Terpenes and Terpenoids

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What You Will Learn in This Chapter

Terpenes and their derivatives, the terpenoids, are synthetized from a five C atom isoprene unit. These units are added to each other to give rise to compounds of different complexities. The synthesis takes place either in the cytosol (MVA pathway) or within the plastids (MEP pathway). Monoterpenes, diterpenes and tetraterpenes are products from the MEP pathway, whereas sesquiterpenes, sterols and triterpenes are derivatives from the MVA pathway. Tailoring enzymes leads to the variations observed. End products are important for plant growth such as hormones (gibberellin and brassinosteroids) or carotenoids. They can be simpler structures, which are sometimes also volatile (such as menthol or ß-caryophyllene) or more complex, even polycyclic (such as ryanodine or stigmasterol).

10.1 Introduction

The building units of terpenes are isoprene units (Dewick 2002; Vranova et al. 2012). These precursors with five carbon atoms (C5) are present in the cell as diphosphates, either isopentenyl pyrophosphate (IPP) or its allylic isomer dimethylallyl pyrophosphate (DMAPP). Their fusion is mostly head-to-tail (a 1,4 link) but can also be head-to-head (a 1,1 link) or tail-to-tail (a 4,4 link) and leads to the formation of terpenes of different length (Fig. 10.1). These structures can be further modified at the methyl groups or by adding oxygen atoms and are then called terpenoids or isoprenoids. It is estimated that between 20,000 and 40,000 different structures are present in plants.

Two main pathways exist for the formation of DMAPP and IPP, the mevalonate pathway (MVA) and a mevalonate-independent pathway called MEP (after the first component 2-C-methyl-D-erythritol 4-phosphate) or DOXP pathway (after 1-deoxy-D-xylulose 5-phosphate). The MVA pathway can be found in all organisms, and in plants, it takes place mainly in the cytosol, whereas the MEP pathway resides in plastids (Tholl 2015). Therefore, the MEP pathway and its end products are specific for plants but can also be found in some bacteria. Due to the lack of the MEP pathway in humans, enzymes of this pathway are good targets for the treatment of human pathogens. One example is fosmidomycin, which acts on the DXP reductoisomerase of bacteria and *Plasmodium falciparum* and is used against infections and malaria (Rodriguez-Concepcion 2004).

In animals, the MVA pathway produces components such as cholesterol, dolichol and ubiquinone, which are important for membrane integrity and electron transport. In plants, six enzymes are needed for the MVA pathway (Fig. 10.2). The first steps are the condensation of two acetyl-CoA via acetoacetyl-CoA thiolase to form acetoacetyl-CoA and the addition of another acetyl-CoA by the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase to form HMG-CoA. The HMG-CoA reductase then synthetizes MVA, one of the control steps of the pathway. This enzyme consists of an endoplasmic reticulum (ER) membrane anchor and a conserved cytosolic catalytic domain. It is tightly regulated via phosphorylation and protein stability, stressing the importance of this pathway (Leivar et al. 2011; Doblas et al. 2013). Two phosphorylation steps are needed to produce mevalonate 5-pyrophosphate (MVAPP) and the diphosphomevalonate decarboxylase catalyzes the conversion of MVAPP to IPP. Components of the MVA pathway are distributed between the cytosol and peroxisomes, especially the enzymes for the two last steps could be localized to the peroxisome, while the first phosphorylation step is cytosolic (Pulido et al. 2012).

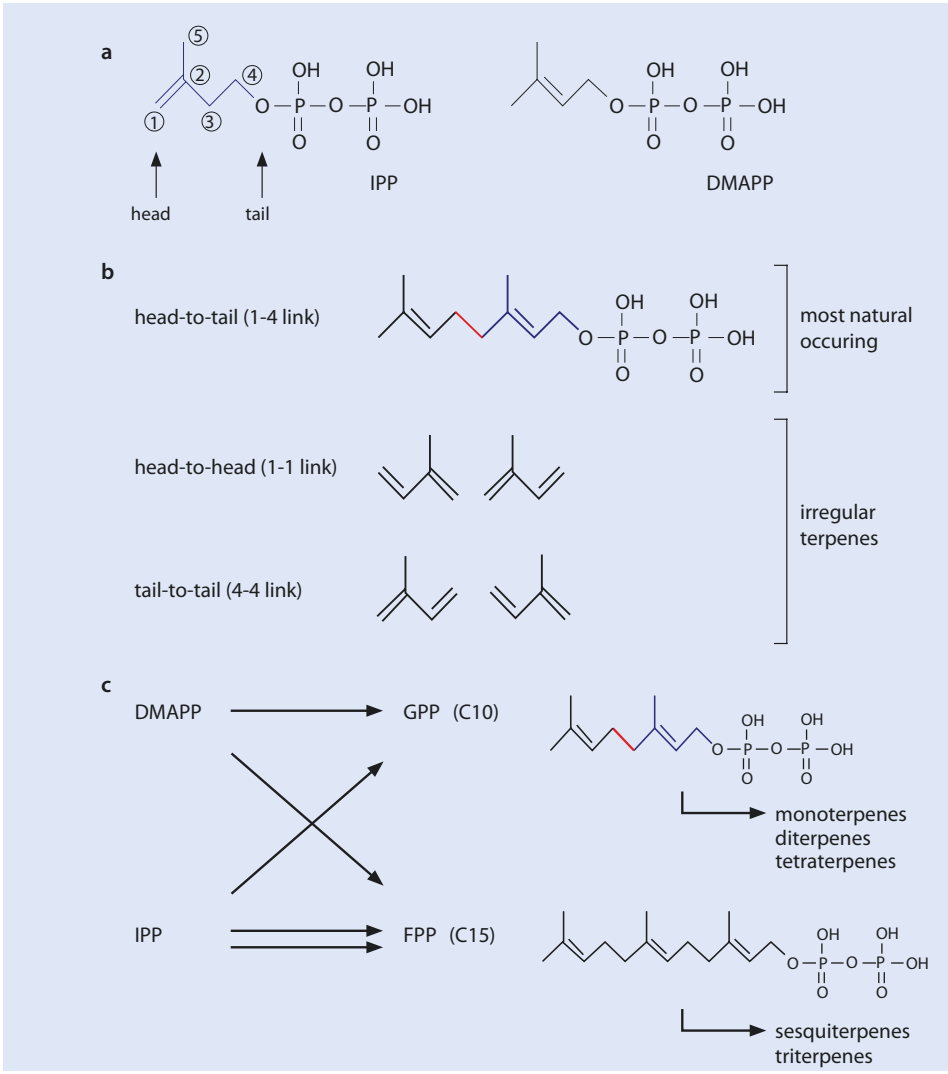

Fig. 10.1 The building units of terpenes are isoprene units. Isopentenyl pyrophosphate (IPP) and its allylic isomer dimethylallyl pyrophosphate (DMAPP) are the precursors of all terpenes. They have five carbon atoms (C5) and are present as reactive pyrophosphates (PP) **a**. They fuse mostly head-to-tail (1,4 link) but can also be head-to-head (1,1 link) or tail-to-tail (4,4 link; irregular terpenes) **b**. These fusion lead to the formation of terpenes of different length, the first product being geranyl pyrophosphate (GPP, C10) and farnesyl pyrophosphate (FPP, C15) **c**

The MEP pathway needs seven enzymes to produce IPP, starting from glyceraldehyde 3-phosphate and pyruvate (Fig. 10.2). The first step is mediated by 1-deoxy-D-xylulose 5-phosphate synthase (DXS) and leads to the formation of 1-deoxy-D-xylulose 5-phosphate (DXP), followed by the 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) step yielding 2-C-methyl-D-erythritol 4-phosphate (MEP). Four additional steps are needed to produce 1-hydroxy-2-methyl-2-(*E*)-butenyl 4-pyrophosphate (HMBPP),

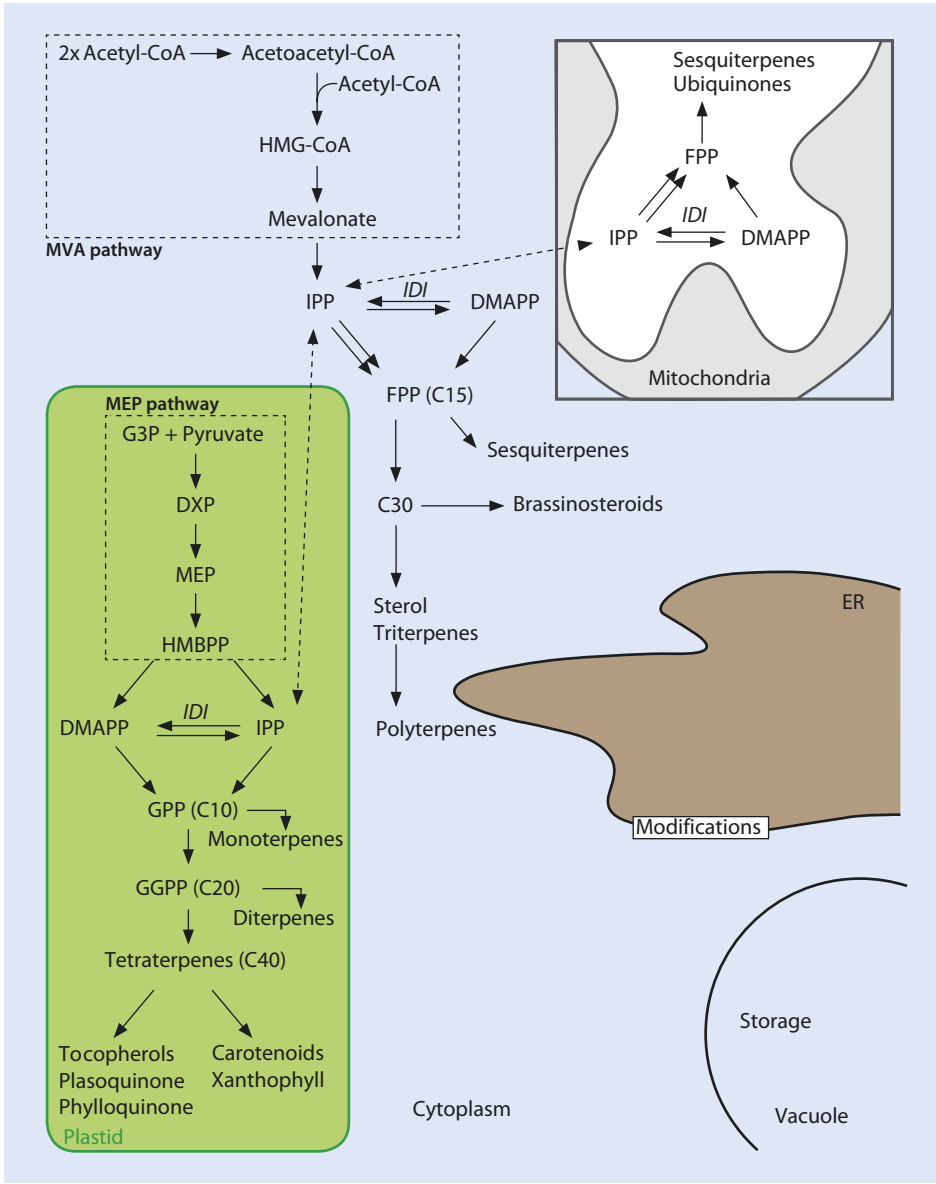

Fig. 10.2 Schematic overview of the biosynthetic pathways and their compartmentalization leading to terpenoids in plants. Several subcellular compartments are involved in the synthesis of terpenoids, especially plastids, mitochondria and the cytoplasm. The mainly cytosolic compartmentalized mevalonate (MVA) pathway and plastid localized mevalonate-independent (MEP) pathway both produce the C5 precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). There is an exchange between these compartments, mainly via isopentenyl pyrophosphate (IPP). The enzymes and end products are different between the MEP and MVA pathways. The C5 units are condensed to generate C10 (geranyl pyrophosphate, GPP), C15 (farnesyl pyrophosphate, FPP), C20 (geranylgeranyl pyrophosphate, GGPP), C30 and C40 precursors. At the ER, many enzymes reside that modify the basic structures (e.g. Cyt P450), and terpenoids are often stored in the vacuole. Enzymatic steps are represented by arrows, one arrow can account for multiple enzymatic steps. Additional abbreviations: *HMG-CoA* Hydroxymethylglutaryl-coenzyme A, *G3P* glyceraldehyde-3-phosphate, *DXP* 1-deoxy-D-xylulose 5-phosphate, *MEP* 2-*C*-methyl-D-erythritol 4-phosphate, *HBMPP* 1-hydroxy-2-methyl-2(*E*)-butenyl 4-pyrophosphate, *IDI* isopentenyl diphosphate isomerase

which is further converted into IPP and DMAPP by the HMBPP reductase. All the enzymes of the MEP pathway are localized in the plastid stroma.

The MVA and MEP pathways in the cytosol and plastids are not completely separated since there is exchange via their common intermediates, especially IPP. IPP can also be transferred to the peroxisomes and mitochondria. The isopentenyl diphosphate isomerase (IDI) mediates the equilibrium and supply of IPP and DMAPP (Berthelot et al. 2012). This enzyme appears to localize in the peroxisomes, mitochondria and plastids, although alternative splice forms have also been found in the cytoplasm (Phillips et al. 2008; Clastre et al. 2011; Guirimand et al. 2012). Besides the difference in the biosynthesis of IPP, there is also a difference in the first condensation step between the MVA and the MEP pathways (◘ Fig. 10.2). Head-to-tail condensation of one DMAPP with one IPP molecule in plastids results in geranyl pyrophosphate (GPP; C10, with 10 carbon atoms; ◘ Fig. 10.1c) formation, the precursor of monoterpenes, and is catalyzed by the GPP synthase in the MEP pathway. For the MVA pathway, the first condensation product is farnesyl pyrophosphate (FPP; C15, ◘ Fig. 10.1c), which is a sequential head-to-tail condensation of two IPPs and one DMAPP molecule by the FPP synthase (FPS) in the cytosol or in mitochondria. Knockout of all FPS genes is lethal for *Arabidopsis*, and embryo development is arrested at the pre-globular stage, demonstrating that this pathway is essential (Closa et al. 2010). In the next condensation step, two GPP or FPP fuse, leading to mainly C30 bodies in the cytoplasm and C20 and C40 bodies in the plastids. Polyprenols comprising C50–C130 bodies are also present in plants; natural rubber can contain more than 10,000 1,4-linked isoprene units.

The primary products of condensation are linear compounds. These are not only precursors for further modifications but also have specific roles in plants. Nevertheless, they also undergo alterations such as reduction, oxidation and side-chain modifications, which lead to terpenoid hydrocarbons, alcohols, ethers, aldehydes, ketones or carboxylic acids and their esters (Tholl 2015). Derivatization often leads to cyclization. Cyt P450 enzymes are involved in many of these steps, which take place mainly in the ER or cytosol. End products from the different compartments vary: in the cytosol mainly sequiterpens (C15), sterols (C30), triterpenes (C30) and polyterpens (>30) are produced, while in the mitochondria especially the coenzyme Q_{10} essential for electron transport is produced and also diterpenes and sesquiterpenes. In plastids, monoterpenes (C10), diterpenes (C20) and tetraterpenes (C40) are synthesized, but many of the end products are then transferred into the cytoplasm.

Many plants produce terpenes in specialized cells or tissues such as the glandular trichomes or the epithelial cells that surround the resin ducts of conifers. This defense strategy allows concentrating terpenoids in areas most likely to be targeted by a predatory organism, such as the surface of leaves (glandular trichomes), in resin ducts or laticifers (see ► Sect. 1.3.7 in ► Chap. 1).

10.2 Monoterpenes (From MEP Pathway, C10, e.g. Menthol, Camphor and Thujone)

Combining two C5 units leads to the most basic terpene, which is the linear monoterpene precursor geranyl pyrophosphate (GPP, C10). This compound is then further processed by monoterpene synthases/cyclases. These mostly belong to the enzyme group of terpene synthases (TPS) (see ► Box 10.1).

Box 10.1 Terpene Synthases (TPS)

Terpene synthases are the class of enzymes with a divalent metal ion cofactor that produce several different terpene end products (C5, C10, C15 and C20 terpenes). One enzyme can hereby generate multiple products from one substrate, but they can also use different substrates, which increase the variation (Tholl 2006; Chen et al. 2011; Pazouki and Niinemets 2016). The first step of the reaction usually involves ionization of the substrate by cleaving off the diphosphate group and the formation of a carbocation. This can lead to cyclization before the reaction is terminated via deprotonation.

Whereas in the moss *Physcomitrella patens* only one *TPS* gene is present, in higher plants over 100 *TPS* genes can be found. Examples are the isoprene synthase, the monoterpene synthases (such as limonene synthase, copalyl diphosphate synthase, pinene synthase, myrcene synthase, ent-kaurene synthase, linalool synthase, sesquiterpene synthases and diterpene synthases).

Monoterpenoids often constitute part of the so-called essential oils. Essential oils (see ► Box 10.2) are defined as non-water-soluble compounds, which usually contain volatile aroma compounds from plants.

The first step in monoterpene formation is the dephosphorylation and ionization of GPP to a geranyl carbocation (► Fig. 10.3). Acyclic monoterpenes are then derived by deprotonation. Those include geraniol (odour of roses, lemon grass and geranium), its isomeric form linalool (in many herbs such as mint, laurels, cinnamon and rosewood and citrus fruits, often acting as olfactory cues for pollinators), myrcene (in bay, thyme, parsley, cardamom and hops) and ß-ocimene (odour of lavender and basil). Many of these compounds can be found in peltate glands from which they are easily released as volatiles upon contact.

Box 10.2 Essential Oils

The term "essential oils" was probably coined in the sixteenth century by alchemists as "*Quinta essential*" (fifth element, universal ether). They occur often in the plant families Alliaceae, Apiaceae, Asteraceae, Lamiaceae, Myrtaceae, Poaceae and Rutaceae and are responsible for the scents of plants especially spices and herbs (Dhifi et al. 2016). This is due to the volatile compounds within the essential oils that are characterized by a high vapor pressure. They are usually stored in secretory glands on leaves (e.g. eucalyptus, mint, thyme, rosemary, sage, basil, marjoram, pine, cypress) and flowers (e.g. orange, lavender, hops, chamomile, clove flower bud), but they are also be found in rhizomes (e.g. ginger), seeds (e.g. coriander, cardamom, pistachios, nutmeg, pepper), fruits (e.g. fennel, anise, citrus, apple) and in wood (e.g. cinnamon, sandalwood, rosewood, balsam fir tree, camphor). They are extracted for industrial (e.g. fragrances) and medicinal purposes by steam distillation, as they are insoluble in water. Essential oils are mixtures of monoterpene and sesquiterpene group but also phenylpropanoids.

In plants, essential oils are important for communication between plants, to attract pollinating insects and to repel predators. Due to their insect repellent and insecticidal activity they are also discussed as biopesticides (Regnault-Roger et al. 2012; Pavela and Benelli 2016).

For the cyclization of monoterpenes, the limonene synthase (a TPS) is the most important enzyme. From the geranyl cation, linalyl pyrophosphate (LPP) is formed, which lacks the trans-double bond that inhibits rotation and thereby cyclization. LPP stays bound to the enzyme. The removal of the pyrophosphate group leads to the linalyl cation, which is then formed into an α-terpinyl cation by cyclization. The α-terpinyl cation can undergo various steps of deprotonation, cyclization and ring closure leading to different cyclic end products (◘ Fig. 10.3). Deprotonation stabilizes the compound and leads to structures

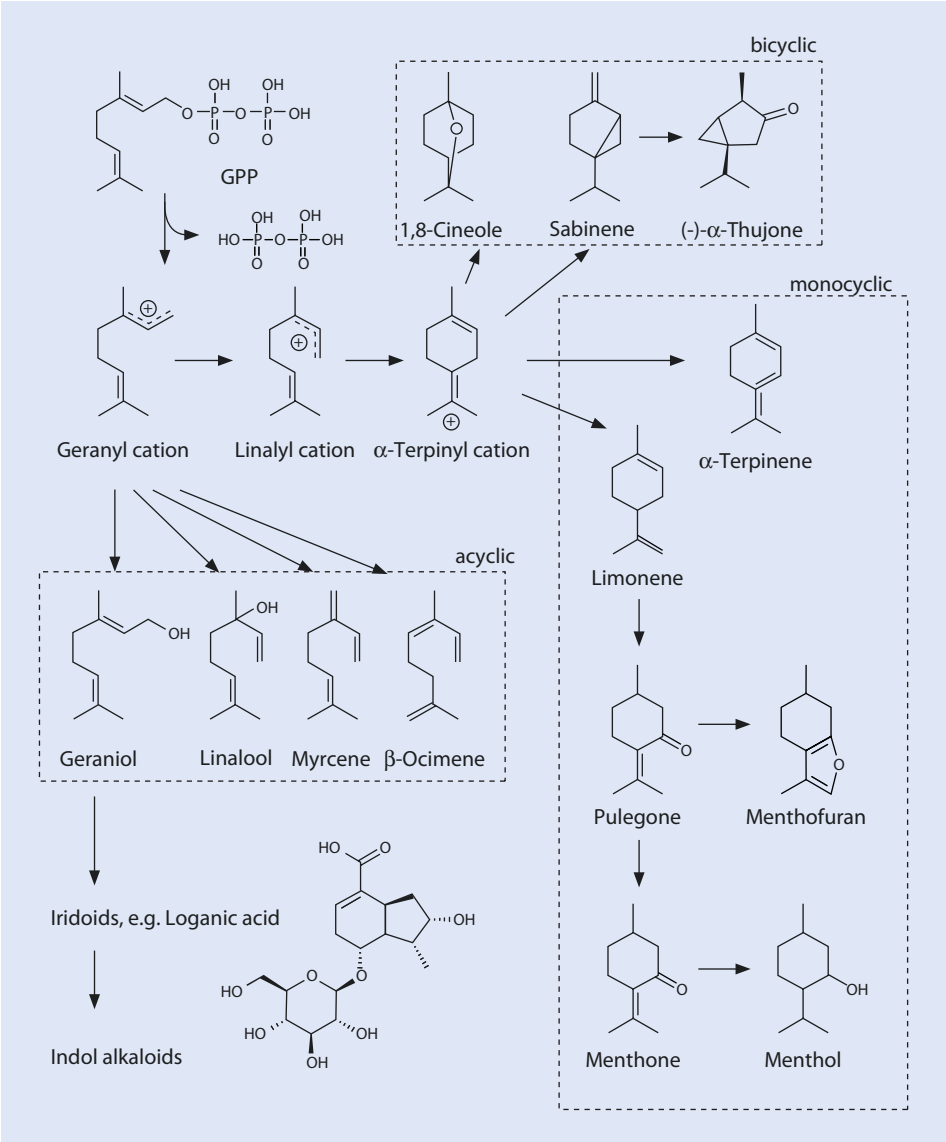

Fig. 10.3 Schematic overview of the biosynthetic pathways and representative members of various monoterpene subfamilies. Abbreviations: *GPP* geranyl pyrophosphate

with one ring (monocyclic, olefins). Among the monocyclic monoterpenes are limonene (odour constituent of citrus), α-terpinene (in cardamom and marjoram) and phellandrene (in eucalyptus, resin of the balsam fir tree, *Abies balsamea,* known as Canada balsam).

Limonene is also an important precursor for the formation of pulegone. The enzyme pulegone reductase then forms menthone from pulegone. Menthol is the oxidized form of menthone (Ahkami et al. 2015). Menthofuran is a side product from pulegone, mainly produced under high light intensities but unwanted in the production of menthol. The main producer of menthol and its derivatives is mint (*Mentha* spp.), making this plant an

important source of essential oils for the flavour, fragrance and aromatherapy industries. Due to its binding to the cold-sensing ion channel TRPM8 menthol can trigger a cooling sensation (Kamatou et al. 2013). The enzymatic reactions occur in specialized anatomical structures of the epidermis called glandular trichomes. The limonene synthase of *Mentha* × *piperita* has been localized only to the leucoplasts (colourless plastids) of gland secretory cells, indicating that the essential oil production occurs within these cells. However, the succeeding steps of monoterpene modification appear to occur outside of the leucoplasts in mint (Turner et al. 1999).

Additional ring closure via the terpinyl cation leads to bicyclic compounds such as careen (in pine, rosemary and cedar), camphene (in turpentine, cypress, camphor, ginger, valerian), 1,8-cineole (in eucalyptus, bay leaves, sage), pinene (in pine resin, pines, spruces and firs, fruits of *Pistacia terebinthus*), sabinene (in *Quercus ilex, Picea abies,* black pepper, nutmeg), fenchol (in rosemary, basil, hops) and camphor. Camphor is biosynthetically produced in plants by way of borneol (Croteau et al. 1981). Besides being present in sage, camphor oil can be isolated by distillation from the wood of the camphor tree (*Cinnamomum camphora,* Lauraceae) or is found in camphor basil (*Ocimum kilimandscharicum*, Lamiaceae). It has served as a fumigant against the Black Death and as an embalming fluid (Chen et al. 2013). These days it is used as an insect repellent and as fragrance, which can induce cold and warm sensations, although it can be toxic at higher dosage. Pinene is discussed as a cannabis antidote as it forms the biosynthetic base for CB2 ligands and has been suggested to act as an acetylcholinesterase inhibitor aiding memory (Russo 2011). Sabinene can further be oxidized to form α-thujone (in *Thuja, Juniperus*, sage, thyme, rosemary), which is known for its presence in the absinthe drink and which acts as a GABA antagonist. All these aforementioned primary products can then be further modified by secondary reactions such as hydroxylation, peroxidation, methylation, acylation, glycosylation or cleavage (often by Cyt P450 enzymes), which increases the diversity of these compounds.

Iridoids, characterized by their cyclopentane (C5) ring fused with a pyran (C6) ring, are formed via a different cyclization step. Geraniol is first oxidized and then undergoes cyclization by the iridoid synthase (De Luca et al. 2014; Ilc et al. 2016). Iridoids, such as aucubin (in *Plantago*) and amarogentin (in gentian, *Gentiana lutea*), are usually stabilized by a sugar moiety. This sugar moiety also allows storage of a less toxic compound. Amarogentin is known for its bitter taste. The iridoid loganic acid (which is also fused to a glucose moiety) is converted to loganine and further secologanin, the last step being an oxidative cleavage of the C5 ring. Secologanin reacts with tryptamine forming strictosidine, leading to indole alkaloids (see ► Sect. 12.2 in ► Chap. 12).

The fusion of two molecules of dimethylallyl pyrophosphate (DMAPP) occurs via c1′–2–3 cyclopropanation by the enzyme chrysanthemyl diphosphate synthase yielding chrysanthemyl pyrophosphate (Yang et al. 2014). Chrysanthemyl pyrophosphate is further converted into chrysanthemol by hydrolysis of the diphosphate moiety, is then oxidized to chrysanthemic acid and further esterified to pyrethric acid. The acids are esterified with one of three alcohols (pyrethrolone, cinerolone or jasmolone), which are probably derived from jasmonate precursors through the oxylipin pathway. This yields the different forms of pyrethroids. The chrysanthemyl diphosphate synthase can be found in glandular trichomes, whereas the later synthesis steps take place in the pericarp. Pyrethrins can be extracted from dried pyrethrum flowers (*Chrysanthemum; Tanacetum cinerariifolium*) and constitute a well-known insecticide, which affects the Na^+-channels of nerve cells especially in insects.

10.3 Diterpene (From MEP Pathway, C20, e.g. Salvinorin A and Ryanodine)

The fusion of two GPPs leads to the formation of geranylgeranyl pyrophosphate (GGPP), the first diterpene with 20 carbons (Fig. 10.4). In contrast to the monoterpenes, diterpens are usually not volatiles due to their larger size. The acrylic phytyl diphosphate, derived from GGPP, is the precursor for tocopherol, phylloquinone and phytol and the side chain of chlorophyll (Zi et al. 2014). All these substances are important for photosynthesis in chloroplasts. Nevertheless, many diterpenoids are produced in leucoplasts.

GGPP, analog to monoterpenes, can by cyclized and rearranged into different forms (Zi et al. 2014). Class I diterpene synthases remove the allylic diphosphate ester bond

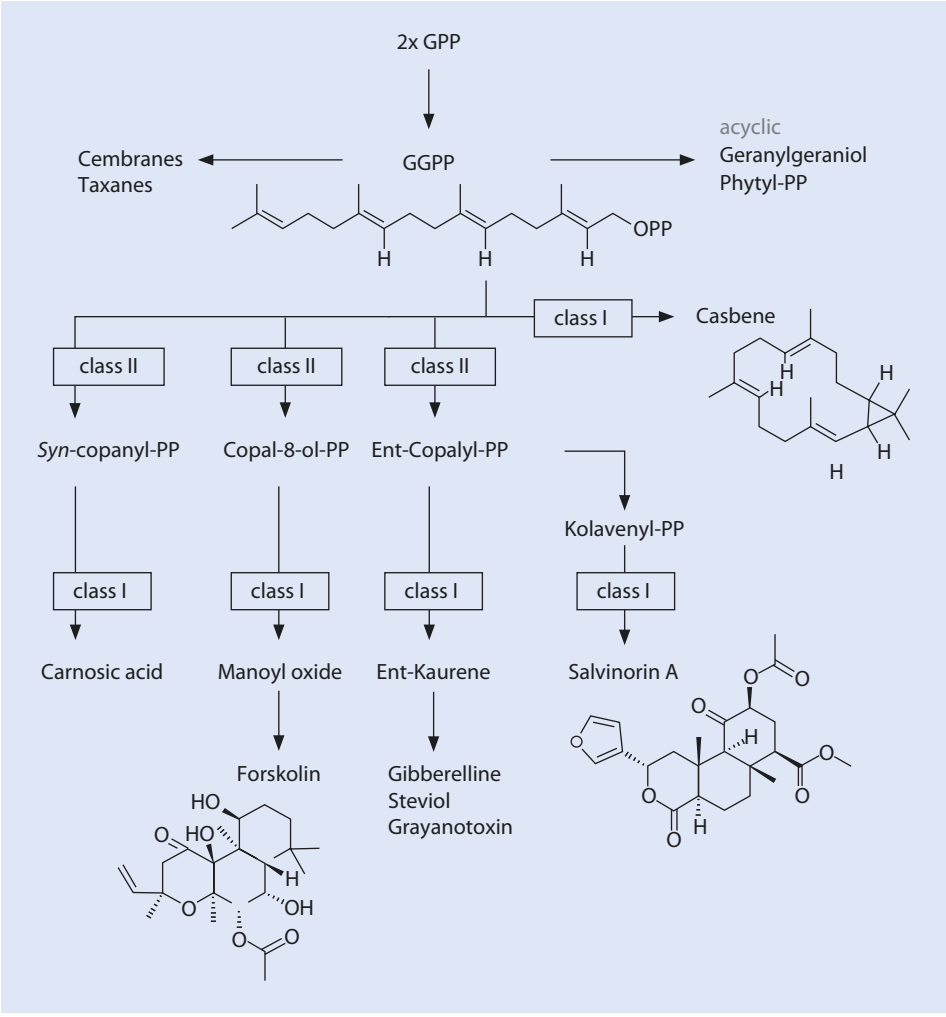

Fig. 10.4 Schematic overview of the biosynthetic pathways and representative members of various diterpene subfamilies. Diterpene synthetases belong to either the class I or the class II type. Abbreviations: *GPP* geranyl pyrophosphate, *GGPP* geranylgeranyl pyrophosphate

present in GGPP whereas class II diterpene cyclases leave this bond intact to be modified by subsequently acting class I diterpene synthases. Both belong to the enzymatic group of TPS (▶ Box. 10.1). In the most common pathway GGPP gives rise to the bicyclic ent-copalyl pyrophosphate via a class II cyclase which forms the bicyclic labdane carbon skeleton (Peters 2010). A type I cyclase closes the third ring leading to ent-kaurene, which is a precursor for the hormone gibberellin.

Other components derived from ent-kaurene are *steviol*, an intermediate for the biosynthesis of the sweetener stevioside (which is a glycosylated derivative) found in *Stevia rebaudiana*; cafestol and kahweol, which are found in coffee, adding to the effect of caffeine; and kauralexins, which are phytoalexins from maize. Furthermore, diterpene resin acids are characterized by tricyclic parent skeletons and are major components of the defense system of pine trees, often accumulating in the resin ducts of the phloem and cortex. In addition, grayanotoxins, toxins found in rhododendrons and other Ericaceae, are suggested to derive from ent-kaurene. The toxins can also be found in the nectar, which when used by honey bees can lead to contamination of the honey ("mad honey") (see ▶ Box 1.1). Grayanotoxins can bind to the group II receptor site in voltage-gated sodium channels within the cell (see ◘ Fig. 4.2b). Forskolin also has a labdane skeleton formed via Copal 8-ol pyrophosphate from the intermediate 13*R*-manoyl oxide. This compound is found in *Plectranthus barbatus* (Syn. *Coleus forskohlii*, Lamiaceae), where it accumulates in the root cork. It has been shown to activate the enzyme adenylyl cyclase and thereby increases intracellular levels of cAMP in humans.

Salvinorin A biosynthesis in *Salvia divinorum* (Lamiaceae), a κ-opiate receptor agonist, is not completely understood but seems to happen via the formation of (-)-kolavenyl diphosphate (Chen et al. 2017). Salvinorin A is a highly oxidized diterpene that is also methylated and acetylated. Peltate glandular trichomes were identified as the major site of salvinorin A accumulation in *S. divinorum* (Siebert 2004).

Derivatives of class I diterpene synthases are, for example, casbene, the antifungal component in castor bean (*Ricinus communis*). Bicyclic diterpenes can be further changed into polycyclic diterpenes, such as the pentacyclic ryanodine (from *Ryania speciosa*, Salicaceae), which interacts with the ryanodine receptor. Others are the tetracyclic phorbol ester present in the latex of many Euphorbiaceae and Thymelaeaceae, which mimics the action of diacylglycerol, and resiniferatoxin, which is present in the latex of *Euphorbia resinifera* (Euphorbiaceae) and hyperactivates the transient vanilloid receptor 1 (TRPV1). Other diterpenoids are formed by macrocyclic-forming steps leading to cembranes and taxanes. Taxol, sold as paclitaxel, is a known anticancer medication.

The diterpene synthases mostly produce olefins, which are highly hydrophobic. Introduction of oxygen increases the solubility and adds hydrogen-bonding potential, a step catalyzed by Cyt P450 enzymes. Some of the compounds, especially if they act as defense compounds, are produced in glandular trichomes such as cembratrieneols in tobacco or carnosol and carnosic acid in rosemary and sage, which seems to have antioxidative and antimicrobial properties.

10.4 Tetraterpene (From MEP Pathway, C40)

Tetraterpenes are produced by the fusion of two GGPP, usually via head-to-head condensation (Ruiz-Sola and Rodriguez-Concepcion 2012; Nisar et al. 2015). This leads to the formation of phytoene by the phytoensynthase. The next step leads to the formation of

lycopene. This is a branch point from which two different cyclases can modify the compound. One branch leads to the α-carotenes, which results in the formation of lutein, whereas the other branch leads to the β-carotenes, resulting in the formation of zeaxanthin, violaxanthin and neoxanthin. Due to the high amount of double bonds, many of these substances are colourful, notably the orange-red coloured carotenoids. Most of the enzymes are attached to the envelope membranes of the chloroplasts. Carotenes and xanthophylls (containing in contrast to carotenes oxygen atoms in form of hydroxyl groups and/or epoxide bridges) are mainly known for their protective role in photosynthesis. β-carotin (or β-carotene) is also degraded into retinal and further to retinol, better known as Vitamin A. Vitamin A is an essential vitamin for humans and animals because they cannot produce β-carotin and need to take it up with the diet. β-carotin accumulates in many fruits, vegetables and fungi, which can be recognized by their orange colour, especially carrots (*Daucus carota*, Apiaceae). Additionally, carotenoid cleavage dioxygenases produce carotenoid cleavage products that have been shown to be important for the formation of hormones such as abscisic acid (ABA) and strigolactones (McQuinn et al. 2015; Hou et al. 2016). Additionally these enzymes can produce cleavage products such as bixin (from Annatto seeds), crocin and safranal in crocus (*Crocus sativus*, Iridaceae; important for taste, colour and smell of saffron), or mycorradicin, which is responsible for colouring of arbuscular mycorrhizal roots.

10.5 Sesquiterpenes (From MVA Pathway, C15, e.g. Valerenic Acid, Anisatin and Tutin)

The first condensation product of sesquiterpenes in the cytosol consists of three isoprene units and is called farnesyl pyrophosphate. It is the result of sequential head-to-tail condensations of two IPP and one DMAPP molecule catalyzed by farnesyl pyrophosphate (FPP) synthase (Fig. 10.5). Sesquiterpenoids are upregulated upon defense and emitted from vegetative tissues in response to herbivore feeding and function as an indirect defense by attracting natural enemies of herbivores. On the other hand, they can be volatile constituents of floral odours that attract pollinators (▶ Box. 10.2).

The sesquiterpene pathway is based on TPS-dependent reactions and works along the same mechanisms shown for monoterpenes (▶ Box. 10.1). Deprotonation of the farnesylation leads to (*E*)-β-farnesene, or there is an additional isomerization via the tertiary diphosphate intermediate nerolidyl diphosphate (Fig. 10.5). The two main acyclic forms are farnesene (green apple odour, aphid alarm pheromone) and nerolidol (in citrus, ginger, jasmine, lavender and lemon grass; woody aroma and reminiscent of fresh bark).

Caulerpenyne, a highly toxic acetylated linear sesquiterpenoid, is found in large amounts in the green algae *Caulerpa taxifolia* (Brunelli et al. 2000; Jung et al. 2002). This is a tropical seaweed and an invasive species in the Mediterranean Sea. Each alga is a single, supersized cell up to several meters in length. When this cell is damaged, caulerpin is metabolized by an enzyme to produce oxytoxin 2, which immediately cross-links proteins forming a gummy gel inside the cell that can plug the break in just 30 s and hardens into protective scar tissue.

The farnesylation is cyclized to yield two major monocyclic forms, which are humulane and bisabolane. Humulane has as an intermediate, the humulyl cation, giving rise to the derivatives humulene and E-β-caryophyllene present in *Syzygium aromaticum*

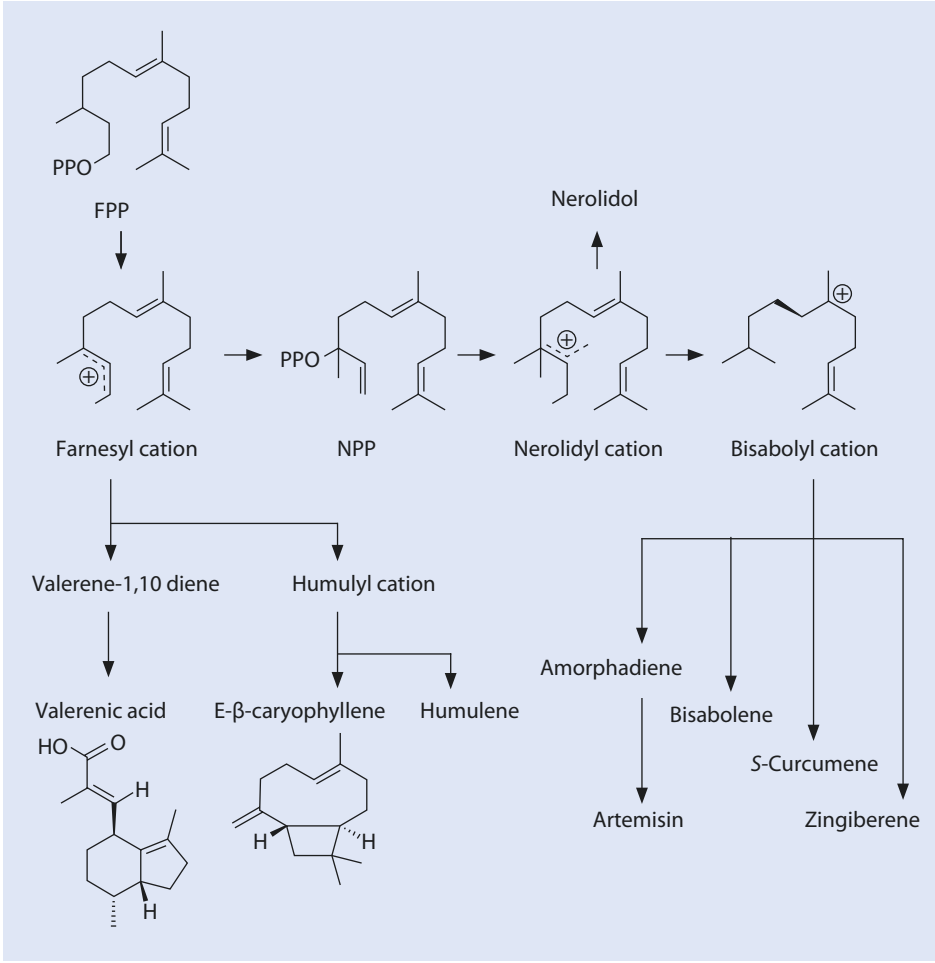

Fig. 10.5 Schematic overview of the biosynthetic pathways and representative members of various sesquiterpene subfamilies. Abbreviations: *FPP* farnesyl pyrophosphate, *NPP* Nerolidyl pyrophosphate

(cloves, Myrtaceae), *Cannabis sativa* (Cannabaceae), *Rosmarinus officinalis* (rosemary, Lamiaceae), *Piper nigrum* (black pepper, Piperaceae) and hops (*Humulus lupulus*, Cannabaceae). β-caryophyllene is emitted from plants, e.g. from maize roots that are attacked by insects. This in turn attracts nematodes that prey on the attacking insect larvae (Rasmann and Turlings 2016). β-caryophyllene is also an agonist of cannabinoid receptor type-2 (CB2) in humans, which perhaps contributes to the calming effect of cloves on toothache (Gertsch et al. 2010; Alberti et al. 2017).

Cyclization via the nerolidyl cation leads to the bisabolyl cation, which gives rise to the stereoisomers zingiberene and *S*-curcumene (in *Zingiber officinale,* Zingiberaceae, ginger; repellant to white flies). Other products of this pathway are bisabolene, which is part of the essential oil from chamomile (*Matricaria recutita L.,* Asteraceae). The bisabolyl cation can be further cyclized to the bicyclic amorpha-4,11-diene. This is an important bicyclic intermediate for the formation of artemisinin, an antimalaria drug. For the isolation of artemisinin and its characterization as a remedy against the disease,

the Chinese scientist Youyou Tu won the Nobel Prize in 2015. Artemisinin is produced in the glandular trichomes of *Artemisia annua* (Asteraceae). Cyt P450 enzymes catalyse the additional modifications, but the last step, the formation of an unusual peroxide bridge, is a photooxidation.

The bicyclic valerena-1,10 diene is the starting product for the synthesis of valerenal and valerenic acid, the latter being the active component in Valerian extracted from the roots of of *Valeriana officinalis* (Ricigliano et al. 2016). The bicyclic picrotoxane is modified into the tricyclic picrotoxin, an equimolar mixture of picrotoxinin and picrotin (found in the fruit of the *Anamirta cocculus*, Menispermaceae). Picrotoxin binds GABA-gated ion channels. Several other plant-derived sesquiterpenes can cause convulsions or other symptoms reminiscent of GABA-gated channel blockade. This is the case with tutin (from *Coriaria arborea*, Coriariaceae), anisatin (from star anise, *Illicium floridanum*, Schisandraceae) and jiadifenolide (from *Illicium jiadifengpi*, Schisandraceae). Many of these products contain lactone motifs and show similarity with GABA (◘ Fig. 8.4). In New Zealand, honey poisoning with tutin was observed (► Box 1.1). This was pinpointed to bees that fed on exudates from an insect that itself had been feeding on *Coriaria sp.* bushes (Larsen et al. 2015).

Englerin A (from *Phyllanthus engleri*, Phyllanthaceae), another compound with a polycyclic terpene skeleton, is a selective activator of TRPC4 and TRPC5 calcium channels, and this activation of transient receptor potential canonical (TRPC) calcium channels selectively kills renal cancer cells (Carson et al. 2015).

10.6 Sterols and Triterpene (From MVA Pathway, C30)

Two FPP are fused (tail-to-tail) by the squalene synthase to form squalene, which is further oxidized to 2,3-oxidosqualene (Thimmappa et al. 2014; Valitova et al. 2016). 2,3-Oxidosqualene is the precursor for sterols and triterpenes and a substrate for several oxidosqualene cyclases.

To form sterols 2,3-oxidosqualene is cyclized to lanosterol (in fungi and animals) or cycloartenol (in plants) via the chair-boat-chair conformation and the protosteryl cation (◘ Fig. 10.6). Lanosterol gives rise to cholesterol, whereas cycloartenol gives rise to phytosterols such as β-sitosterol, stigmasterol and the plant hormone brassinosteroid. Because this backbone is very similar to cholesterol, these compounds can displace low-density lipoprotein (LDL) cholesterol in the human intestine. In some plants, cholesterol formation is also possible, starting from cycloartenol, which is converted into cycloartanol and further into cholesterol. Additionally, in Solanaceae and *Euphorbia* lanosterol was detected, which might contribute to cholesterol biosynthesis. Cholesterol is also a precursor for steroidal alkaloids (see ► Sect. 12.11 in ► Chap. 12).

Stigmasterol is present in many plant fats, which are enriched in various vegetables such as seeds (e.g. rape seeds), soy bean and nuts. Stigmasterol is also present in milk. Pasteurization inactivates stigmasterol, a fact that led to its discovery as the Wulzen factor (antistiffness factor). It had been suggested that stigmasterol could lessen symptoms of arthritis, and this would have asked for avoiding pasteurized milk. Stigmasterol can be used as a precursor in the manufacture of semisynthetic progesterone and vitamin D3.

β-Sitosterol is found in vegetable oil, nuts and avocados. Another derivative of 2,3-oxidosqualene is cucurbitadienol and further cucurbitacins from pumpkin, squash or

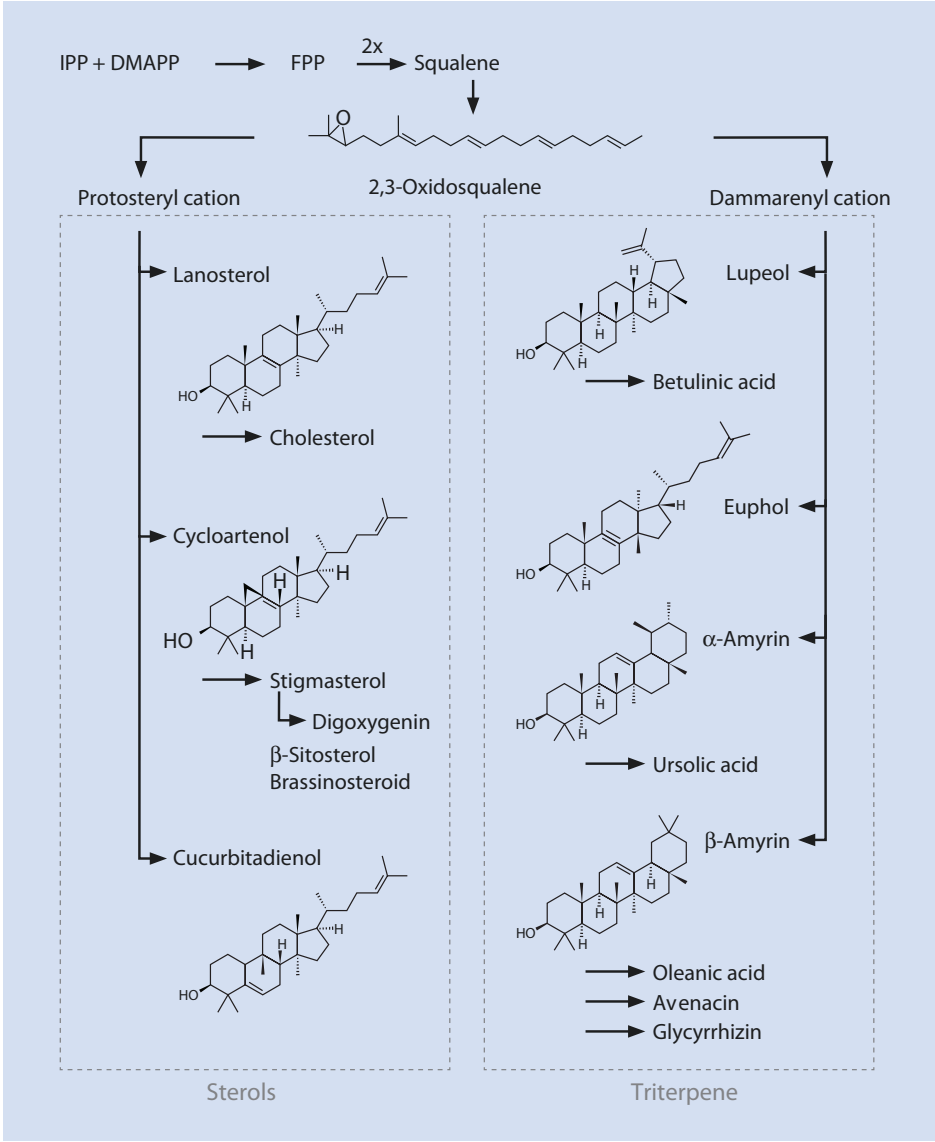

Fig. 10.6 Schematic overview of the biosynthetic and representative members of sterols and triterpene subfamilies. Abbreviations: *IPP* isopentenyl diphosphate, *DMAPP* dimethylallyl diphosphate, *FPP* farnesyl pyrophosphate

cucumber. These substances have been bred out from commercially available varieties, as they taste very bitter and are toxic.

In *Digitalis sp.* (foxgloves, Plantaginaceae) stigmasterol can be converted into pregnenolone and further to cardenolides such as progesterone, which can be converted in several steps into digoxigenin and further to digitoxin and lanatoside by adding sugar moieties. These are also called cardiac glycosides because they control the heart rhythm

(Lindemann 2015; Gurel et al. 2017). Another toxic cardiac glycoside is oleandrin, which is found in laticifers especially of oleander (*Nerium oleander*, Apocynaceae).

Triterpenes are folded into the chair-chair-chair conformation prior to cyclization, leading to a dammarenyl carbocation intermediate (◘ Fig. 10.6). The triterpene synthases catalyse the synthesis of tricyclic, tetracyclic and pentacyclic molecules by concerted reaction steps of single enzymes. As in the previous groups, Cyt P450 enzymes are essential in modifying the scaffolds further to give rise to more elaborate molecules (Ghosh 2017). Triterpenes are often found in plants in a glycosylated form, the so-called saponins. Glycosylation increases the polarity and therefore the water solubility of the triterpenes, which are per se hydrophobic. These amphiphilic properties of the compounds lead to the formation of foam in water, which explains the name saponin (soap). Furthermore, they allow the molecules to insert themselves into membranes, often due to interaction with cholesterols (Lorent et al. 2014). This perturbs the membranes and induces tensions leading to membrane perforation. When acting on red blood cells, which contain high amounts of cholesterol, this can lead to haemolysis. Saponins can also influence the micelle formation between sterols and bile acids, which is necessary for sterol absorption in the intestine, thereby interfering with cholesterol uptake. Saponins are defensive secondary metabolites that allow plants to cope with unfavourable environmental conditions (storing and conserving water, resisting predators and surviving severe weather conditions).

The dammarenyl carbocation can also give rise to protopanaxadiol and further the saponin ginsenoside (from the root of ginseng (*Panax ginseng*, Araliaceae)). Ginsenosides activate apoptosis in animals by triggering fast-mediated cell death through interference with membrane lipid rafts. Moreover, their structures are similar to steroid hormones, and they have been indicated as agonists for multiple steroidal receptors in mammalians (Kennedy and Scholey 2003; Park et al. 2017). Other prominent triterpenes are amyrin and lupeol. Lupeol gives rise to the potential anticancer agent betulinic acid, found in the bark of several trees including birch. α-Amyrin gives rise to ursolic acid, which is found in peels of fruits such as apples and could be acting on the growth-controlling pathways in mammalians. β-Amyrin is transformed into oleanic acid, which possibly has anti-cancer properties, and the saponines avenacin and glycyrrhizin. Avenacin is an antifungal component in the oat root (*Avena sativa,* Poaceae) and glycyrrhizin is the sweet-tasting constituent of liquorice (in *Glycyrrhiza glabra*, Fabaceae) that accumulates in root and stolon. From the sap of the fire tree (*Euphorbia tirucalli*, Euphorbiaceae), the tetracyclic euphol, an anti-inflammatory drug, can be extracted (Dutra et al. 2012).

Take-Home Messages

- Terpenes and terpenoids are synthetized by two different pathways, the cytosolic MVA pathway and the plastid MEP pathway.
- Terpene synthetases are involved in many enzymatic steps of biosynthesis.
- Monoterpenes include menthol and thujone and provide the precursors for indole alkaloids.
- Diterpenes include salvinorin A, ryanodine and resiniferatoxin.
- Sesquiterpenes include ß-caryophyllene and GABA antagonists such as picrotoxin and tutin.
- Especially monoterpenes and sesquiterpenes can be extracted as essential oils.

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