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Terpenoids for medicine

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Chapter 1

General Introduction

Terpenoids, also known as isoprenoids or terpenes, are a large class of natural products found in nearly all living organisms (Oldfield and Lin, 2012). Over 60,000 terpenoid structures have been identified from natural sources making them one of the largest classes of natural products known (Köksal et al., 2011; Berthelot et al., 2012). Terpenoids are perhaps most familiar to us as major components of essential oils produced by various aromatic plants, tree resins such as turpentine, or as cholesterol found in animal cell membranes. Essential oils are composed of mixtures of volatile compounds which are produced by physical processes such as distillation or pressing from the organisms producing them. Due to their volatile nature along with a wide variety of scents and flavors terpenoids are important ingredients in the cosmetic and flavor industries (Schmidt, 2010). It is the purpose of this thesis to investigate aspects of plant terpenoid chemistry relevant to medicine.

History of terpenoid chemistry

Humankind has for thousands of years made use of plants containing terpenoids as medicines, incenses, foods, intoxicants, and even natural rubber. Devices and documents that appear to resemble a water distillation apparatus have been dated back to ancient Mesopotamia from around 3,000 BCE. The ancient Egyptians adopted such primitive distillation practices however the Arabs in the middle ages are often credited as the inventors of distillation. Ad-Dimaschki a 13th century Arabian writer described the distillation of rose water and early improvements on condensation of the vapors. In the 15th century, German physician and scientist Hieronymus Brunschwyk in his book *Liber de Arte Distillandi* (The true art to distill) described the production of 25 essential oils (Figure 1). It should be noted that many of the products of such early distillation practices would not be considered essential oils but rather water or alcohol solutions enriched with volatile compounds (Schmidt, 2010). The medicinal use of aromatic plants is recorded in ancient herbal texts such as *De Materia Medica* written by the Greek herbalist Pedanius Dioscorides in the first century CE. Resins such as myrrh, from *Communphora* and *Balsamodendron* species as well as sandalwood oils from *Santalum* species have been used as far back as ancient Egypt for embalming and cleansing rituals. Aromatic plants are also featured in both traditional Chinese medicine and Ayurvedic medicine (Maffei et al., 2011). Systematic investigations into selection criteria of medicinal plants suggest that smell and taste are important characteristics for incorporation of a plant into traditional medicine systems (Leonti et al., 2003).

Modern terpenoid chemistry began in the 1800's. The German chemist Otto Wallach who was awarded the Nobel Prize in chemistry in 1910 for his achievements in the structure elucidation of monoterpenoids is often regarded as the father of terpenoid chemistry. Wallach began researching terpenoids while working under the direction of another Nobel Prize laureate, August Kekulé in Bonn, Germany (Christmann, 2010). At the time hydrocarbons isolated from plant essential oils with the molecular formula

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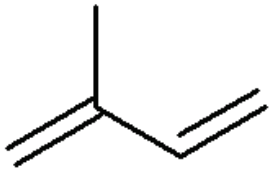
$C_{10}H_{16}$ which stayed liquid even at low temperatures were known as terpenes, a term coined by Kekulé due to their presence in turpentine oil. Compounds with the molecular formulas $C_{10}H_{16}O$ or $C_{10}H_{14}O$ containing an alcohol or a ketone respectively and could be crystallized or precipitated as solids were known as camphors (Kubeczka, 2010). Through the use of distillation techniques, particularly vacuum and fractional distillation, Wallach and his contemporaries were able to isolate individual essential oil components. By synthesizing terpenoid derivatives which could be crystallized and physical properties such as melting point, optical rotation, boiling point etc., characterized a systematic way of identifying terpenoids was developed. It was also discovered that natural terpenoids could be converted into other natural terpenoids. This sort of chemistry made it possible for quality control of natural essential oils and for the development of the synthetic fragrance and flavor industry. Furthermore, this research revealed that plants within the same genus could produce essential oils of different composition and that species taxonomically far apart could produce the same compounds (Wallach, 1910).

Figure 1. Inside title page of Hieronymus Brunschwyk's *Liber de Arte Distillandi*. Image downloaded from National Library of Medicine website (http://www.nlm.nih.gov).

In 1887 Wallach proposed that monoterpenoids were constructed from two isoprene units (Figure 2). Further development of the isoprene rule was accomplished

by the work of L. Ruzicka, in Zurich, through the structure elucidation of higher terpenes for which he also won the Nobel Prize in 1939 (Kubeczka, 2010). Ruzicka described the main terpene chemical classes as monoterpenoids composed of two isoprene units, sesquiterpenes three isoprene units (C_{15}), diterpenes four isoprene units (C_{20}), and triterpenes six isoprene units (C_{30}) (Ruzicka, 1953). We now know that hemiterpenoids made up of a single isoprene unit (C_5), tetraterpenes (C_{40}), as well as isoprenoid polymers with thousands of units such as rubber are also classes of terpenoids (Berthelot et al., 2012). Since the development of chromatographic techniques and nuclear magnetic spectroscopy (NMR) in the 2nd half of the 20th century the isolation and structure elucidation of thousands of terpenoids has been accomplished. Through improvements in analytical techniques such as gas chromatography (GC), mass spectrometry (MS), GC and MS in tandem (GC-MS), and high performance liquid chromatography (HPLC) identification and quantification of terpenoids from natural sources has become routine.

Figure 2. Isoprene



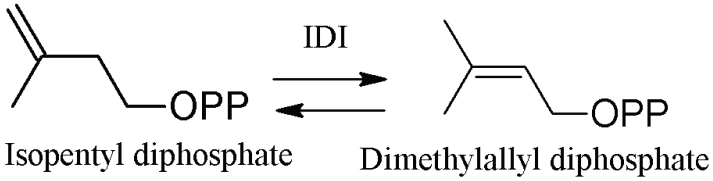
Biosynthesis

The isoprene used in the biosynthesis of terpenoids consists of isopentyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). The enzyme isopentyl diphosphate isomerase (IDI), also known as IPP isomerase, converts IPP to DMAPP in a reversible isomerization reaction, regulating the pool of these 2 metabolites (Figure 3) (Berthelot et al., 2012). The mevalonate (MVA) pathway, discovered in the 1950's, was the first biosynthetic pathway elucidated that lead to IPP and DMAPP. The MVA pathway was first identified in yeast and mammals (Rodríguez-Concepción and Boronat, 2002). The MVA pathway is located in the cytosol of most eukaryotes, archaebacteria, as well as some gram positive and gram negative bacteria (Oldfield and Lin 2012; Berthelot et al., 2012). However in the 1990's, another pathway the 2-*C*-methyl-D-erythritol 4 phosphate (MEP) pathway was discovered. The MEP pathway is known to occur in the plastids of terrestrial plants, algae, some protozoa such as malaria parasites, and many eubacteria including *Escherichia coli* (Phillips et al., 2008). Both the MVA and MEP pathways leading to IPP and DMAPP are shown in Figure 4.

Terpenoid biosynthesis begins with the condensation of IPP and DMAPP, except in the case of the hemiterpenoids. The reaction occurs through a 1'-4 so called "head to tail" process beginning with the ionization of DMAPP to remove the diphosphate group forming a carbocation intermediate. The carbocation through nucleophilic attack with double bond in IPP and removal of H^+ forms geranyl diphosphate (GPP). Geranyl diphosphate can be further condensed with IPP to farnesyl

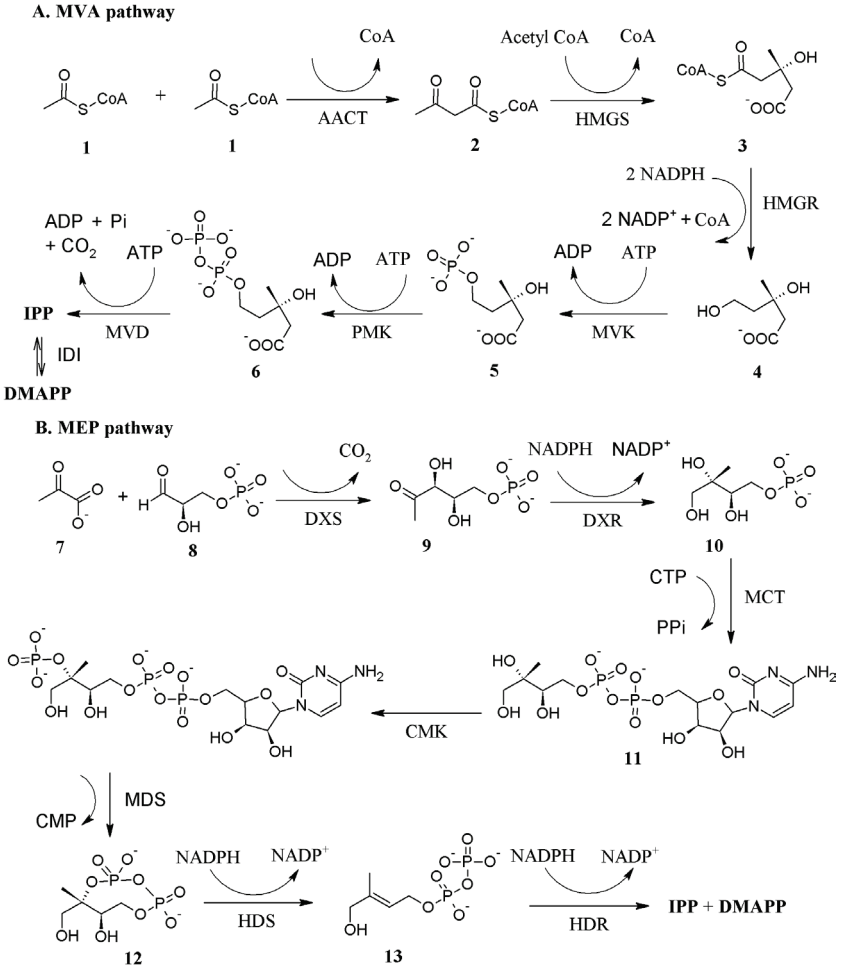
diphosphate (FPP), and again to geranylgeranyl disphosphate (GGPP) (Figure 5). Geranyl diphosphate, FPP, and GGPP are the precursors to the C_{10} monoterpenoids, C_{15} sesquiterpenoids and C_{20} diterpenoids respectively. Both FPP and GGPP can also be condensed in a 1'-2,3 so called "head to head" manner to form C_{30} triterpenoid and C_{40} tetraterpenoid precursors. The C_{30} is used in plants mainly for the production of sterols from squalene while C_{40} for the production of carotenoids from phytoene (Figure 6) (Oldfield and Lin, 2012; Chen et al., 2011). Monoterpenoids, diterpenoids, and tetraterpenoids are mainly synthesized by the MEP pathways while sesquiterpenoids and triterpenoids are mainly synthesized by the MVA pathway, although there are exceptions and cross talk between the 2 pathways occurs (Rodríguez-Concepción and Boronat, 2002; Maffei et al., 2011). The hemiterpene (C_5) isoprene is also biosynthesized in plants mainly from DMAPP by loss of diphosphate group. Terpenoids and their precursors can also be used to post translationally modify proteins (Oldfield and Lin, 2012).

Figure 3. Isopentyl diphosphate isomerase mediated isomerization of IPP and DMAPP.



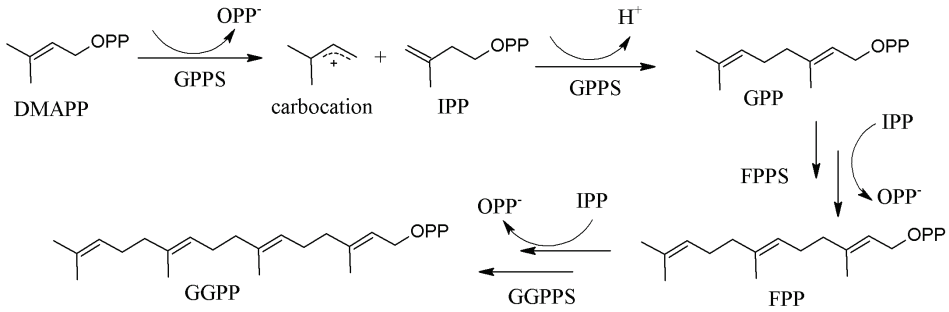
Terpene synthases, also known as terpene cyclases, catalyze the reactions which lead to the wide variety of terpenoid structures. Typically terpene synthases function by ionizing the respective terpenoid precursor, removing the phosphate groups leading to the formation of carbocation intermediates which undergo cyclizations, hydride shifts, or other rearrangements. The reaction is terminated by nucleophile capture often with water or deprotonation (Figure 7). An interesting aspect of terpene synthases is that a single enzyme can sometimes produce multiple terpenoid products and enantiomers (Chen et al., 2004; Degenhardt et al., 2009; Chen et al., 2011). Terpenoid structures can then be further enzymatically altered by hydroxylation, oxidation, reduction, isomerization, further cyclization, or be used as substrates to modify other compound classes (Dewick, 2002; Sell, 2010). Cytochrome P450 enzymes are an important class of proteins known to further modify terpenoids skeletons after they have been formed by terpene synthases (Keeling and Bohlmann, 2006; Mizutani, 2012).

Figure 4. MVA and MEP pathways. **1** Acetyl CoA, **2** acetoacetyl-CoA, **3** 3-hydroxy-3-methylglutaryl-CoA, **4** mevalonate, **5** mevalonate-5-phosphate, **6** mevalonate-5-diphosphate, **7** pyruvate, **8** D-glyceraldehyde 3-phosphate, **9** 1-deoxy-D-xylulose 5-phosphate, **10** 2-*C*-methyl-D-erythritol 4-phosphate, **11** 4-(cytidine 5'-diphospho)-2-*C*-methyl-D-erythritol, **12** 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate, **13** (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate. AACT: acetoacetyl-CoA thiolase, HMGS: 3-hydroxy-3-methylglutaryl-CoA synthase, HMGR: 3-hydroxy-3-methylglutaryl-CoA reductase, MVK: mevalonate kinase, PMK: 5-phosphomevalonate kinase, MVD: mevalonate-5-diphosphate decarboxylase, DXS: 1-deoxy-D-xylulose 5-phosphate synthase, DXR: 1-deoxy-D-xylulose 5-phosphate reductoisomerase, MCT: 2-*C*-methyl-D-erythritol 4-phosphate cytidylyltransferase, CMK: 4-(cytidine 5'-diphospho)-2-*C*-methyl-D-erythritol kinase, MDS: 2-*C*-methyl-D-erythritol 2,4-cyclodiphosphate synthase, HDS: (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate synthase, HDR: (*E*)-4-hydroxy-3-methylbut-2-enyl diphosphate reductase.



Terpenoid secondary metabolites are often produced and secreted by specialized plant cells in anatomical structures such as glandular trichomes, secretory cavities, and resin ducts. Glandular trichomes are made up of groups of cells which form an outgrowth of the epidermis. The morphology of glandular trichomes can vary considerably (Figure 8) (McCaskill and Croteau, 1998). Glandular trichomes can be divided into two groups peltate and capitate. Peltate trichomes contain a basal cell on the epidermis, a stalk or neck cell, and 4-16 cells on the head which secrete terpenoids into a large subcuticular space. Capitate trichomes contain a smaller subcuticular space and typically 1-4 secreting cells (Franz and Novak, 2010). Citrus fruits secrete essential oils into secretory cavities surrounded by isolated cells located beneath the epicarp. Similar secretory cavities are found in eucalyptus leaves which are located beneath the epidermis (Schmidt, 2010). Pine trees produce terpenoids in layers of cells found within the intercellular space of their bark which can be excreted through resin ducts (Keeling and Bohlmann, 2006).

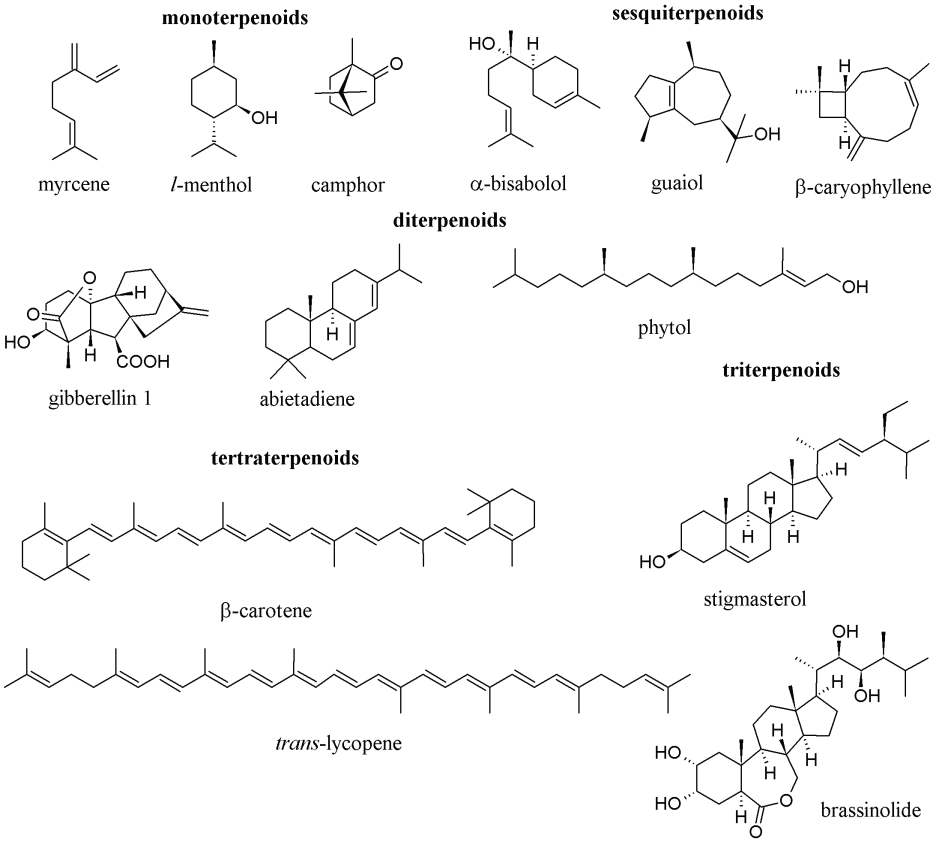
Figure 5. Biosynthesis of important terpenoid precursors.



Roles in plant physiology

Certain terpenoids are ubiquitous in plants having essential functions in their physiology and are thus often considered part of primary metabolism. Protein prenylation by C_{15} and C_{20} units is important for transferring and anchoring of cytosol proteins to membranes. Carotenoids make up part of the photosynthetic apparatus and are important plant pigments. Chlorophylls contain a diterpene side chain derived from phytol (Figure 6). Ubiquinones, which are part of the mitochondrial electron transport chain and plastoquinone in the photosynthetic electron carrier chain, both contain polyprenyl isoprenoid units (Lohr et al., 2012). Sterols are important constituents of plant cell membranes and are required for various cellular processes including cell division, elongation, and polarity (Figure 6). Although the mechanisms of plant sterols function remain largely unknown. Plant sterols are also precursors to the steroid hormones, brassinosteroids which are involved in plant growth and development (Figure 6) (Boutte and Grebe, 2009). Another important group of plant hormones are the diterpenoid carboxylic acids known as gibberellins which act as endogenous plant growth regulators (Figure 6) (Hedden and Thomas, 2012).

Figure 6. Examples from terpenoid classes.



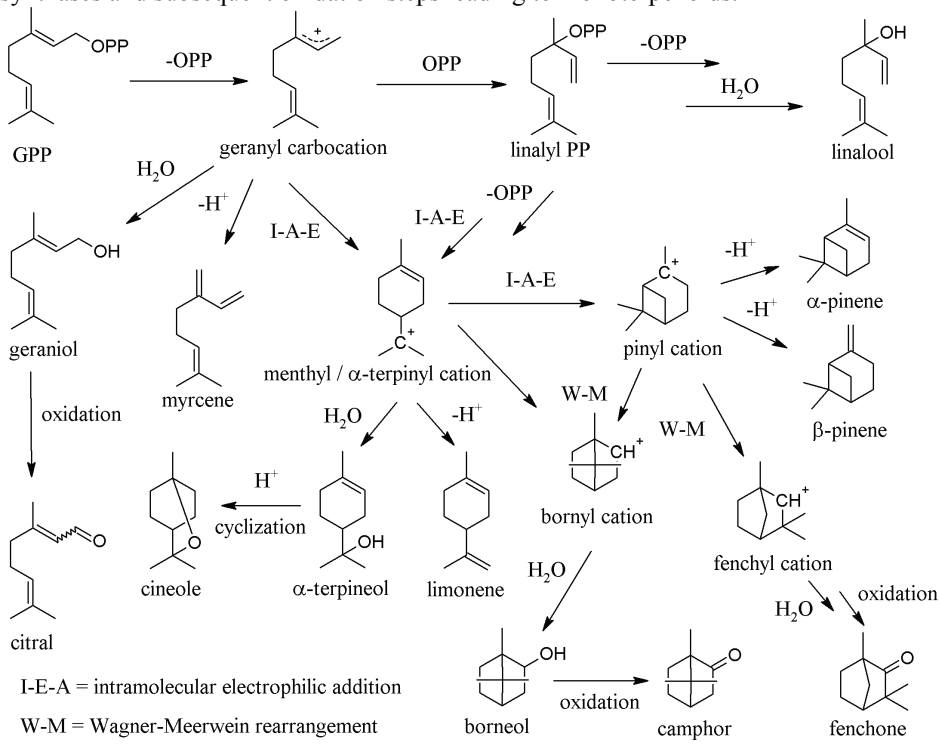
Chemical ecology

The majority of terpenoid chemical diversity found in nature is the result of secondary metabolism. Plants produce terpenoids and other volatile compounds to interact with the surrounding environment. Volatile terpenoids from flowering plants can serve as important environmental cues to attract or deter pollinators. Allelopathic effects from a terpenoid producing plant on other plants in the environment such as growth inhibition or inhibition of seed germination are also known (Maffei et al., 2011). Terpenoids and other plant volatiles can act as defense compounds against herbivores by either direct action against the attacking organism or by attracting natural predators of herbivores (Lucas-Barbosa et al., 2011).

Examples of direct terpenoid defenses against insects include the triterpene glycosides (eg. cardenolides) found in the latex of the milkweed *Asclepias curassavica*. The larvae of *Trichoplusia ni* (Lepidoptera) after feeding on *A. curassavica* have spasms resulting in immobility, taking around 3 days to recover. *Aradopsis thaliana*

genetically modified to produce large amounts of the monoterpene alcohol linalool are more resistant to *Myzus persicae* (aphids) then non-engineered plants which produce very low levels of linalool (Gershenzon and Dudareva, 2007). Conifer resins which often contain mixtures of monoterpenoids, sesquiterpenoids, and diterpenoids have been shown to have important roles in defending trees against bark beetles and pathogenic fungi (Langenheim, 1994; Keeling and Bohlmann, 2006). Saponins (triterpenoid glycosides) can act as detergents disrupting fungal cell membranes leading to toxicity. *Avena strigosa* an oat species which normally produces saponins has mutant varieties which do not produce saponins. Such plants are significantly more susceptible to fungal pathogens (Gershenzon and Dudareva, 2007).

Figure 7. Generalized scheme showing typical reaction mechanisms of monoterpene synthases and subsequent oxidation steps leading to monoterpenoids.

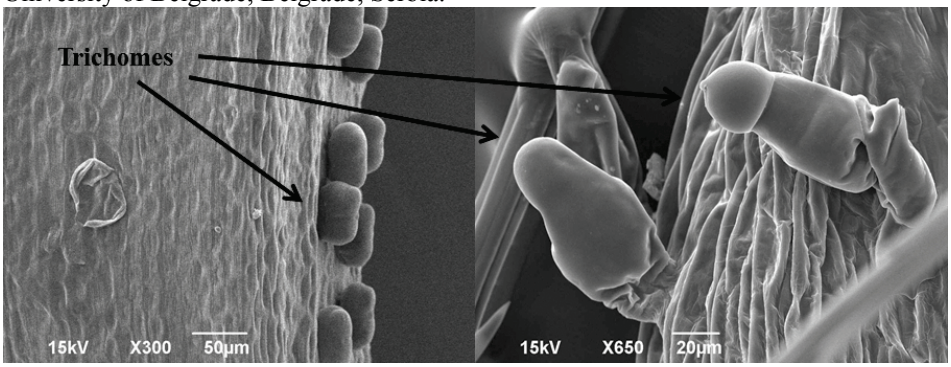


Biological activity

Due to the historical use of terpenoids in medicine and the evolutionary fine tuning of their interactions in natural systems it is no surprise that so many biological activities have been reported. Since there are hundreds of studies dealing with the biological activity of terpenoids the aim of the following sections is not to provide a comprehensive survey of their activity but rather to provide an overview of biological activities important to medicine and understanding subsequent discussions in later

chapters. Throughout the rest of this thesis the focus will be on monoterpenoids, sesquiterpenoids, and diterpenoids. Tripterpenoids and carotenoids are excluded because they are not discussed or studied in subsequent chapters. The term essential oil will be used to refer to essential oils dominated by monoterpenoid and sesquiterpenoid constituents while those containing mostly phenylpropanoids or other compounds will not be discussed. One common chemical feature of many but not all terpenoids is their lipophilic nature. Due to their hydrophobic nature it is often assumed that the biological activity of certain terpenoids is a result of their tendency to partition into cellular membranes and either disrupt membrane integrity or interact with membrane bound proteins (Edris, 2007; Maffei et al., 2011; Solórzano-Santos et al., 2012).

Figure 8. Scanning electron microscope photos of trichomes on floret surfaces of *Tanacetum parthenium* (left) and *Inula britannica* (right). Image generously donated by Dr. Slađana Todorović from Institute for Biological Research "Siniša Stanković", University of Belgrade, Belgrade, Serbia.



Antimicrobial

One of the most commonly reported biological activities of terpenoids and especially those found in essential oils is their *in-vitro* antimicrobial activity. Thymol and carvacrol, phenolic monoterpenoids commonly found in *Thymus vulgaris* and *Origanum vulgare* essential oils as well as menthol found in peppermint oils are among some of the best studied antimicrobial monoterpenoids (Pauli and Schilcher, 2010; Solórzano-Santos et al., 2012). Due to the fact that essential oils occur in mixtures and their chemical composition can vary so can their antimicrobial effects. This makes clinical trials and pharmaceutical research into essential oils difficult. For example rosemary oil's minimum inhibitory concentration (MIC) against *Staphylococcus aureus* in nine *in-vitro* studies ranged between 20-50,000 µg/mL (Pauli and Schilcher, 2010). Many diterpenoids also exhibit antibacterial and antifungal activities (Hanson, 2007). Antiviral effects of monoterpenoids, sesquiterpenoids, and diterpenoids have also been reported (Sun et al., 2003; Wang et al., 2005; Buchbauer, 2010).

One reason why so many essential oils have *in-vitro* antimicrobial activity is likely due to their non-specific membrane disrupting properties. *In-vitro* antimicrobial data of terpenoids must therefore be interpreted carefully (Maffei et al., 2011).

Furthermore weak or strong *in-vitro* antibacterial data of essential oils does not always correlate well with *in-vivo* results complicating the interpretation of *in-vitro* data even further (Pauli and Schilcher, 2010). While the complex chemical profile of essential oils and weak to moderate antimicrobial activity makes them unlikely candidates for systemic treatment of infections, essential oils do have uses as topical and gastrointestinal antimicrobial agents as well as antiseptics (Maffei et al., 2011). Clinical data regarding the antimicrobial activity of essential oils is unfortunately scarce. Regarding topical application of essential oils studies have shown positive effects against *Propionibacterium acnes* (acne) infections from treatment with *Ocimum gratissimum* (rich in thymol) and tea tree oil (*Melaleuca alternifolia*). A number of studies have also shown promising results against topical fungal infections from various essential oils. However the best clinically studied use of topically administered essential oils is against infectious bacteria of the oral cavity. The commercial mouthrinse Listerine contains 1,8-cineole (cineol), menthol, and thymol as active ingredients (Harris, 2010).

Artemisinin, a sesquiterpene lactone found in *Artemisia annua* (Asteraceae) is an example of a potent antimicrobial terpenoid with specific activity against the malaria parasite, *Plasmodium falciparum* (Figure 9). Artemisinin and its analogues can kill most stages of the parasites life cycle and are currently the main treatments against malaria worldwide (White, 2008). The endoperoxide functionality is necessary for artemisinin's antimalarial activity. Artemisinin's mechanism of action is not entirely understood although there is evidence that once inside the parasite the endoperoxide can be cleaved by an iron source, such as Fe^{2+}. This reaction leads to the formation of radical ion intermediates which bind to various proteins one of which is the sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA) thereby killing the organism (O'Neill, 2010).

Inflammation and pain

Many plants containing terpenoids are used in traditional medicine for their anti-inflammatory and pain relieving properties. The Asteraceae family contains many species used traditionally against inflammatory conditions which produce sesquiterpene lactones. One well known example is parthenolide from *Tanacetum parthenium* commonly known as feverfew (Figure 9). The pro-inflammatory transcription factor, nuclear factor kappa B (NF-$_K$B) is inhibited by sesquiterpene lactones which may explain their mechanism of action (Salminen et al., 2008). Many essential oils have been evaluated for anti-inflammatory and analgesic activity in a variety of cellular and animal models (Buchbauer, 2010). Small clinical trials performed with essential oils have demonstrated positive effects against dysmenorrhea (menstrual pain), infantile colic, traumatic or surgical joint related pain, headaches, postherpetic neuralgia, and irritable bowel syndrome (Harris, 2010). Important anti-inflammatory diterpenoids include triptolide found in the traditional Chinese medicinal plant *Tripterygium wilfordii* (Figure 9). Traditionally the plant is used in the treatment of autoimmune conditions. Triptolide may also exert its effects via NF-$_K$B mediated mechanisms (Salminen et al., 2008). The labdane diterpenoid, andrographolide from *Andrographis panuculata,* exhibits anti-inflammatory effects through inhibition of pro-inflammatory mediators tumor necrosis factor α (TNF-α) and interleukin-12 (Figure 9) (Hanson,

2007). Abietane diterpenoids carnosic acid and carnosol found in *Rosmarinus officinalis* (rosemary) and *Salvia officinalis* (sage) also have anti-inflammatory properties. They have been shown to activate the peroxisome proliferator-activated receptor gamma (PPARγ) (Rau et al., 2006) as well as suppress pro-inflammatory responses of human polymorphonuclear leukocytes (Peockel et al., 2008).

Other potential protein targets of terpenoids are the transient receptor potential channels (TRP) also known as vanilloid or capsaicin receptors. These membrane bound receptors are known to be involved in sensing hot or cold temperatures and other noxious stimuli. Compounds such as (-)-menthol and camphor exhibit their cooling sensations by interacting with TRP's (Maffei et al., 2011). The TRP vanilloid-3 (TRPV3) is known to be involved in skin inflammation and peripheral nerve injuries. Camphor and cineol not only activate the TRPV3 but desensitize the receptor after long term exposure which may explain their mechanism as topical analgesics (Sherkheli et al., 2009). Sesquiterpene dialdehyes which are pungent ingredients in certain spices also activate vanilloid receptors (Szallasi et al., 1998). The cannabinoid receptors, CB1 and CB2 are G protein coupled receptors. They are the primary targets of cannabinoids, part terpenoid and part polyketide compounds found in *Cannabis sativa* L (cannabis). The CB1 receptor is located primarily on central and peripheral neurons while the CB2 receptor is located primary on immune cells. Many of the pain relieving, anti-inflammatory, and psyschoactive effects of cannabinoids are mediated through the CB1 and CB2 receptors (Pertwee, 2009). Interestingly the dietary sesquiterpenoid, β-caryophyllene is a potent and selective CB2 receptor agonist with anti-inflammatory properties. Many herbs and spices including cannabis, cinnamon, and black pepper contain significant amounts of β-caryophyllene (Gertsch, 2008).

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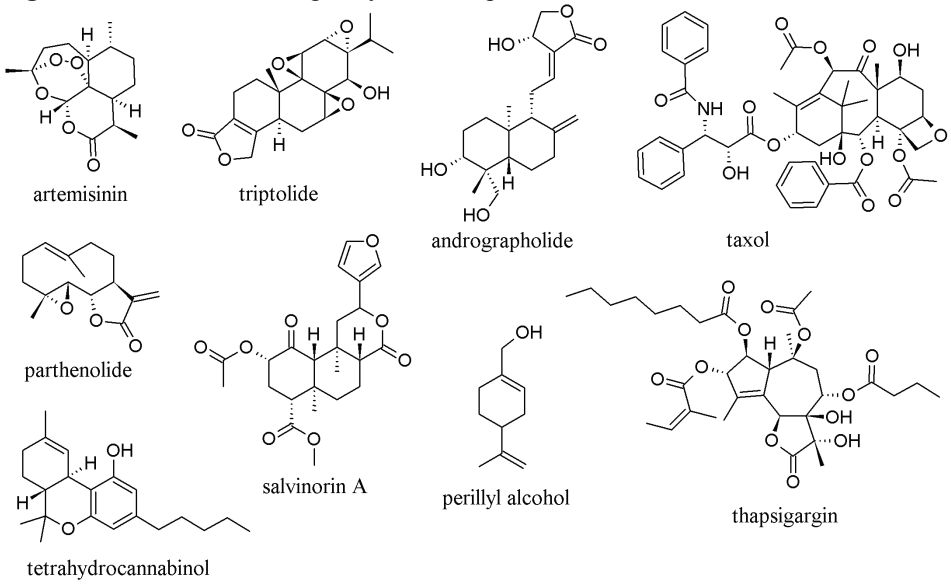
Figure 9. Structures of biologically active terpenoids.



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Cytotoxic, antitumor, and anticancer

In the following section cytotoxic compounds will refer to compounds which inhibit cancer cells *in-vitro*, antitumor to those which inhibit tumor growth *in-vivo*, and anticancer to those being used to treat cancer in humans. An important anticancer diterpene is taxol, also known as paclitaxel isolated from the bark of *Taxus* species (Figure 9). Taxol is used in the treatment of a variety of cancers such as ovarian, breast, and lung cancer (Wang et al., 2005). The anticancer effects of taxol are due to its ability to promote polymerization of tubulin into stable microtubules, which thereby prevent mitosis. Structure activity relationships of taxols anticancer properties revealed that the oxetane ring and aromatic substituents are important for the drugs activity (Kingston, 1994). Many other diterpenoids also exhibit cytotoxic or antitumor properties (Hanson, 2007). Interestingly many of the same diterpenoids mentioned above as having anti-inflammatory activity also have cytotoxic and antitumor activities. Andrographolide has antitumor properties which may also be mediated by inhibition of NF-$_{K}$B (Gunn et al., 2011; Kuttan et al., 2011). Carnosic acid and carnosol have cytotoxic and antitumor properties (Johnson, 2011; Ngo et al., 2011).

Many sesquiterpenoids have been reported to have cytotoxic activity (Modzelewska et al., 2005). Sesquiterpene lactones are among the most well studied cytotoxic sesquiterpenoids. Extensive structure activity relationships have demonstrated that the α-methylene-γ-lactone moiety is often, although not always necessary for cytotoxicity. Through a Michael addition reaction the α-methylene-γ-lactone functionality can act as a nucleophile reacting with cysteine residues on proteins forming a protein adduct. As with anti-inflammatory activity an important target protein for sesquiterpene lactones cytotoxic activity is NF-$_{K}$B. Parthenolide is an example of an antitumor sesquiterpene lactone containing the α-methylene-γ-lactone (Ghantous et al., 2010). However it is important to note that the α-methylene-γ-lactone group is also responsible for certain sesquiterpene lactones causing contact dermatitis (Lepoittevin et al., 2009) and toxicity in mammals (Robles et al., 1995). Thapsigargin, from *Thapsia garganica* and *Thapsia gymnesica* is an example of a sesquiterpene lactone that does not contain an unsaturated lactone yet still possesses antitumor activity (Figure 9). The main mechanism of inducing apoptosis by thapsigargin is inhibition of SERCA which causes an increase in cytoplasmic Ca^{2+} leading to cell death (Drew et al., 2009). Artemisinin also lacks the unsaturated lactone functionality yet also exhibits anti-tumor activity through a similar mechanism as against malaria. Interestingly parthenolide, thapsigargin, and artemisinin all seem to exhibit selectively towards cancer cells *in-vivo*. Analogues of all 3 compounds are being developed as anti-cancer drugs (Ghantous et al., 2010).

Monoterpenoids such as limonene and its main metabolite in humans, perillyl alcohol exhibit antitumor activities (Figure 9). Other monoterpenoid alcohols such as geraniol, linalool, carveol prevent tumor formation in chemically induced tumor animal models. Many essential oils have also been tested for cytotoxic and antitumor properties (Buchbauer, 2010). Despite much research *in-vitro* and in animals, clinical trials with

monoterpenoids are scarce. Perillyl alcohol has been tested in phase II clinical trials however no anti-cancer effects were observed in advanced ovarian, colorectal, or breast cancer. A reduction in tumor size was noted in a trial with recurrent malignant gliomas (Harris, 2010). A phase I and II trial to assess anticancer potential of limonene demonstrated that limonene was well tolerated however was only able to exhibit a partial response in one patient. The researchers note however that the dose which was the maximum tolerated dose for each patient may not be appropriate and suggested further research (Vigushin et al., 1998).

Central nervous system

The most obvious effect of terpenoids can have on the central nervous system (CNS) is odor perception. Why exactly humans maintain the evolutionary traits which allow us to sense and discriminate so many volatile terpenoids is not entirely clear. Terpenoids are not directly linked to the nutritional value of a plant when compared to other plant volatiles derived from essential nutrients such as fatty acids or carotenoids. The antimicrobial effects of many spices containing terpenoids suggest that cultural preferences for certain flavors may have developed throughout history due to health promoting or food preservation benefits (Goff and Klee, 2006). Olfactory receptors are located primarily on neurons in nasal cavities. About 380 genes encode for human olfactory receptors. Another, approximately 400 non-functional pseudogenes can be found in the human genome suggesting that olfactory genes were lost throughout primate evolution (Niimura and Nei, 2003; Schmiedeberg, 2007). In contrast with other senses, olfactory information can bypass the thalamus and directly link with areas of the brain involved in emotion and memory such as the amygdala, frontal cortex, hypothalamus, and hippocampus (Kandal et al., 2000). Furthermore due to their lipophilic nature terpenoids are able to pass the blood brain barrier (BBB) and interact directly with the brain. Both mechanisms are important because odorous terpenoids can have a direct pharmacological action in the brain, such as interaction with a neural receptor, and a psychological component through the olfactory system (Heuberger, 2010).

Essential oils used commonly in folk medicine and pure monoterpenoids have been demonstrated to cause sedative and stimulating effects in mice (Buchbauer et al., 1993). Psychopharmacological effects of essential oils and pure monoterpenoids in animals include anxiolytic, anticonvulsant, antidepressant, and hypnotic effects (Nunes et al., 2010). Mechanisms of action have been investigated. Lemon oil vapor increases metabolism of dopamine in the hippocampus and serotonin in the prefrontal cortex and striatum which may explain its anxiolytic and antidepressant effects in mice (Komiya et al., 2006). The anticonvulsant effects of linalool may be mediated by interactions with NMDA receptors (Brum et al., 2001). The effects of essential oils and pure fragrances on human cognition have also been investigated. Examples of higher cognitive functions studied include alertness and attention as well as learning and memory. Unfortunately most studies do not report chemical composition of the essential oil under investigation and dose response curves are not established. Therefore it is difficult to draw clear relationships between essential oils and their effects on higher cognitive functions in humans (Heuberger, 2010).

Terpenoids can also have profound effects on human consciousness. *Salvia divinorum* is used by Mazatec traditional healers for divination. The plant is often chewed or smoked to produce hallucinations necessary for their healing rituals. The main active ingredient is salvinorin A, a neoclerodane diterpenoid (Figure 9). Salvinorin A is one of the most potent non-nitrogenous natural hallucinogens known with doses as little as 200-500 μg inducing hallucinatory experiences. The psychoactive effects of salvinorin A are due to its ability to act as a selective K-opioid receptor agonist (Vortherms and Roth, 2006). The cannabinoid, Δ^9-tetrahydrocannabinol (THC) is the primary psychoactive ingredient in cannabis (Figure 9). The psychoactive effects of THC are mediated mainly through the CB1 receptor (Pertwee, 2009). Clinical studies demonstrate potential of cannabinoids and cannabis in treating chronic neuropathic pain, as appetite stimulants in cancer patients undergoing chemotherapy as well as AIDS patients, and treatment of multiple sclerosis (Amar, 2006; Hazekamp and Grotenhermen, 2010).

Thesis outline and goals

Throughout this discussion a number of issues become apparent with regards to complications in the development of terpenoids and plants containing them as drugs. One major issue is that plants and products derived from them such as essential oils or extracts are often of variable or unknown chemical composition. Another issue concerns administration forms of volatile terpenoids. Often protocols for administering essential oils vary between studies and controlled dosing is difficult. Finally for many terpenoids the mechanisms of action and structure activity relationships are still unknown. Therefore the major goals of this thesis are:

1. Investigate vaporization as an administration form for volatile terpenoids in various medicinal plants.
2. Determine if plants can be standardized to produce reproducible levels of active components in order to improve clinical research.
3. Isolate and study the biological activity of medically interesting sesquiterpenoids and diterpenoids.

In chapter 2 a device designed to administer plant volatile compounds called a vaporizer was investigated. Some common terpenoid producing plants with medicinal properties were selected and tested in the device. The essential oil content and terpenoid content of the vapor was analyzed with GC and GC-MS. In chapter 3, cannabis was used as a model plant to test the vaporizer in more detail, compare it with cannabis smoke, and test whether any components in the smoke or vapor altered the CB1 binding of THC *in-vitro*. In order to determine if plants producing terpenoids can be standardized for their chemical content cannabis was again chosen as a model plant. In chapter 4 a number of cannabis varieties grown in multiple batches under the same environmental conditions were analyzed with GC-FID and GC-MS. Multivariant data analysis was used to chemically classify the varieties. The reproducibility of the chemical profiles was determined. The plants studied in chapters 5-7 were investigated

as part of a larger projet to further study their biological activity and their biosynthesis called the Terpmed project (EU grant number 227448). Chapter 5 focuses on the isolation and biological activity of sesquiterpenoids from *T. parthenium*. The isolated compounds were tested as activators of a biological pathway emerging as a potential drug target for neurodegenerative disease, the nuclear factor E2-related factor 2 / Kelch-like ECH-associated protein 1 pathway. This same pathway is investigated for diterpenoids isolated from *S. officinalis* in chapter 7. In chapter 6 sesquiterpene lactones isolated from *Inula britannica* were studied for their cytotoxic activity in drug susceptible cancer cell lines and multi drug resistant cancer cell lines. Finally chapter 8 discusses the overall conclusions from these investigations and future perspectives.

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