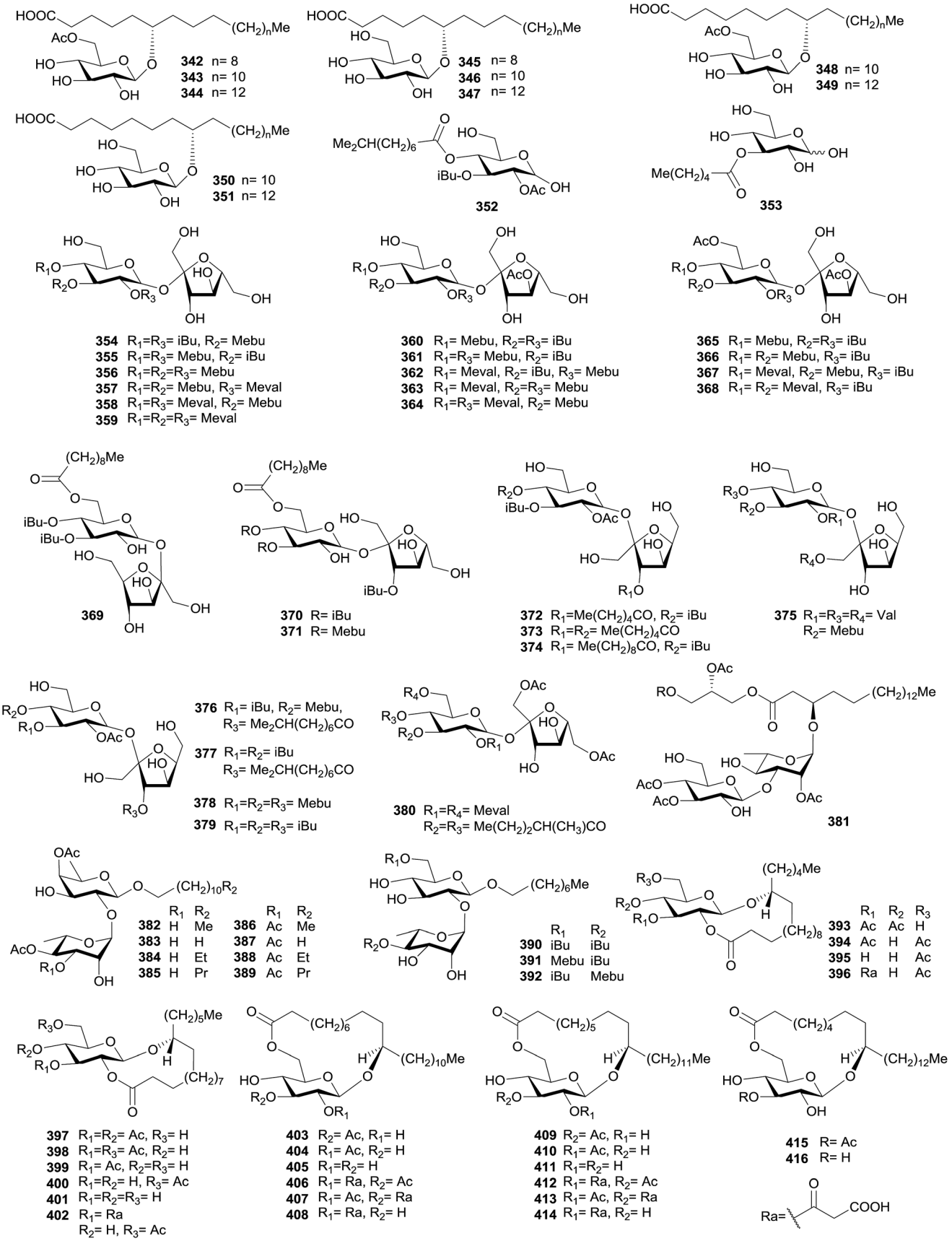


Fig. 16 The chemical structures of acylsugars 342–416 from plant GTs.

mortality of *Heliothis zea*.[214,215] Palmitic acid, stearic acid, linoleic acid, and linolenic acid were identified in the leaf extracts of *Clytostoma callistegioides* (Bignoniaceae) and further localized to the GTs by histochemical staining.[216] A series of *N*-(3-methylbutyl)amides of saturated C_{14}–C_{18} aliphatic acids were characterized as major components of the GTs extracts of an alfalfa genotype, *Medicago sativa* G98A, resistant to potato leafhopper (*Empoasca fabae*). Bioassays revealed that the *N*-(3-methylbutyl) amide of linoleic acid exhibited deterrence against leafhopper settling in a dose-dependent manner.[217]

Alkaloids are rarely found in plant GTs. *N*-Hydroxyacylnornicotine was detected in the trichome exudate of *N. stocktonii*, and was suggested as an *N*-acylated product of nornicotine.[55] Six *N*-acyl nornicotines (**484–489**, Fig. 18, Table S10†) were then isolated from the GTs secretions of *N. repanda* and identified by GC-MS, which were more toxic than nicotine to the nicotine-adapted insect herbivore, *Manduca sexta*.[218] In addition, the content of the well known antineoplastic alkaloid camptothecin in leaves of *Camptotheca acuminata* (Nyssaceae) was shown to be positively correlated to its trichome density and size, but further study announced that this compound was produced in resin ducts rather than in the GTs.[11,219]

4. Biosynthesis and regulation of non-volatile natural products in plant GTs

The biosynthesis of plant natural products has long been of great interest to both biologists and chemists. However,

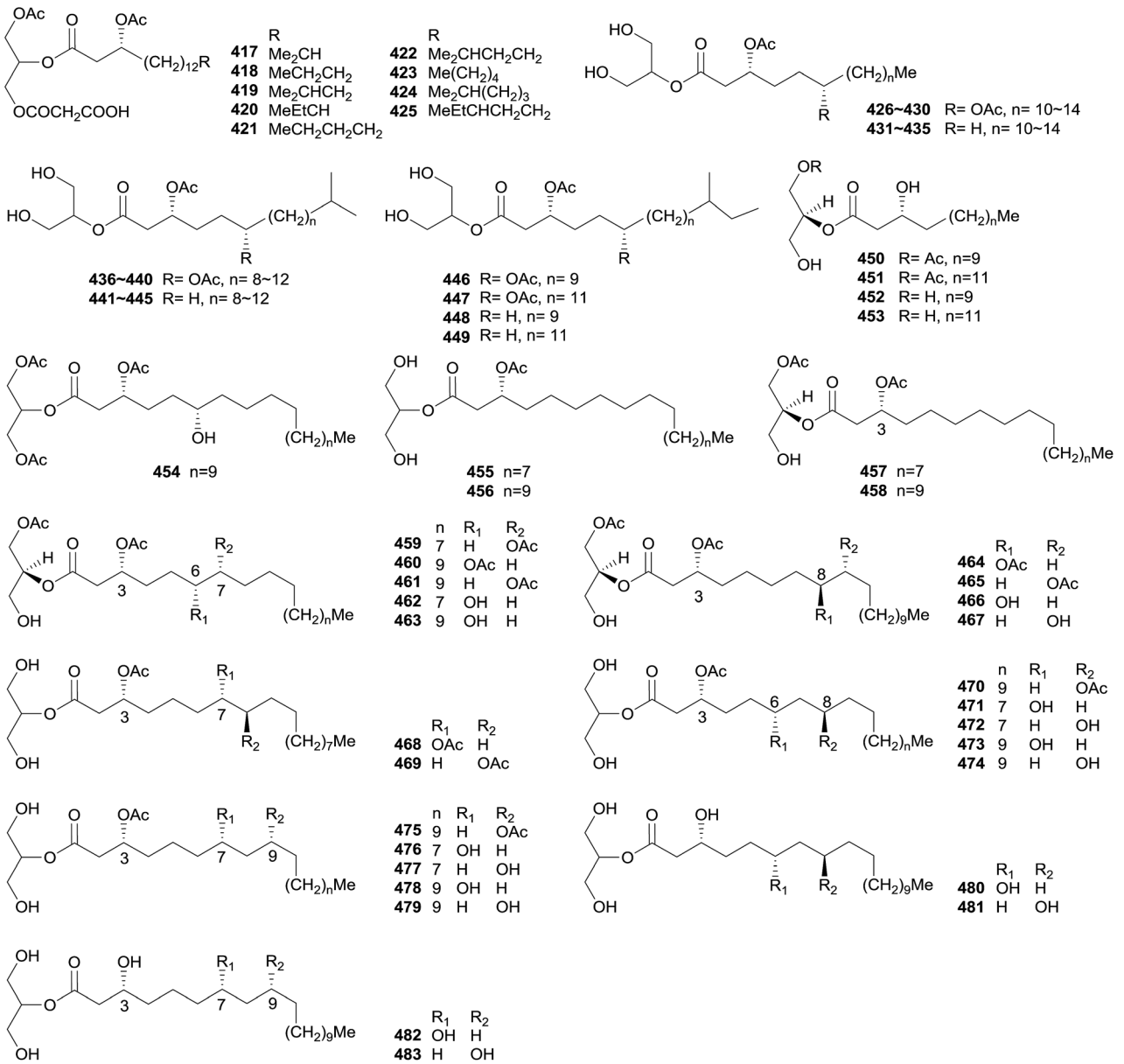


Fig. 17 The chemical structures of glycerides **417–483** from plant GTs.

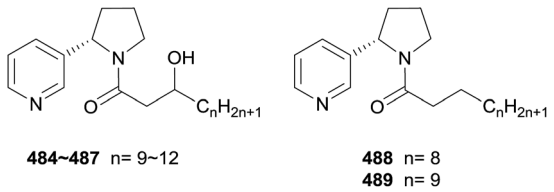


Fig. 18 Chemical structures of *N*-acyl nornicotines **484–489**.

elucidating the biosynthetic pathways of plant natural products has been frequently challenging due to the huge size of a plant genome, the large protein family, the lack of any genomic information in the vast majority of plant species, and very limited knowledge of the extent of gene clustering in plant secondary metabolism. Natural products accumulated in plant GTs have been well known to also be biosynthesized there, which provide tremendous opportunities to explore the biosynthesis and regulation of these special compounds through the isolation of GTs and the mRNA and proteins they contain. In particular, due to the benefits from the rapid development of "omics" techniques, such as transcriptomics and metabolomics, a large number of genes and enzymes involved in the biosynthesis and regulation of plant natural products have been discovered and characterized, which provides an avenue for the large-scale production of bioactive compounds using synthetic biology.

4.1 Non-volatile terpenoid biosynthesis and regulation

Terpenoids represent the largest and structurally most diverse class of natural products and are constructed from two fundamental isoprene units, isopentenyl diphosphate (IDP, C_5) and its isomer dimethylallyl diphosphate (DMADP, C_5). In plants, IDP and DMADP are synthesized *via* either cytosol-localized mevalonic acid (MVA) pathway or plastidial 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway. Condensation of IDP and DMADP leads to the formation of linear precursors, which then undergo cyclization and subsequent post-modification reactions to generate an enormous diversity of terpenoids, including monoterpenoids (C_{10}), sesquiterpenoids (C_{15}), diterpenoids (C_{20}), sesterterpenoids (C_{25}), triterpenoids (C_{30}), and so on. Using isolated GTs incubation with labelled precursors, such as $^{14}CO_2$, ^{14}C-sucrose, ^{14}C-acetate, or ^{14}C-mevalonic acid, GTs were suggested to be the sites of terpenoid biosynthesis as well as secretion in several plants.[29,54,220] Recently, GTs-specific omics studies on genes or proteins involved in the early steps of terpenoid biosynthetic pathways further supported that GTs are likely the major sites of terpenoid biosynthesis in many plants.[5,221–223] The biosynthetic pathways of some GTs-specific terpenoids have also been illuminated, which opens up opportunities for the metabolic engineering of terpenoids with high value, as exemplified by artemisinin (**119**), carnosic acid (**186**) and cannabinoids.

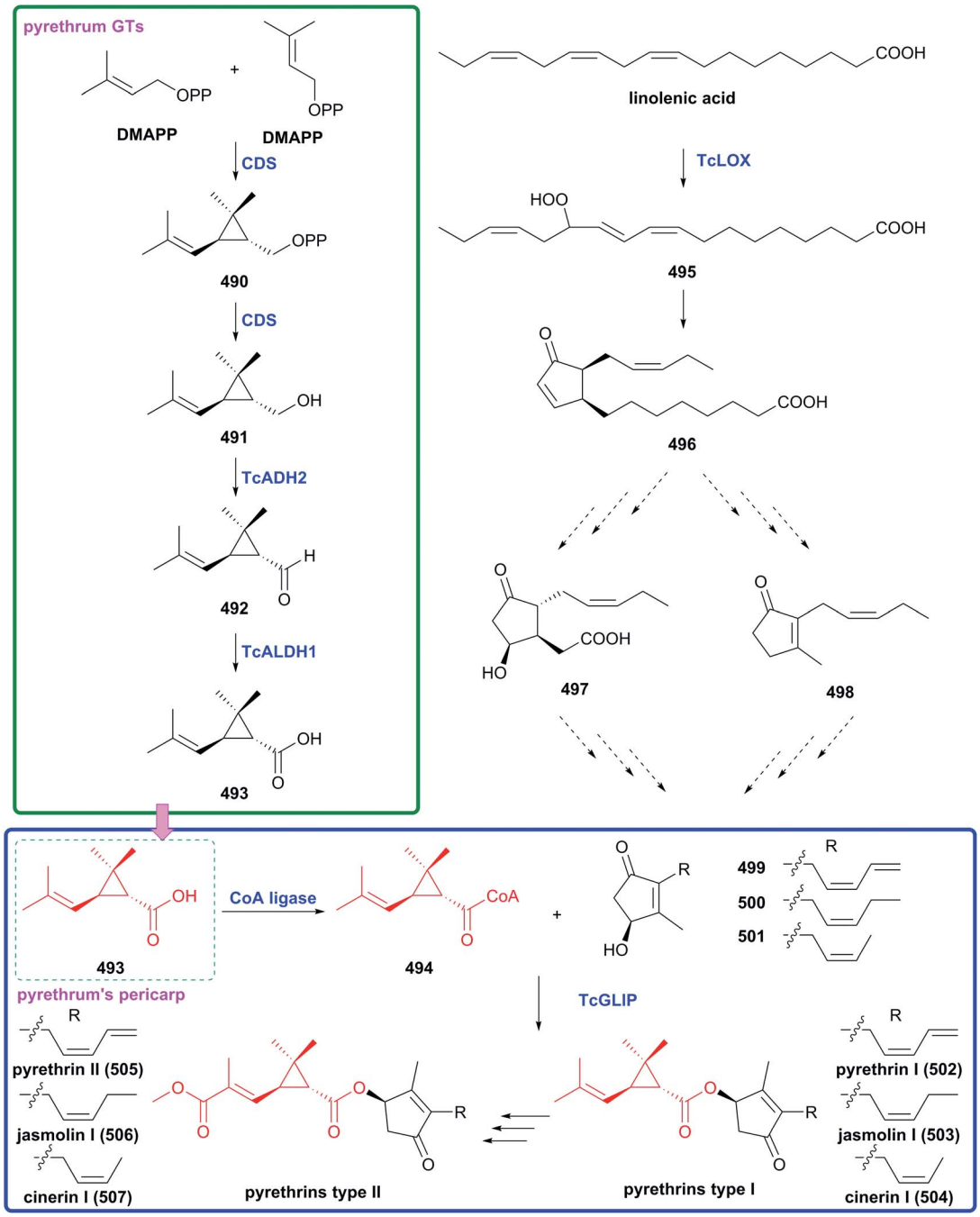
4.1.1 Monoterpenoid biosynthesis. Pyrethrins (**502–507**) are a group of irregular monoterpenoid esters produced predominantly in the flower achenes of pyrethrum (*Tanacetum cinerariifolium*), and are economically important botanical insecticides due to their safety and effectiveness. The monoterpenoid acid moiety, *trans*-chrysanthemic acid (**493**) or pyrethric acid, possessing a cyclopropane ring is biosynthesized from two molecules of DMADP (rather than the condensation of DMADP and IDP found in classic monoterpenoid biosynthesis) derived from the plastid-localized MEP pathway.[224] The first dedicated step is an intermolecular C1′-C2-C3 cyclopropanation reaction of DMADPs (head-to-middle linkage) to form *trans*-chrysanthemyl diphosphate (**490**). The enzyme responsible for this step was characterized as chrysanthemyl diphosphate synthase (CDS) from pyrethrum and sagebrush (*Artemisia tridentata*), which belonged to the chain elongation prenyltransferase family of the terpenoid synthase superfamily.[225–227] The resulting **490** could be immediately dephosphorylated by CDS to form *trans*-chrysanthemol (**491**).[228] Recently, two oxidoreductases, TcADH2 and TcALDH1, responsible for two sequential oxidation reactions of **491** first to *trans*-chrysanthemal (**492**) and then to **493**, were cloned and identified from pyrethrum (Scheme 1).[229]

The alcohol moiety, pyrethrolone (**499**), jasmolone (**500**) or cinerolone (**501**), shows structural resemblance to plant hormones jasmonic acid (JA) and *cis*-jasmone (**498**), and is likely biosynthesized from linolenic acid *via* the oxylipin pathway.[224] Hydroperoxidation of linolenic acid at the C13 position to generate 13-hydroperoxylinolenic acid (**495**) is considered as the first step of the alcohol moieties biosynthesis, and a lipoxygenase TcLOX catalyzing this reaction was functionally characterized from pyrethrum.[230] The remaining steps for the alcohol moieties biosynthesis are still unknown, which were supposed to be biosynthesized either from 7-hydroxyjasmonic acid (**497**) by desaturation and decarboxylation or from **498** by hydroxylation and desaturation (Scheme 1).[224]

The final esterification that transferred a chrysanthemoyl group from the coenzyme A (CoA) thioester to one of the alcohol moieties was performed by the *T. cinerariifolium* GDSL lipase-like protein (TcGLIP), a member of the GDSL lipase family (Scheme 1).[231] Intriguingly, both pyrethrum CDS gene and **491** were found to be exclusively present in the GTs, suggesting that the biosynthesis of **491** and **493** should occur in the GTs.[232] However, GTs only contained one-quarter of **493** with the remaining three quarters in the pericarp, and thus **493** was supposed to be transported to the pericarp where it was used by the pericarp-specific GLIP to synthesize pyrethrins (**502–507**). Accordingly, pyrethrum GTs could selectively biosynthesize the monoterpenoid moiety of pyrethrins, which was then transported in the basipetal direction towards the pericarp where it could be further esterified into pyrethrins (**502–507**) and accumulated in intercellular spaces. The dominant expression of *TcLOX1* in the GTs suggested the possibility that the alcohol moieties of pyrethrins might also be biosynthesized in pyrethrum GTs and then exported to the pericarp in a similar mechanism to the monoterpenoid moiety biosynthesis.

4.1.2 Sesquiterpenoid biosynthesis and regulation

4.1.2.1 Artemisinin biosynthesis and regulation in A. annua GTs. Artemisinin (**119**, also called Qinghaosu) is one of the most famous and representative terpenoids found to be biosynthesized and accumulated in the plant GTs. The linear



Scheme 1 Summary of the biosynthetic pathway of pyrethrins (**502**–**507**) in pyrethrum GTs. Dashed arrows indicate putative steps.

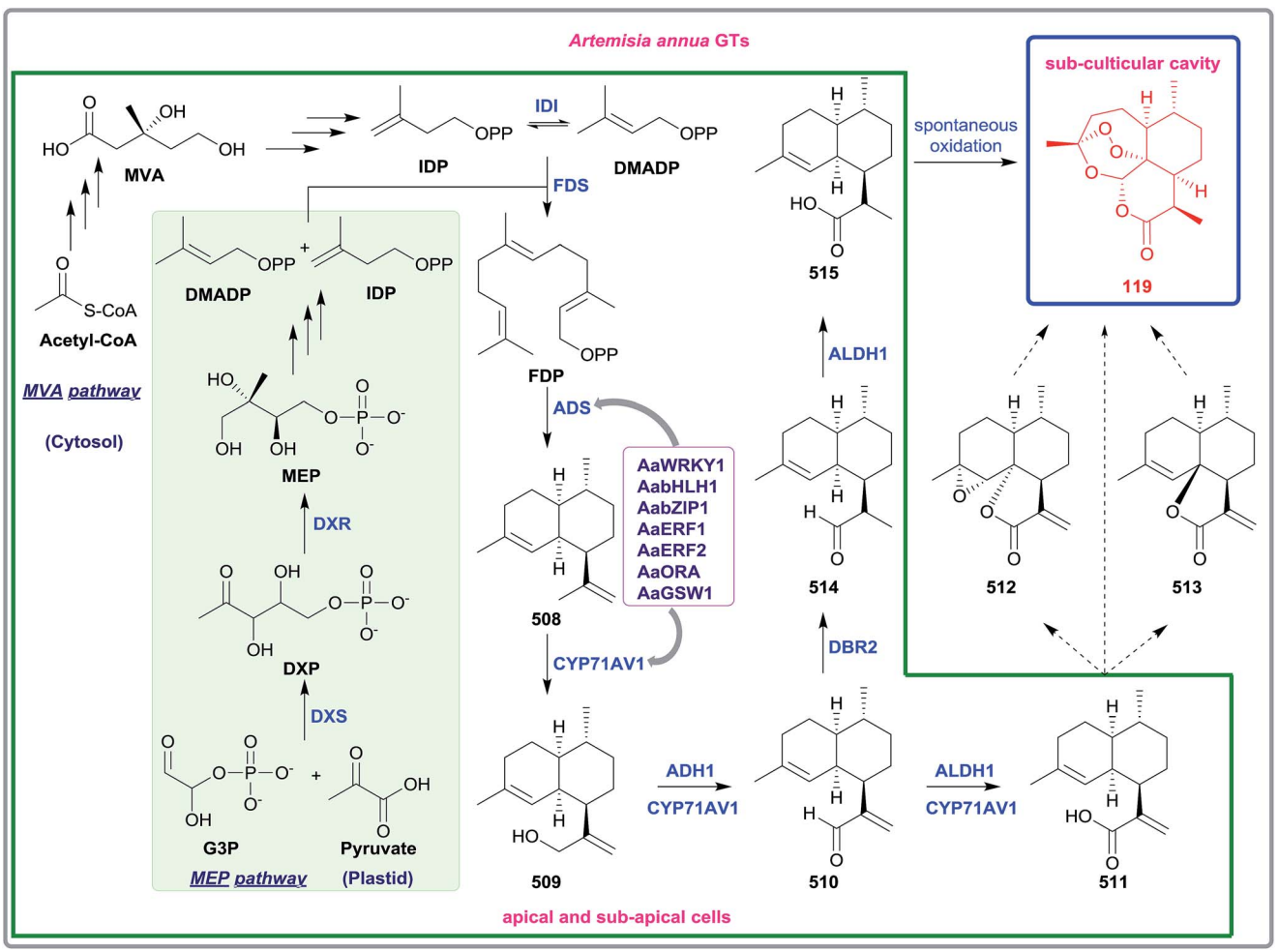
precursor of **119**, (*E*,*E*)-farnesyl diphosphate (FDP), is synthesized from IDP and DMADP derived from both the cytosol-localized MVA pathway and the plastid-localized MEP pathway.[233,234] The first step is the cyclization of (*E*,*E*)-FDP to amorpha-4,11-diene (**508**), catalyzed by terpene cyclase amorpha-4,11-diene synthase (ADS).[235–238] Subsequently, a multifunctional cytochrome P450 amorpha-4,11-diene 12-hydroxylase (CYP71AV1) catalyzes the regioselective hydroxylation of **508** at the C12 position yielding artemisinic alcohol (**509**), followed by oxidization of the alcohol to the corresponding aldehyde (**510**) and acid (**511**).[59] The ADH1 gene encoding an alcohol dehydrogenase is specific toward **509** for its oxidation to the aldehyde.[239]

The next steps after the successive oxidation of **508** still remain controversial. The early isotope feeding experiments with plant homogenates or cell-free systems indicated that artemisinic acid (**511**) might be the key intermediate of **119** biosynthesis.[240,241] However, the *in vivo* feeding experiments conducted by Brown and Sy indicated that **511** could not be converted directly into **119**.[242] Instead, arteannuin B (**512**) and *epi*-deoxyarteannuin B (**513**) were considered as the possible biogenetic precursors of **119** by Nair and Basile,[243] and Wang

et al.,[244] respectively. Nevertheless, Wang *et al.* found that **512** could not be converted into **119** by a leaf homogenate of *A. annua*.[245] In addition, dihydroartemisinic acid (**515**), the 11,13-dihydro analogue of **511**, was also considered as a possible biogenetic precursor of **119**.[245] Accordingly, artemisinic aldehyde (**510**) produced by CYP71AV1 was supposed to be released from the enzyme after the second oxidation, and then the $\Delta^{11(13)}$ double bond was reduced to form dihydroartemisinic aldehyde (**514**), which was further oxidized to **515**. These steps were well supported by the cloning and characterization of *DBR2* and *ALDH1* genes encoding a carbon double-bond reductase and an aldehyde dehydrogenase homologue from *A. annua*, respectively.[60,61] In addition, ALDH1 also exhibited oxidative activity on **510** as well as short linear aldehydes to form the corresponding acid.[61] The final step in the conversion of **515** to **119** was considered to be a non-enzymatic reaction and may occur extracellularly.[246,247]

It is noteworthy that the genes involved in **119** biosynthesis, including part of MVA and MEP pathway genes, FDP synthase gene (*FDPS*), *ADS*, *CYP71AV1*, *DBR2*, and *ALDH1*, were all expressed mainly in the apical and sub-apical cells of *A. annua* GTs (Scheme 2),[32,33] and **119** was exclusively localized to the subcuticular cavity of the GTs.[70] Therefore, the entire biosynthetic pathway of **119** should be localized to the GTs of *A. annua*, and different cell types of the GTs execute particular tasks in the production and storage of **119**.

Moreover, a number of transcription factors that regulate **119** biosynthesis were either identified from the cDNA library of *A. annua* GTs or found to be highly expressed in the GTs. These include a WRKY transcription factor AaWRKY1, a basic helix-loop-helix (bHLH) transcription factor AabHLH1, a basic leucine zipper transcription factor AabZIP1, and two JA-responsive AP2/ERF transcriptional factors AaERF1 and AaERF2, which positively regulated **119** biosynthesis by binding to the *cis*-elements present in *ADS* and *CYP71AV1* promoters.[248–251] AaORA is a trichome-specific AP2/ERF transcription factor that can up-regulate the expression levels of *ADS*, *CYP71AV1*, *DBR2* and *AaERF1*.[252] A GTs-specific WRKY transcription factor AaGSW1 was reported to enhance **119** biosynthesis by interacting with W-box motifs of *CYP71AV1* and *AaORA* promoters.[253] Moreover, an MYB transcription factor AaMIXTA1 responsible for prompting GTs initiation was



Scheme 2 Summary of the biosynthetic pathway and regulation of artemisinin (**119**) in *Artemisia annua* GTs. Dashed arrows indicate putative steps.

expressed predominantly in the basal cells of *A. annua* GTs.[9] As a consequence, *A. annua* GTs is not only the site for **119** biosynthesis and storage but also an enriched material for studying the regulation mechanism of **119** biosynthesis, which plays important roles in the metabolic engineering of the plant itself to obtain a high yield of **119**.

In 2006, by engineering the FDP biosynthetic pathway and introducing *ADS* and *CYP71AV1*, an engineered yeast (*Saccharomyces cerevisiae*) producing 100 mg L^{-1} artemisinic acid (**511**) was obtained.[254] With the discovery and characterization of five important enzymes including *CYP71AV1*, CPR1 (*CYP*71AV1 cognate reductase), CYB1 (cytochrome b_5), *ADH1*, and *ALDH1* from the *A. annua* GTs-specific cDNA library, the entire biosynthetic pathway was then reconstructed in yeast in 2013, resulting in the production of around 25 g L^{-1} **511** under optimized fermentation conditions.[239] Together with an efficient chemical process for the conversion of artemisinic acid to **119**, the engineered yeast strain paved a new way for the industrial-scale production of **119**.[239]

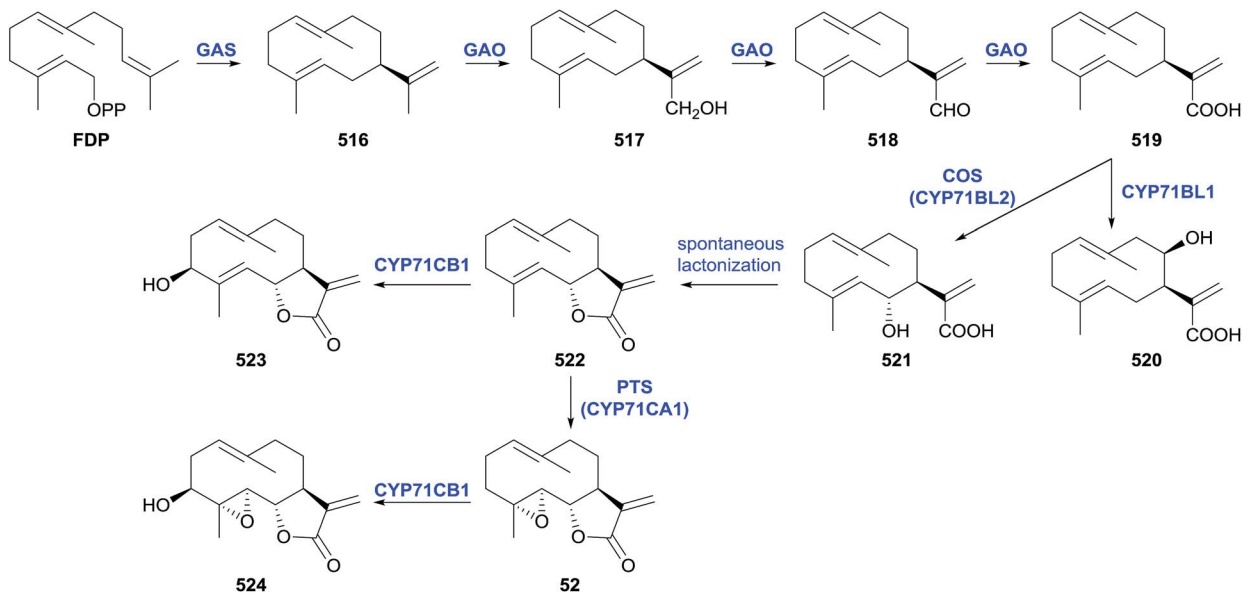
4.1.2.2 Sesquiterpene lactone biosynthesis in Asteraceae plant GTs. Sesquiterpene lactones (STLs) are a sub-class of terpenoids characterized by their α-methylene γ-lactone moiety, which endowed STLs with diverse biological activities. STLs are the characteristic constituents in Asteraceae plants and are frequently found to be biosynthesized and accumulated in the capitate GTs of this plant family.[255] Two germacrene A synthases (GASs) responsible for the cyclization of FDP to germacrene A (**516**) were identified to be predominantly expressed in the stalk cells of the capitate GTs of sunflowers.[256] Subsequently, the C12 methyl group of **516** underwent three consecutive oxidations to yield germacrene A acid (**519**), which was catalyzed by a single cytochrome P450 monooxygenase, germacrene A oxidase (GAO) (Scheme 3). GAO was first identified from lettuce (*Lactuca sativa*) and was later found to be evolutionarily conserved across the Asteraceae family.[257] Intriguingly, *GAO* was one of the most abundant transcripts in the sunflower's trichome EST database,[258] and was found in both the secretory and stalk cells of sunflower capitate GTs.[259]

Furthermore, an 8β-hydroxylase (CYP71BL1), catalyzing the 8β-hydroxylation of **519** was isolated from the sunflower trichome EST database. Unfortunately, 8β-hydroxy-germacrene A acid (**520**) could not undergo spontaneous lactonization but a costunolide synthase (COS, CYP71BL2) catalyzing the 6α-hydroxylation of **519** was then identified by examining the CYP71BL1 homologs in lettuce.[258] The resulting 6α-hydroxy-germacrene A acid (**521**) could be spontaneously lactonized to costunolide (**522**), which is a key precursor for the C6-C7 costunolide-type STLs (Scheme 3).[258] TcGAS, TcGAO (CYP71AV2) and TcCOS (CYP71BL4) were then identified from the pyrethrum trichome EST library, but the relatively poor efficiency of TcCOS on **519** implied that TcCOS might prefer C7-C8 lactonized substrates rather than **519**.[260]

Moreover, a TpGAS was identified to be highly expressed in the feverfew GTs, which contained a high concentration of parthenolide (**52**).[92] Further investigation of the feverfew trichome EST database led to the identification of a parthenolide synthase (TpPTS, CYP71CA1) and a costunolide and parthenolide 3β-hydroxylase (CYP71CB1) besides TpGAO and TpCOS (Scheme 3). Then, through the co-expression of TpGAS, TpGAO, TpCOS, TpPTS and CYP71CB1, together with a soluble *Arabidopsis thaliana* HMG-CoA reductase (AtHMGR) in *N. benthamiana*, **52** and its derivatives were produced.[261]

4.1.3 Diterpenoid biosynthesis

4.1.3.1 Cembrane diterpenoid biosynthesis in tobacco GTs. The 14-membered macrocyclic diterpenoid cembrenoids are principal components of the trichome exudate of *N. tabacum*,[262] and tobacco GTs were suggested to be the primary site of 2,7,11-cembratriene-4,6-diol (CBT-diol, **164**) biosynthesis through



Scheme 3 Summary of the biosynthetic pathway of sesquiterpene lactones in Asteraceae plant GTs.

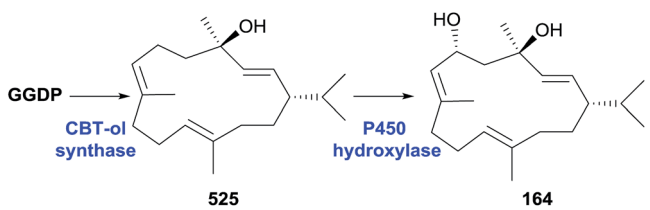
incubation of the GTs with ^{14}C-acetate and ^{14}C-mevalonate.[54] The isolated glandular heads were shown to be capable of synthesizing **164** from acetate and carbonate in the light, suggesting that the chloroplasts in the glandular heads were involved in the biogenesis of these compounds.[25,56] The labelled geranylgeraniol (GGOH) and cembrane were also reported to be incorporated into the **164**.[263] However, the biosynthesis of cembratriene-4-ol (CBT-ol, **525**) from geranylgeranyl diphosphate (GGDP) was observed in the cell-free extracts of tobacco GTs, and a GTs-localized CBT-ol synthase was partially purified and characterized, suggesting that **525** rather than cembrane was the probable precursor of **164** *in vivo* (Scheme 4).[264] Conversion of **525** to **164** was catalyzed by a GTs-specific cytochrome P450 hydroxylase.[58] The function of this gene was characterized by the suppression of its activity in tobacco plants using antisense and co-suppression strategies. P450-suppressed transgenic plants had decreased CBT-diols, increased CBT-ols in trichome exudate, enhanced exudate toxicity to aphids, and reduced aphid colonization, thus demonstrating the feasibility of using metabolic engineering to manipulate natural product synthesis in plant GTs to enhance its resistance against pests.[58,265]

4.1.3.2 Labdane diterpenoid biosynthesis in plant GTs. Biosynthesis of labdane diterpenoids from GGDP involves two steps. The first step is the protonation-initiated cyclization, performed by class II terpene synthases, resulting in the production of copalyl diphosophate (CDP). The second step is the ionization-initiated reaction of CDP, catalyzed by class I terpene synthases.

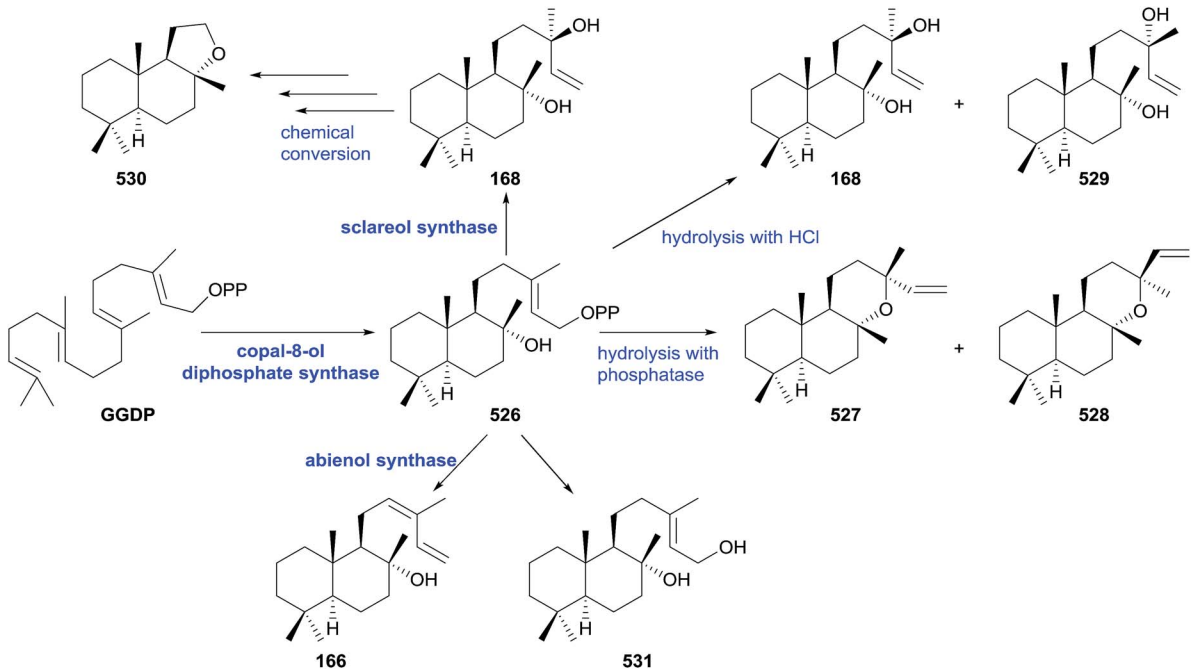
Our understanding of the biosynthesis of oxygenated labdane diterpenoids comes largely from studies of plant GTs. It was shown that *cis*-abienol (**166**), labdene-diol (**531**) and (−)-sclareol (**168**) could be biosynthesized from GGDP by the cell-free extracts from GTs of *N. tabacum* and *N. glutinosa*.[266,267] The first angiosperm copal-8-ol diphosphate synthase was identified from the trichome-specific cDNA library of *Cistus creticus*, which could catalyze the formation of copal-8-ol diphosphate (**526**) from GGDP (Scheme 5).[268] A copal-8-ol diphosphate synthase (NtCPS2) and an abienol synthase (NtABS) governing **166** biosynthesis were isolated from *N. tabacum* GTs. **526** synthesized by NtCPS2 could be sequentially cyclized to produce **166** by NtABS (Scheme 5).[269] A similar pathway employing **526** intermediate was reported for the biosynthesis of **168** in the GTs of *S. sclarea*. The reaction of recombinant *S. sclarea* copal-8-ol diphosphate synthase with GGDP resulted in the formation of a diastereomeric mixture of manoyl oxide (**527**) and 13-*epi*-manoyl oxide (**528**) after hydrolysis with alkaline phosphatase, and formation of **168** and 13-*epi*-sclareol (**529**) after hydrolysis with aqueous HCl (Scheme 5). Incubation of the enzymatic reaction in the presence of $H_2{}^{18}O$ or ^2H_2O resulted in the incorporation of ^{18}O or ^2H in the products respectively, suggesting the generation of a carbocation intermediate that was ultimately quenched by water capture.[130] A class I terpene synthase (sclareol synthase) was characterized from *C. creticus*, which could convert **526** into **168** (Scheme 5).[270,271] In addition, the biosynthetic pathway for **168** was reconstructed in engineered *Escherichia coli*, resulting in a yield of *ca.* 1.5 g L^{-1}, which could be used as a synthetic precursor for ambrox (**530**), which that is one of the most appreciated substitutes for ambergris.[270]

The biosynthetic pathway of labdane diterpenoids in the chaste tree, *V. agnus-castus* (Lamiaceae), a popular phytomedicine in Europe for the treatment women's hormone imbalance syndromes, was investigated.[47] The diterpenoids were localized to the GTs distributed on the surface of fruits and leaves by MALDI-MSI, and seven candidate genes involved in their biosynthesis, including three class II diterpene synthases (VacTPSs1, 3, 5), three class I diterpene synthases (VacTPSs2, 4, 6), and a CYP (VacCYP76BK1), were identified from the GTs-specific transcriptome database. VacTPS1 was identified as a peregrinol diphosphate (**532**) synthase capable of producing the precursor of C9 hydroxylated diterpenoids, while VacTPS3 was characterized as a *syn*-CDP synthase (Scheme 6). The co-expression of *VacTPS2* with *VacTPS1* in *N. benthamiana* resulted in two main products, viteagnusin D (**533**) and 9,13(*R*)-epoxy-labd-14-ene (**534**) (Scheme 6). VacTPS2 was also found to couple with VacTPS3 in *N. benthamiana* to produce vitexifolin A (**536**). VacTPS6 could accept the product of VacTPS1 to yield labda-13(16),14-dien-9-ol (**537**). VacTPS4 was not found to couple with any of the *V. agnus-castus* class II enzymes but could accept the substrate *ent*-CDP to produce *ent*-kaurene. VacCYP76BK1 was found to catalyze 16-hydroxylation of the diol-diterpene, peregrinol (**538**), to form labd-13*Z*-ene-9,15,16-triol (**539**), which is a potential intermediate in the biosynthetic pathway towards the furan- and lactone-containing bioactive diterpenoids (**176** and **542**) characteristic of this plant.

4.1.3.3 Clerodane diterpenoid biosynthesis in S. divinorum GTs. Salvinorin A (**182**) is a neo-clerodane diterpenoid with potent agonistic activity on brain κ-opioid receptors, which has only been isolated from a medicinal herb *S. divinorum*. Although the total chemical synthesis of **182** was achieved,[272] multistep chemical synthesis routes made it commercially unviable. Elucidation of its biosynthetic pathway would provide the alternative production of **182** through synthetic biology. Labelling studies demonstrated that **182** was biosynthesized *via* the MEP pathway rather than the MVA pathway.[273] A localization study using MALDI-MSI indicated that **182** was almost exclusively found in the peltate GTs on the abaxial side of leaves, informing the feasibility of using GTs-specific transcriptome to mine its biosynthetic genes.

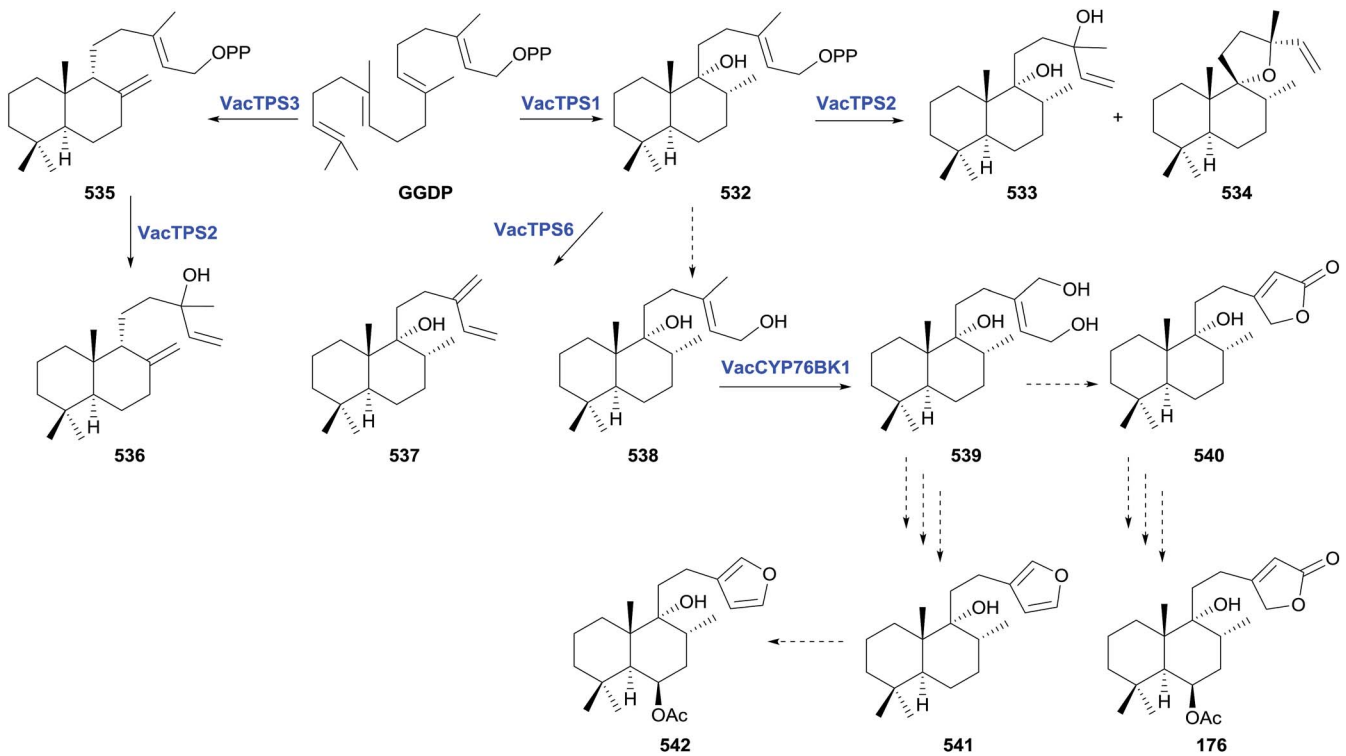


Scheme 4 Pathway for cembrane diterpenoid biosynthesis in tobacco GTs. Only the α-epimers of CBT-ol (**525**) and CBT-diol (**164**) are shown.



Scheme 5 Summary of the biosynthetic pathway of *cis*-abienol (**166**), labdene-diol (**532**) and (−)-sclareol (**168**) in plant GTs.

A (−)-kolavenyl diphosphate synthase (SdKPS, also termed as SdCPS2) was suggested to be the most likely enzyme catalyzing the first reaction of **182** biosynthesis owing to its biochemical function and trichome-specific expression in *S. divinorum*. Coincidently, a kolavenyl diphosphate synthase (VacTPS5) was also characterized using the GTs-specific transcriptome database of *V. agnus-castus*.[48] Structure-guided mutagenesis revealed that four catalytic residues including F255, A314, C359 and

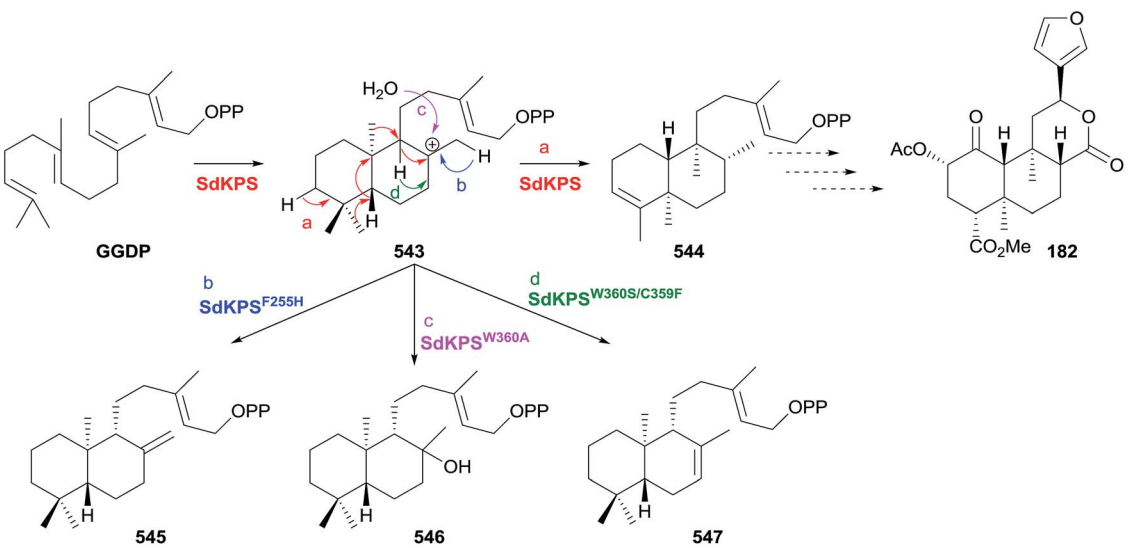


Scheme 6 Proposed pathway for the formation of furan- and lactone-containing labdane diterpenoids **176** and **542** in *Vitex agnus-castus*. Dashed arrows indicate putative steps.

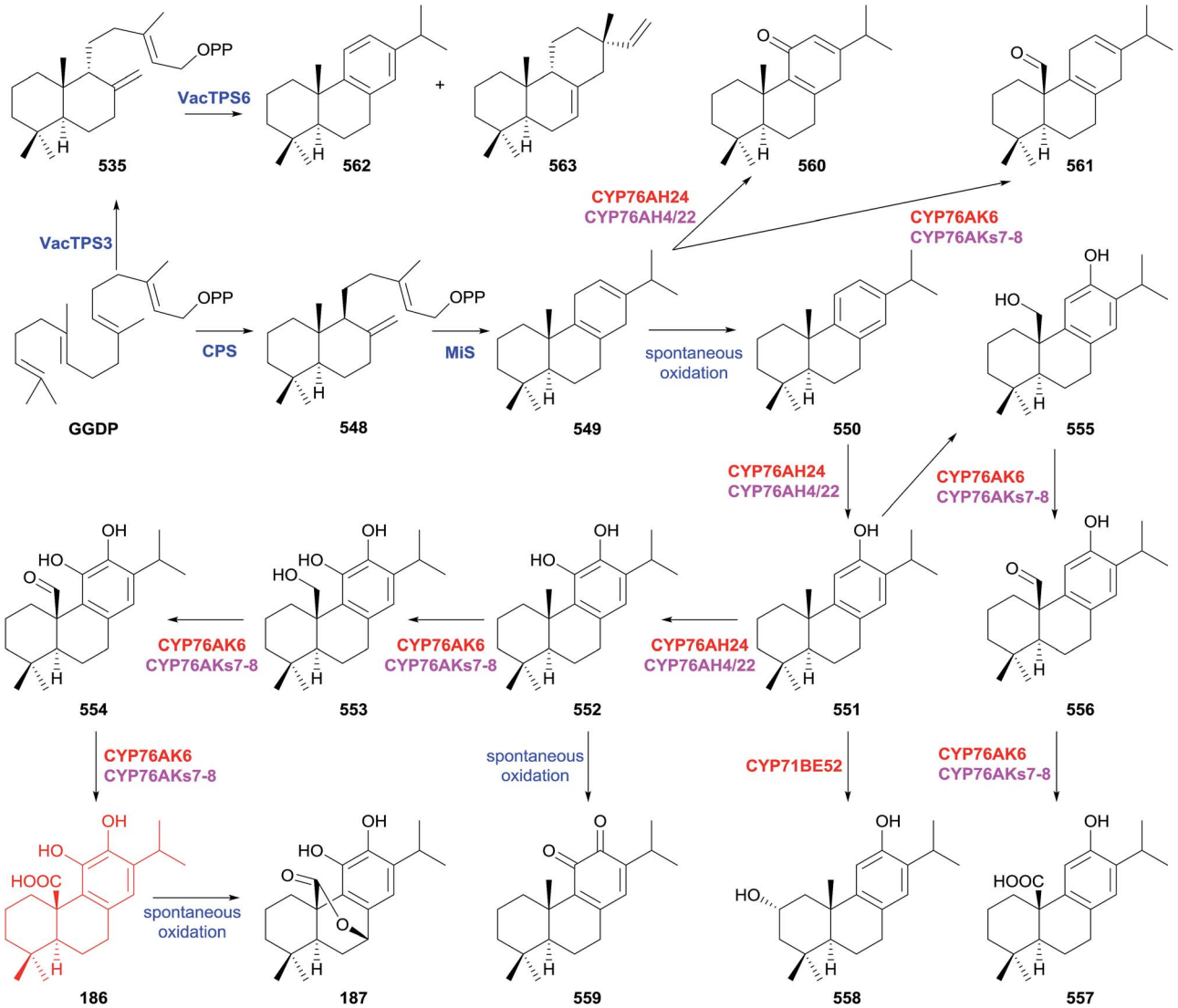
W360 participated in the modulation of the product specificity of SdKPS (Scheme 7). The mutant SdKPS^{F255H} showed a complete switch of product specificity from (−)-kolavenyl diphosphate (**544**) to *ent*-CDP (**545**), indicating the importance of this position for controlling the fate of the common labda-13-en-8-yl^{+} intermediate (**543**) toward 1,2-hydride shifts en route to either **544** or terminal deprotonation at C8 to form **545**.[48,274] The double-mutant SdKPS$^{A314V/F255H}$ led to the recovery of the ability to produce **544** as well as **545**.[48] W360 was identified as another 'hot spot' for altering SdKPS product specificity. The mutant SdKPS^{W360A} led to the formation of *ent*-8α/β-labda-13-en-8-ol diphosphate (**546**), while the mutant SdKPS^{W360S} resulted in multiple products, including **546** and *ent*-7,13*E*-CDP (**547**). The double-mutant SdKPS$^{W360S/C359F}$ improved the catalytic specificity toward producing only **547**. Moreover, an *ent*-CDP synthase (SdCPS1) and three class I enzymes (SdKSLs1–3) were also found from leaf- and GTs-specific transcriptiomes of *S. divinorum*. *SdKSL2* and *SdKSL3* were dominantly expressed in the GTs; unfortunately, no product was detected when SdKSL2 or SdKSL3 were co-expressed with either SdCPS1 or SdKPS. Therefore, the remaining biosynthetic pathway of **182** is worthy of further investigation.

4.1.3.4 Abietane diterpenoid biosynthesis in Lamiaceae plant GTs. The abietane diterpenoids carnosic acid (**186**), carnosol (**187**), pisiferic acid (**557**) and salviol (**558**) were reported to be preferentially stored in the GTs of several Lamiaceae plants, including rosemary (*Rosmarinus officinalis*), *S. pomifera* and *S. fruticose*, and showed diverse biological activities such as antioxidative, anti-inflammatory and anti-cancer effects. Biosynthetic pathways leading to the formation of **186** and other related diterpenoids were uncovered from GTs transcriptome data of these plants based on transcript abundance in the trichome cDNA library and similarity with other plant diterpenoid biosynthesis. The first steps for the skeleton biosynthesis of these diterpenoids are catalyzed by a CDP synthase (CPS) and a kaurene synthase-like enzyme called miltiradiene synthase (MiS), which cyclize GGDP to miltiradiene (**549**) through the intermediate (+)-CDP (**548**) (Scheme 8).[134,275,276] **549** could be spontaneously oxidized to abietatriene (**550**). The development of a modular yeast terpenoid production platform led to the functional characterization of the sequential oxidases for carnosic acid-related biosynthesis.[277,278] The bifunctional enzymes CYP76AH4, CYP76AH22, and CYP76AH24 catalyzed two successive oxidations at C12 and C11 of **550** to afford ferruginol (**551**) and 11-hydroxyferruginol (**552**);[277,278] they could also catalyze the successive oxidations of **549** to generate 11-keto-miltiradiene (**560**). Three additional oxidases CYP76AKs6–8 were responsible for the sequential oxidations of **552** at the C20 position to form the major diterpenoids including **186** in rosemary and *S. pomifera* (Scheme 8). **186** could be spontaneously oxidized to **187**. Meanwhile, CYP76AKs6–8 could also oxidize **549** and **551** to generate miltiradien-20-al (**561**) and pisiferic acid (**557**), respectively, but with much lower efficiency.[278] In addition, CYP71BE52, which catalyzed the oxidation of **551** at position C2α to yield **558**, was also identified from *S. pomifera*. Interestingly, the diterpene synthase VacTPS6 that catalyzed the cyclization of *syn*-CDP (**535**) to afford dehydroabietadiene (**562**), as well as *syn*-isopimara-7,15-diene (**563**), was also characterized from the GTs-specific transcriptome database of *V. agnus-castus* (Scheme 8).[48]

4.1.4 Sesterterpenoid biosynthesis. Plant sesterterpenoids are a special group of terpenoid natural products with complex structures and wide biological activities but our knowledge of their biosynthesis is still in infancy. In our laboratory, a plant geranylfarnesyl diphosphate synthase (LcGFDPS), an enzyme producing the C_{25} prenyl diphosphate precursor to all sesterterpenoids, was cloned and functionally characterized from the GTs of the aforementioned woody Lamiaceae plant with a colored nectar, *L. canum* (Scheme 9).[144] Lc-GFDPS was reported to be localized to the plastids, and the plastidial MEP pathway



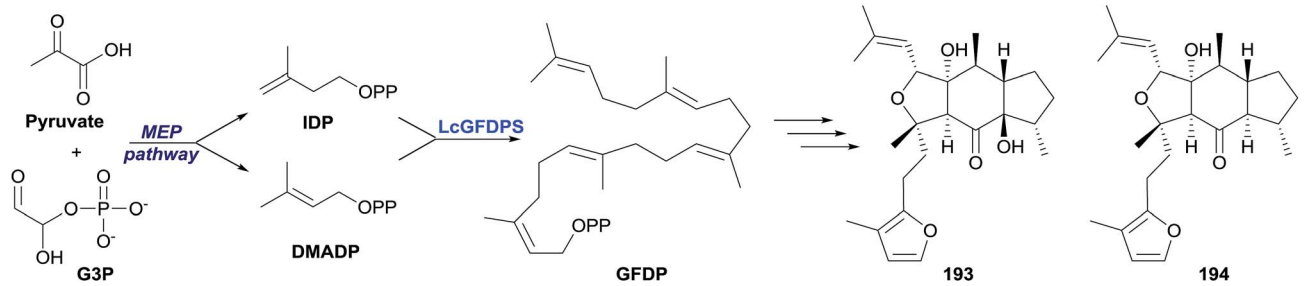
Scheme 7 Conversion of the common intermediate **543** into distinct diphosphate products observed in SdKPS and its mutants. Dashed arrows indicate putative steps.



Scheme 8 Summary of the biosynthetic pathway of carnosic-acid-related diterpenoids in the GTs of *Rosmarinus officinalis* (magenta) and *Salvia pomifera* (red).

was the primary supplier of isoprenyl diphosphate precursors for sesterterpenoid biosynthesis. Interestingly, GFDPS was suggested to have evolved from the plant GGDP synthase through gene duplication and neofunctionalization driven by positive selection.[144] The sesterterpenoid synthases should also exist in the GTs of *L. canum* but are yet to be discovered.

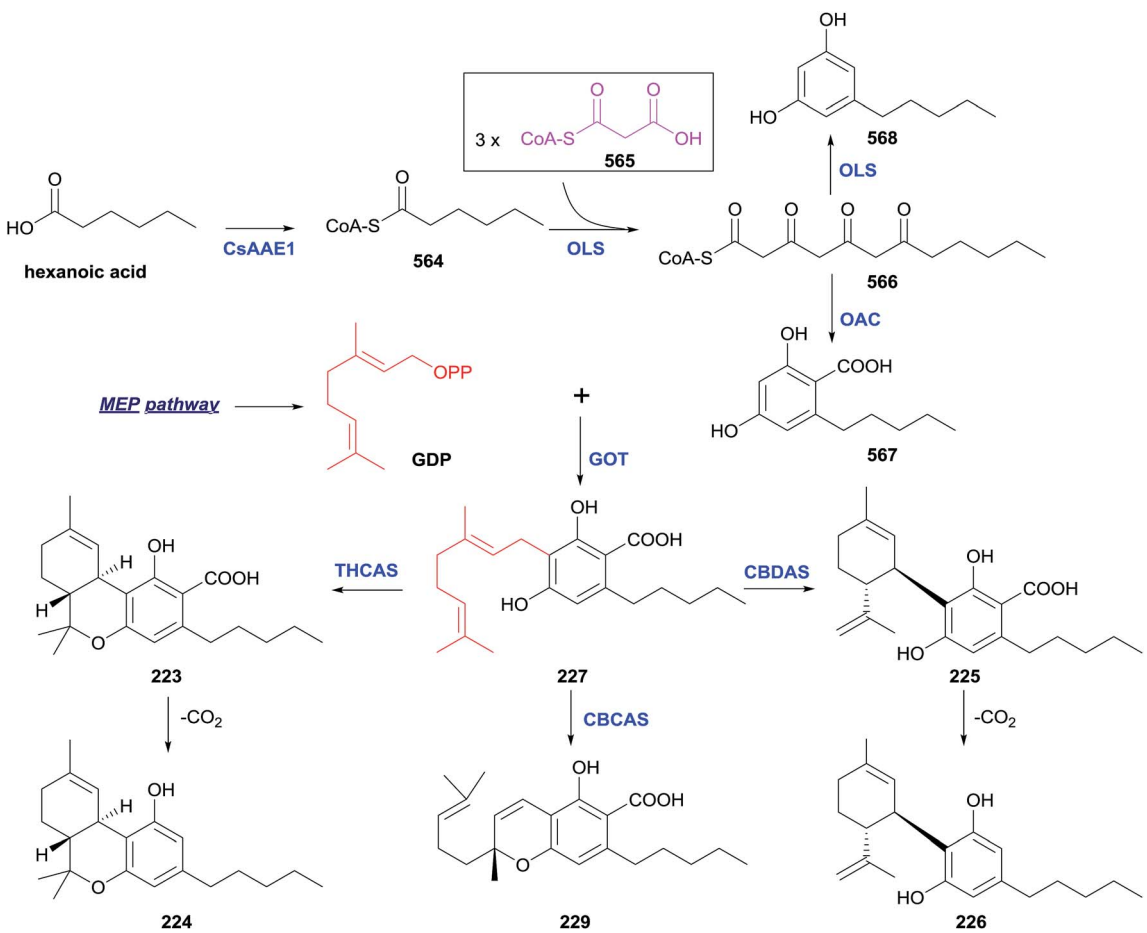
4.1.5 Meroterpenoid biosynthesis. Cannabinoids are terpenophenolic compounds consisting of an alkylresorcinolic acid and a monoterpenoid moiety. Because of their important pharmacological activity, the biosynthesis of cannabinoids has attracted much attention since the 1970s.[279] The early radiolabelling experiment showed that ^{14}C-labelled mevalonate and malonate could be incorporated into tetrahydrocannabinolic acid (THCA, **223**) and cannabichromenic acid (CBCA, **229**) at low rates.[279] However, subsequent labelling studies using [1-^{13}C] glucose or [U-$^{13}C_6$] glucose indicated that the C_{10}-terpenoid moiety was biosynthesized mainly *via* the MEP pathway and the phenolic moiety was generated *via* the polyketide pathway.[280] Biosynthesis of the phenolic moiety olivetolic acid (**567**) might start with hexanoic acid derived from either *de novo* fatty acid biosynthesis or breakdown of linoleic acid *via* lipoxygenase pathway, and candidate genes encoding the enzymes needed for the two routes in GTs-specific cDNA library were found (Scheme 10).[4] *C. sativa* acyl-activating enzyme 1 (CsAAE1), identified from the GTs-specific transcriptome was a hexanoyl-CoA synthetase responsible for the conversion of hexanoic acid into hexanoyl-CoA (**564**).[281] The olivetol synthase (OLS) belonging to type III polyketide synthases (PKS) catalyzed aldol condensation of **564** with three molecules of malonyl-CoA (**565**) to generate olivetol (**568**) rather than **567**.[282] An olivetolic acid cyclase (OAC) that could function in concert with PKS to catalyze a C2 → C7 intramolecular aldol condensation with carboxylate retention to generate **567**, was identified from GTs-specific EST of *C. sativa* (Scheme 10).[283] The studies on the crystal structures of OAC and



Scheme 9 Proposed biosynthetic pathway for leucosceptrane sesterterpenoids in *Leucosceptrum canum* GTs.

the OAC-**567** complex indicated that OAC belonged to the α + β barrel (DABB) superfamily and possessed a unique active-site cavity containing a pentyl-binding hydrophobic pocket and a polyketide binding site.[284]

Alkylation of **567** with GDP could generate cannabigerolic acid (CBGA, **227**), the central branch-point intermediate for the biosynthesis of various cannabinoids (Scheme 10). This reaction was performed by an aromatic prenyltransferase named geranylpyrophosphate:olivetolate geranyltransferase (GOT).[285] Subsequently, the geranyl group of **227** could be stereoselectively cyclized by three oxidocyclases, tetrahydrocannabinolic acid synthase (THCAS), cannabidiolic acid synthase (CBDAS), and cannabichromenic acid synthase (CBCAS), to afford THCA (**223**), cannabidiolic acid (CBDA, **225**), and CBCA (**229**), respectively.[286–288] Both THCAS and CBDAS are flavin adenine dinucleotide (FAD)-dependent oxidases and require molecular oxygen as an electron acceptor for the re-activation of flavin.[289,290] The crystal structure of THCAS was reported in 2012, which was divided into two domains with a FAD coenzyme positioned between each domain and covalently bound to His114 and Cys176.[291] Finally, **223** and **225** could undergo nonenzymatic decarboxylation to yield Δ^9-

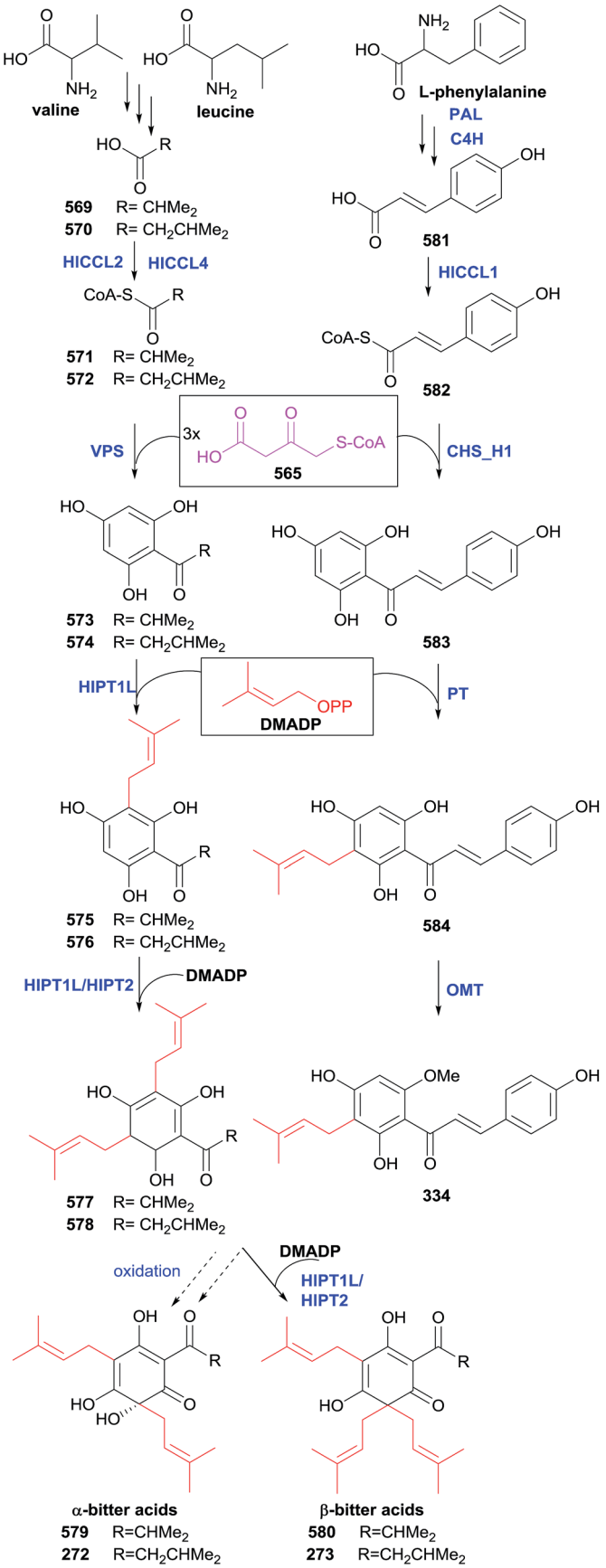


Scheme 10 Summary of the biosynthetic pathway of cannabinoids in *Cannabis sativa* GTs.

tetrahydrocannabinol (Δ^9-THC, **224**) and cannabidiol (**226**), respectively. It is noteworthy that besides CsAAE1 and OAC, GOT and THCAS were also found to be exclusively expressed in the secretory cells of GTs,[292] suggesting that the entire biosynthesis pathway of cannabinoids might take place in *C. sativa* GTs. Recently, an engineered yeast, *Komagataella phaffii*, co-expressing a prenyltransferase gene with *THCAS* was constructed with the capability for producing 82 ± 4.6 pmol L^{-1} OD^{-1} h^{-1} of **223** from OA and GDP, which paved a new way for the production of **224** through synthetic biology.[293]

4.2 Prenylated phenolic biosynthesis

Prenylated phenolics are predominantly produced in hops GTs, which not only contribute to the bitter taste of beer but also display broad biological activities; thus, their biosynthesis and regulation have received wide attention. Recently, several hops GT-specific cDNA libraries were constructed and sequenced, which have accelerated the functional identification of genes involved in prenylated phenolic biosynthesis in hops.[13] The branched-chain acyl-CoA precursors are derived from the degradation of branched-chain amino acids. Specifically, isobutyl-CoA (**571**) and isovaleryl-CoA (**572**) for bitter acids (**579**/**580** and **272**/**273**, respectively) biosynthesis are produced by the degradation of leucine and valine, respectively, while 4-coumaroyl-CoA (**582**) for xanthohumol (**334**) biosynthesis originates from phenylalanine catabolism (Scheme 11).[294] Both carboxyl CoA ligases (CCLs) and thioesterases involved in branched-chain acyl-CoA biosynthesis were identified from the hops GTs.[295] Recombinant HlCCL2 showed high specific activity for isovaleric acid (**570**), whereas HlCCL4 specifically utilized isobutyric acid (**569**) as its substrate. HlCCL1 catalyzed the conversion of 4-coumaric acid (**581**) to **582**. Besides, 11 additional ligases, including HlCCL3 and HlCCLs5–13, were also reported from the hops GTs. **571**, **572** or **582** could be condensed with malonyl-CoA (**565**) to produce phlorisobutyrophenone (**573**), phlorisovalerophenone (**574**) or naringenin chalcone (**583**), respectively (Scheme 11). This step was performed by a type III polyketide synthase such as valerophenone synthase (VPS) or chalcone synthase (CHS_H1).[296–301] Subsequently, aromatic prenyltransferases (PTs) catalyzed the transfer of DMADP derived from the MEP pathway to **573**, **574** and **583** (Scheme 11). HlPT1 could transfer one molecule of DMADP to these prenyl acceptors,[302] while the recently identified HlPT1L and HlPT2 could physically interact to form a complex catalyzing the major prenylation in bitter acid formation.[168] HlPT1L catalyzed the first prenylation step and HlPT2 catalyzed the last two prenylation steps. Meanwhile, an engineered yeast that produced β-bitter acids was constructed by expressing the entire pathway genes, including CCLs for bitter acid biosynthesis.[168] Additionally, an *S*-adenosyl-L-methionine-dependent *O*-methyltransferase (OMT), which performed the final reaction in **334** biosynthesis was also identified.[8] OMT showed high specificity for only a few substrates, methylating desmethylxanthohumol (**584**) to form **334**. Recently, two non-catalytic chalcone isomerase (CHI)-like proteins, HICHIL1 and HICHIL2, were characterized to be involved in the production of **584** and

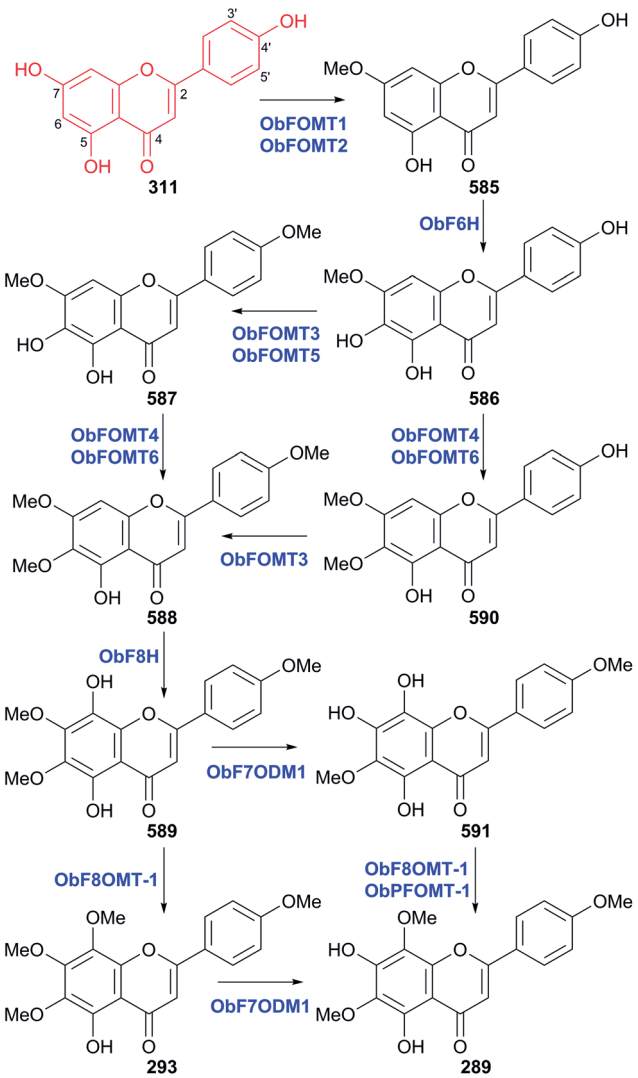


Scheme 11 Summary of the biosynthetic pathway of terpenophenolics in hops GTs.

334.[303] HICHIL2 enhanced the activities of CHS_H1 and HIPT1L through direct protein–protein interactions, while HICHIL1 appeared to bind **583** and **584** to stabilize the ring-open configuration of these chalconoids. Moreover, an engineered yeast constructed for the functional identification of these two genes could produce 4.59 μmol L^{-1} OD^{-1} of **584**.[303]

4.3 Flavonoid biosynthesis

Polymethoxylated flavonoids (PMFs) of peppermint (*Mentha piperita*) and sweet basil (*Ocimum basilicum*) are mainly stored in their peltate GTs, which provides a good opportunity for the investigation of the PMF biosynthetic pathway. *O*-Methylation is a common modification of natural flavonoids, performed by flavonoid *O*-methyltransferases (FOMTs) that transfer the *S*-adenosyl-*l*-methionine-dependent methyl group to the hydroxyl group of the flavonoids (Scheme 12). A systematic study of FOMTs came from the GTs-specific EST of peppermint. MpFOMTs showed very low substrate specificity but high regiospecificity and were active on a broad range of flavonoid substrates at the hydroxyl groups at specific positions. Here, both MpFOMT1A and MpFOMT1B could methylate 3-OH, while MpFOMT2 acted as 8-*O*-methyltransferase, MpFOMT3 as 3′-*O*-methyltransferase and MpFOMT4 as 4′-*O*-methyltransferase.[304] The sequence of MpFOMTs was then used to search the EST database constructed using cDNA from the peltate GTs of four basil chemotypes, and six cation-independent FOMTs were identified. Two 7-*O*-methyltransferases, ObFOMT1 and ObFOMT2, could accept a broad range of substrates but showed high affinity for apigenin (**311**) to generate genkwanin (**585**). ObFOMT3 and ObFOMT5 executed both 6- and 4′-*O*-methylations, but preferred the 4′-OH of flavonoids like scutellarein-7-methyl ether (**586**) to convert it to labanein (**587**). In contrast, ObFOMT4 and ObFOMT6 only functioned as 6-*O*-methyltransferases, catalyzing **586** and **587** to produce cirsimaritin (**590**) and salvigenin (**588**), respectively (Scheme 12).[305] Homology modelling and site-directed mutagenesis experiments revealed that changing three amino acid residues was sufficient to convert 4′-*O*-methyltransferase into 6-*O*-methyltransferase, but the reciprocal mutations did not result in a comparable activity shift.[305] Two flavonoid 8-*O*-methyltransferases were also identified.[306] *O. basilicum* phenylpropanoid and flavonoid *O*-methyltransferase-1 (ObPFOMT-1), belonging to the cation-dependent *O*-methyltransferase family, which are promiscuous enzymes. ObPFOMT-1 might act as flavone 3′-*O*-methyltransferase, but its 8-*O*-methylating activity was higher than the 3′-*O*-methylating activity. ObF8OMT-1 is a cation-independent *O*-methyltransferase and it displayed the most favorable kinetics with 7,8,4′-hydroxyflavone. Interestingly, ObF8OMT-1 was specifically inhibited by gardenin B (**293**), the 8-*O*-methylation product of 8-hydroxysalvigenin (**589**).



Scheme 12 Summary of the biosynthetic pathway of polymethoxylated flavonoids in plant GTs.

Besides multiple regiospecific *O*-methylation, hydroxylations at positions C6 and C8, performed by flavone-6-hydroxylase (ObF6H) and flavone-8-hydroxylase (ObF8H), respectively, were also characteristic in basil PMFs (Scheme 12). ObF6H belongs to the CYP82D family and can use **585** rather than **311** as its substrate.[307] However, ObF8H is a membrane-bound and ferredoxin-dependent Rieske-type oxygenase that displayed high affinity for **588**.[308] Notably, 7-*O*-methylation appeared to be a prerequisite for 6-hydroxylation of flavones in basil, implying that nevadensin (**289**) found in basil was produced *via* the late removal of the 7-*O*-methyl group from **293**.[307] Subsequently, a 2-oxoglutarate-dependent dioxygenase (2-ODD) encoding flavone 7-*O*-demethylase 1 (ObF7ODM1) was identified from the EST database of the basil GTs.[309] ObF7ODM1 was found to be active with **293** and **589**. The treatment of basil plants with a 2-ODD inhibitor, prohexadione-calcium, significantly reduced the formation of **289** but increased the amounts of **293** and **589**, confirming that 2-ODD was involved in **289** biosynthesis in basil.[309]

Additionally, a recent study on *Solanum* GTs addressed four myricetin *O*-methyltransferases (MOMTs) responsible for the formation of myricetin derivatives (**593–598**) that are universally methylated at 3-OH; some are additionally methylated at the 3′-, 4′-, 5′-, and 7-hydroxyls.[310] *S. habrochaites* MOMT1 (ShMOMT1) and MOMT2 (ShMOMT2) were capable of methylating

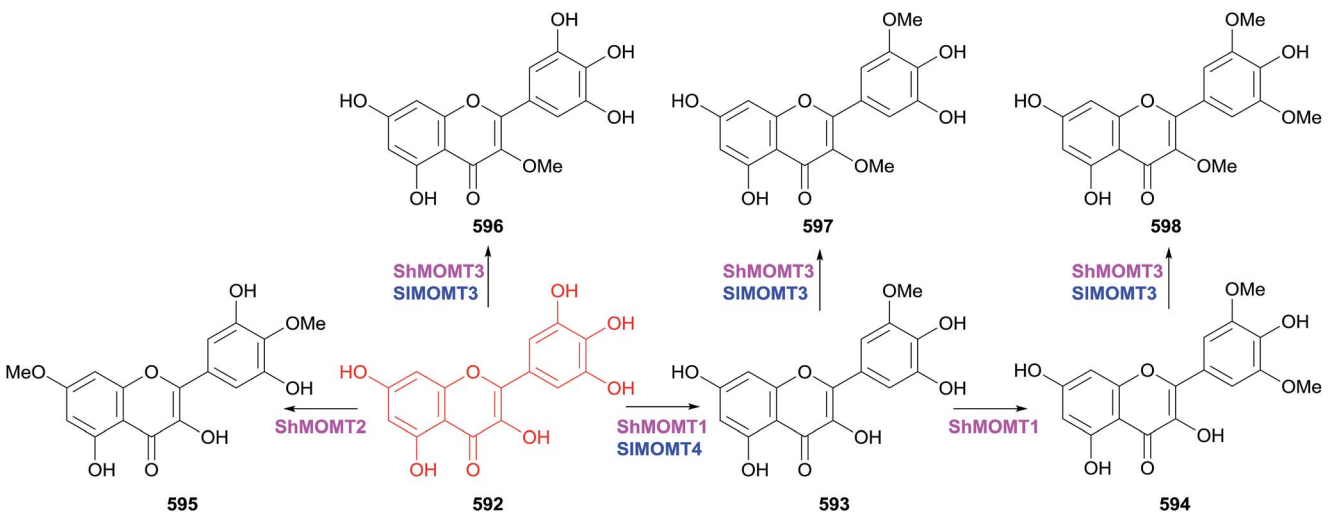
myricetin (**592**) at the 3′/5′- and 4′/7-hydroxyls, respectively.[310,311] ShMOMT3 was a myricetin 3-*O*-methyltransferase, while *S. lycopersicum* MOMT4 (SlMOMT4) was a 3′-*O*-methyltransferase (Scheme 13).[312]

4.4 Acylsugar biosynthesis

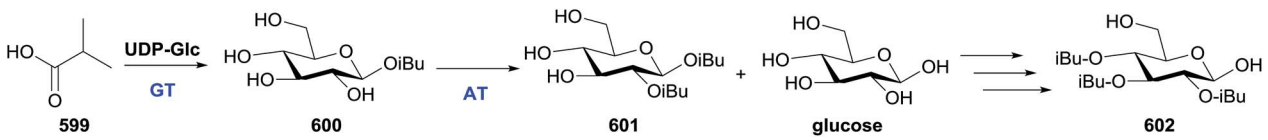
Acylsugars, typically consisting of either a sucrose or glucose backbone esterified with varying numbers of acyl groups, are biosynthesized in the GTs of many Solanaceae species. Radiolabelling experiments have suggested that 6-methylheptanoic acid and 5-methylheptanoic acid (6MeC7:0 and 5MeC7:0), two predominant acyl groups of acylsugars in the tobacco GTs, were derived from modified branched-chain amino acid metabolism rather than fatty acid metabolism.[37] Likewise, the branched short-chain acyl groups of acylsugars in wild tomato (*L. pennellii*) were suggested to be formed *via* the oxidative decarboxylation of α-keto derivatives of branched-chain amino acids.[313] However, two distinct modes for the elongation of branched-chain fatty acids (BCFAs) in different species of Solanaceae have been reported. In wild tomato, the C_{10}–C_{12} medium-chain fatty acids were produced through the two-carbon elongation of short-chain acyl acids by fatty acid synthases.[313–315] In contrast, several tobacco species and the genus *Petunia* were shown to produce extended BCFAs *via* α-keto acid elongation-mediated one-carbon increments.[315–317]

Esterification of the hydroxyl groups at various positions of the sugar by acyl groups varying in length and branching is a unique step in acylsugar biosynthesis. Intriguingly, investigation of the biosynthesis of 2,3,4-tri-*O*-acylglucoses (*e.g.* 2,3,4-tri-*O*-isobutyryl-glucose, **602**) in wild tomato GTs suggested that the fatty acids were activated to form high-energy 1-*O*-acyl-β-glucose (*e.g.* 1-*O*-isobutyryl-β-glucose, **600**) derivatives *via* UDP-glucose-dependent fatty acid transglucosylation rather than the well-known thioester-activated intermediates.[318] Two UDP-glucose:fatty acid glucosyltransferases (GTI and GTII) were separated and partially purified from wild tomato.[57] Both GTI and GTII showed similar affinities for UDP-glucose but GTI possessed greater specificity toward short-chain fatty acids (4 : 0) while GTII preferred medium-chain fatty acids (8 : 0 and 12 : 0).[57] Further additions of acyl groups were catalyzed by a glucose acyltransferase (AT) catalyzing both the disproportionation of two equivalents of 1-*O*-acyl-β-glucoses to form 1,2-di-*O*-acyl-β-glucoses and the anomeric acyl exchange between 1-*O*-acyl-β-glucose and glucose (Scheme 14).[319,320]

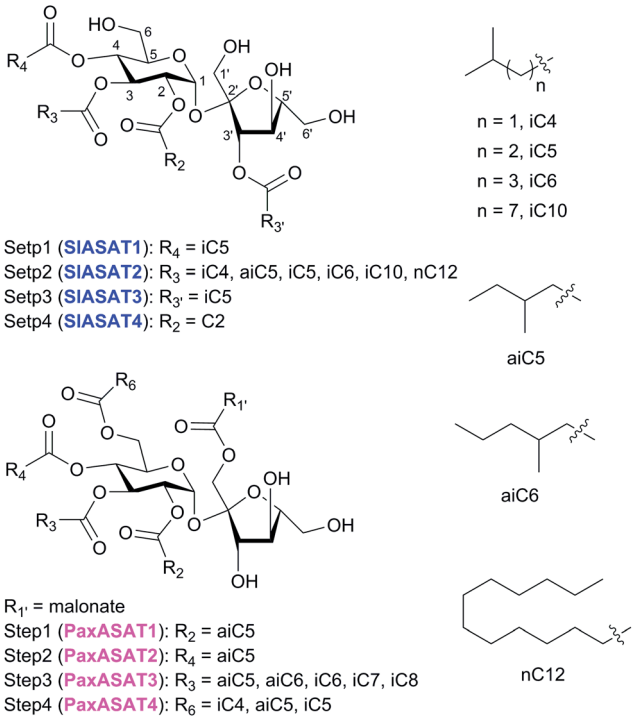
Recently, a series of acylsugar acyltransferases (ASAT) were further identified from the GTs of Solanaceae plants. The assembly of acylsucrose in cultivated tomato (*S. lycopersicum*) was shown to begin with the esterification of sucrose by various acyl-groups catalyzed by a GTs-specific SlASAT1 to make mono-acylsucroses with pyranose R_4 acylation. The addition of the second acyl group to the R_3 position to generate the $R_{3,4}$ di-acylsucrose was catalyzed by a GTs-specific SlASAT2.[321] Subsequently, the GTs-expressed SlASAT3 was responsible for $R_{3'}$ acylation on the five-membered furanose of the di-acylsucroses to make tri-acylsucroses,[322] and SlASAT4 was responsible for



Scheme 13 Summary of the biosynthesis of myricetin derivatives (**593–598**) in *Solanum* GTs.



Scheme 14 The biosynthetic pathway for 2,3,4-tri-*O*-acylglucose in wild tomato. Isobutyrate represents the acyl group but branched and straight short (C_4–C_5) to medium (C_{10}–C_{12}) chain fatty acids could be utilized and activated by chain-length specific UDP-Glc:fatty acid transglucosylases.

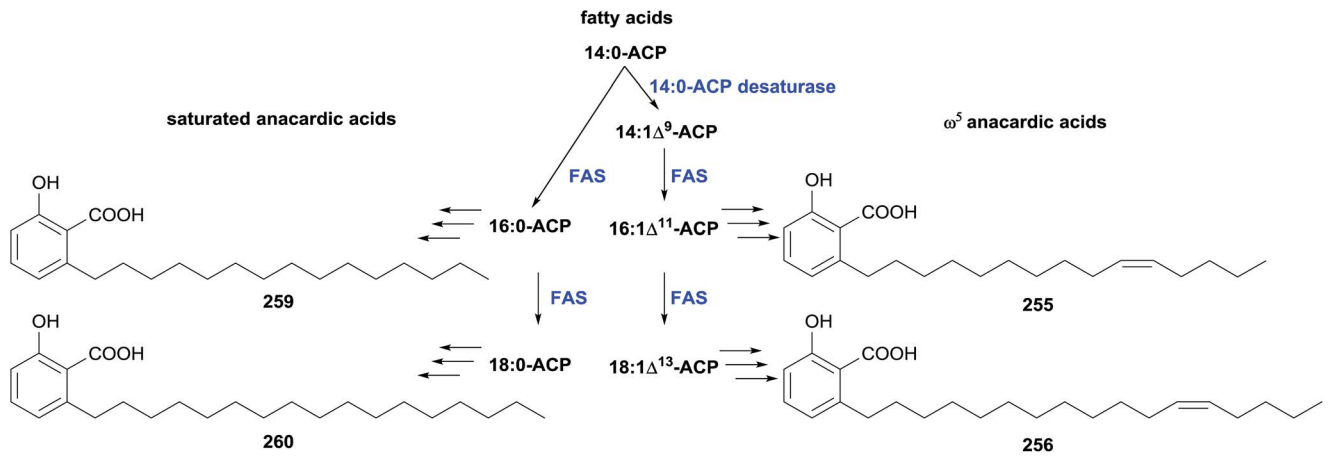


Scheme 15 The assembly of tetra-acylsucroses in tomato and *P. axillaris*. The four steps required for the formation of tetra-acylsucroses in each species are shown. ai: anteiso branched; i: iso branched; n: normal straight chain.

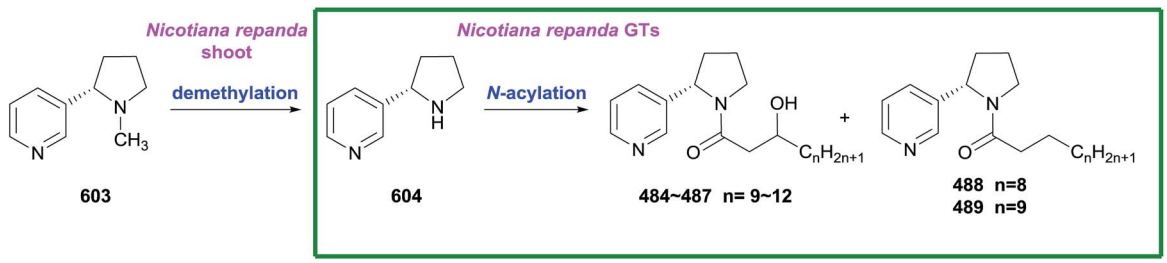
transferring an acetyl moiety to the pyranose ring of the triacylsucroses to form tetra-acylsucroses (Scheme 15).[323,324] Nevertheless, tetra-acylsucroses in *Petunia axillaris* were assembled through four ASATs, which catalyzed sequential additions of acyl groups to the R_2, R_4, R_3 and R_6 positions (Scheme 15).[325] Moreover, acylsugar acylhydrolases (AHSs) that catalyzed the hydrolysis of ester-linked acyl chains from specific positions of acylsugars were also identified from both cultivated and wild tomatoes, suggesting that a mechanism for the turnover or remodelling of acylsugar molecules might exist in the tomato GTs.[326]

4.5 Anacardic acid biosynthesis

Geranium (*Pelargonium xhortorum*) possesses abundant tall GTs that can secrete anacardic acids, especially 22:1ω^5 (**255**) and 24:1ω^5 (**256**) anacardic acids, as defensive chemicals against pests. Fatty acids were shown to be precursors of anacardic acids in the geranium GTs.[327] A Δ^9 14:0-acyl carrier protein (ACP) fatty acid desaturase, which placed a double bond at the Δ^9 position of a 14:0-ACP substrate, was identified from the GTs-enriched cDNA library.[158] The immediate product of this enzyme was 14:1Δ^9 fatty acid, which could be elongated by fatty acid synthases (FASs) to afford 16:1Δ^{11} and 18:1Δ^{13} fatty acids, the direct precursors to **255** and **256** (Scheme 16).[328] In parallel, saturated anacardic acids (**259, 260**) could also be synthesized from 14:0-ACP by FASs *via* intermediates 16:0-ACP and 18:0-ACP.



Scheme 16 Summary of the biosynthetic pathway of anacardic acids (**255**, **256**, **259**, **260**) in geranium GTs.



Scheme 17 Summary of *N*-acyl nornicotines (**484–489**) biosynthesis in *Nicotiana repanda*.

4.6 *N*-Acyl nornicotine biosynthesis

Nicotine was once thought to be a GTs product but later proved to be biosynthesized in the roots and then translocated to all aerial organs of the plant including the GTs.[218] However, *N*-acyl nornicotines **484–489** were suggested to be *N*-acylated in the GTs of *N. repanda* and then excreted to the leaf surface.[55,218] Interestingly, the GTs-specific *N*-acyl nornicotines were likely biosynthesized from nornicotine (**604**), which was produced by demethylation of root-produced nicotine (**603**) in the shoot (Scheme 17).[55,218]

5. Conclusions and outlook

In this review, we summarized the approaches used in the studies of the chemistry and biosynthesis of plant GTs-specific natural products. It is obvious that several modern techniques have been developed, especially LMPC, MSI, LMD coupled with UPLC-MS/MS and/or cryogenic ^{1}H NMR spectroscopy, and high-throughput "omics" techniques, which will undoubtedly accelerate the discovery of GTs-specific natural products and their biosynthetic genes. In particular, the introduction of LMD could pinpoint the natural products in various single types of GTs and their biosynthesis and regulation with a whole new degree of precision. Although all techniques have their own advantages and limitations as discussed in the previous section, the selection of appropriate methods based on research purposes would be helpful for better understanding the chemistry and biosynthesis of these mysterious and fascinating natural products.

Since the 1960s, 489 non-volatile compounds have been reported from the GTs of 81 plant species, mainly including terpenoids, simple phenolics, flavonoids, acylsugars, and glycerides. These compounds frequently function as defensive chemicals for the plant. Broad and significant pharmacological activities of many GTs-specific natural products have also been described. Foremost among them is artemisinin (**119**), which is currently one of the most effective antimalarial drugs, and its potential as an antiviral, antischistosomal, anticancer and immunosuppressive agent is being exploited. Other representative examples include Δ^9-THC (**224**) and cannabidiol (**226**), which were approved by the FDA for the treatment of chemotherapy-induced nausea and vomiting, and Dravet and Lennox-Gastaut syndromes, respectively. In addition, some GTs-specific compounds also have important economic value, as exemplified by carnosic acid (**186**) in the food, nutritional health and cosmetics industries. Despite the identification of many valuable natural products from plant GTs, the chemistry of the GTs of most plants (*ca.* >99%) is yet to be investigated. We are convinced that with the development of more highly sensitive and accurate techniques, a rapidly increasing number of new and bioactive compounds will be discovered from different plant GTs in the near future.

A set of novel genes and enzymes have been functionally characterized from plant GTs, which not only help to illuminate the functions of GTs natural products (*e.g.* the defensive function of CBT-diols in tobacco was disclosed by the suppression of P450 hydroxylase activity), but also contribute to understanding the origin and evolution of plant natural products and their biosynthesis (*e.g.* the evolution of plant GFDPS from GGDPS). Additionally, the elucidation of the biosynthetic pathways of natural products in plant GTs could guide the modification or reconstruction of the specific pathway either in the native plant or in heterologous hosts to produce sufficient quantities of the desired target compounds. The most glaring example is the high-level production of the antimalarial drug precursor artemisinic acid in engineered yeast, which has provided great potential for increasing the stable provision of **119** at a reduced average price. Besides, reconstruction of the biosynthetic pathway of the valuable cannabinoids (**223–231**), (−)-sclareol (**168**) and **186** using the synthetic biology platform has already been well underway.

This review has shown that plant GTs, as an important reservoir of diverse biologically active natural products and a valuable bank of novel biosynthetic genes and enzymes, still have great potential to be tapped into. Meanwhile, GTs provide an excellent system as well as a shortcut for mining original bioactive molecules and their biosynthetic pathways, which are critical for their large-scale production using synthetic biology, as well as their ultimate rational utilization.

6. Conflicts of interest

The authors declare no conflict of interest.

7. Acknowledgements

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8. Notes and references

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