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REVIEW

Plant volatiles: Production, function and pharmacology†

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Plant volatiles typically occur as a complex mixture of low-molecular weight lipophilic compounds derived from different biosynthetic pathways, and are seemingly produced as part of a defense strategy against biotic and abiotic stress, as well as contributing to various physiological functions of the producer organism. The biochemistry and molecular biology of plant volatiles is complex, and involves the interplay of several biochemical pathways and hundreds of genes. All plants are able to store and emit volatile organic compounds (VOCs), but the process shows remarkable genotypic variation and phenotypic plasticity. From a physiological standpoint, plant volatiles are involved in three critical processes, namely plant–plant interaction, the signaling between symbiotic organisms, and the attraction of pollinating insects. Their role in these "housekeeping" activities underlies agricultural applications that range from the search for sustainable methods for pest control to the production of flavors and fragrances. On the other hand, there is also growing evidence that VOCs are endowed with a range of biological activities in mammals, and that they represent a substantially under-exploited and still largely untapped source of novel drugs and drug leads. This review summarizes recent major developments in the study of biosynthesis, ecological functions and medicinal applications of plant VOCs.

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1 Introduction

Volatile organic compounds (VOCs) are released into the atmosphere not only by human activity, but also as a result of a host of physiological processes of plants from marine and terrestrial environments.[1] The biogenic production dwarfs the anthropogenic one: over 90% of the natural emission of VOCs is due to plant species, and the Amazonian rainforest is the single largest sources of these compounds.[2] Plants emit 400–800 Tg C/yr as hydrocarbons, an amount equivalent to the sum of the biogenic and anthropogenic methane emissions,[3] while up to 36% of the assimilated carbon is released as complex mixtures of VOCs.[4–7] VOCs of plant origin are extremely reactive in the troposphere, with life-times in the minutes to hours range,[1] and contribute to the aerosol[8] that scatters light and produces the blue hue of the sky. All compounds contained in essential oils are, in principle, VOCs, although the relatively high boiling point of some of them (>200 °C) makes their atmospheric concentration very low.

VOCs are important components of the plant's chemical phenotype, and their ecological relevance can hardly be underestimated.[7] Leaves, flowers and fruits release them into the atmosphere, and roots into the soil. Pollinator-attracting floral VOCs have been a source of olfactory pleasure for humans since antiquity, and a large number of aromatic plants as flavorings, preservatives, and herbal remedies are also used.[9,10] The primary

functions of airborne VOCs are the defense against herbivores and pathogens, the attraction of pollinators and seed dispersers, and the signaling involved in plant–plant communication.[11] In some plants, released VOCs may also act as wound sealers.[12] Certain less volatile VOCs may be dangerous for man when present at higher concentrations[13] (*vide infra*), and plant-emitted VOCs are also major precursors of tropospheric phytotoxic compounds.[14] Since some VOCs can act as precursors of photochemical smog, their level is one of the fundamental parameters for the assessment of atmosphere quality.[15] VOCs can regulate the oxidative capacity of the troposphere in terms of the concentrations of carbon monoxide, ozone, and the overall aerosol balance. Their role in the generation of phytotoxic ozone in the presence of a high concentration of nitrogen oxides and sunlight has been extensively investigated.[16] Furthermore, VOCs have also been shown to be involved in the formation of secondary aerosols in the atmosphere,[8] a process of great relevance for the radiative balance of the Earth.[17] Routine measurements of VOCs in outdoor air have shown that their day-long average concentrations are significantly lower than those normally used in laboratory experiments to study their effects on plants. Nevertheless, their production undergoes enormous fluctuations during the day, and spikes of concentrations of specific VOCs up to 10-fold higher than the average can be detected for relatively brief (minutes to a few hours) periods of time.[18] Many VOCs produced by plants, in particular the structurally more evolved fragrant constituents of aromatic essential oils, are commercialized as flavors and/or fragrances, and their use in the food and perfume industries has a long tradition.[19] Fragrant plants have always played a central role in folk medicine. The medicinal use of essential oils, as well as of herbal remedies produced from plants containing essential oils, has long been documented for conditions that can benefit from their antimicrobial, anti-inflammatory, bronchodilatory, expectorant, anticonvulsant, cholagogic, analgesic, and spasmolytic activity, and Western Pharmacopoeias list several plant essential oils (*aetheroleum* drugs) (Table 1). Nevertheless, the molecular bases for these activities are still largely unknown. Due to a pronounced lipophilicity and a poor chemical functionalization, VOCs and essential oils have substantially failed to raise interest in the medicinal chemistry community. However, recent insights in the pharmacological action of structurally simple but nonetheless highly bioactive plant VOCs is spurring a growing interest for biological properties that go beyond the odor perception, and encompasses the interaction with specific receptors not directly involved in olfaction.

This review aims at summarizing information on the mechanism of production of VOCs from plants, on the ecological rationale of these processes, and on the potential of plant VOCs for drug discovery, an issue still largely overlooked.

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2 Concepts in the study of plant volatiles

From a chemical standpoint, VOCs belong to various classes of natural products, namely terpenoids (homo-, mono-, di-,

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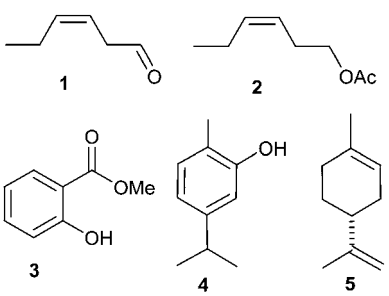
Table 1 VOC containing essential oils with pharmaceutical applications

PH. Eur.[a] 6.5, No	USP-NF 32–27	English Name	Latin Name (Pharmacopoeia)
804	x	Anise oil	*Anisi aetheroleum*
1826	x	Bitter-fennel fruit oil	*Foeniculi amari fructus aetheroleum*
2380		Bitter-fennel herb oil	*Foeniculi amari herbae aetheroleum*
1817	x	Caraway oil	*Carvi aetheroleum*
1496		Cassia oil	*Cinnamomi cassiae aetheroleum*
1501		Cinnamon bark oil, Ceylon	*Cinnamomi zeylanicii corticis aetheroleum*
1608		Cinnamon leaf oil, Ceylon	*Cinnamomi zeylanici folii aetheroleum*
1609		Citronella oil	*Citronellae aetheroleum*
1850		Clary sage oil	*Salviae sclareae aetheroleum*
1091	x	Clove oil	*Caryophylli floris aetheroleum*
1820	x	Coriander oil	*Coriandri aetheroleum*
2377		Dwarf pine oil	*Pini pumilionis aetheroleum*
390		Eucalyptus oil	*Eucalypti aetheroleum*
1629		Fennel oil, sweet	*Foeniculi dulcis aetheroleum*
1832		Juniper oil	*Iuniperi aetheroleum*
1338		Lavender oil	*Lavandulae aetheroleum*
620	x	Lemon oil	*Limonis aetheroleum*
2355		Mandarin oil	*Citri reticulatae aetheroleum*
1836		Matricaria oil	*Matricariae aetheroleum*
1838		Mint oil, partly dementholised	*Menthae arvensis aetheroleum*
1175		Neroli oil	*Neroli aetheroleum*
2468		Niaouli oil	*Niaouli aetheroleum*
1552		Nutmeg oil	*Myristicae fragrantis aetheroleum*
405	x	Peppermint oil	*Menthae piperitae aetheroleum*
1842		Pine sylvestris oil	*Pini sylvestris aetheroleum*
1846		Rosemary oil	*Rosmarini aetheroleum*
1849		Spanish sage oil	*Salviae lavandulifoliae aetheroleum*
2419		Spike lavender oil	*Spicae aetheroleum*
2108		Star anise oil	*Anisi stellati aetheroleum*
1811	x	Orange oil, sweet	*Aurantii dulcis aetheroleum*
1837		Tea tree oil	*Melaleucae aetheroleum*
1374		Thyme oil	*Thymi aetheroleum*
1627		Turpentine oil, Pinus pinaster	*Terebinthinae aetheroleum ab Pino pinastro*
—	x	Cardamon oil	*Elettaria cardamomum* essential oil
—	x	Rose oil	*Rosa canina* essential oil

[a] PhEur Pharmacopeia Europea 6; USP-NF United States Pharmacopeia/National Formulary.

sesquiterpenoids), fatty acid degradation products [*e.g.* (*Z*)-hex-3-enal (**1**), (*Z*)-hex-3-enyl acetate (**2**)], phenylpropanoids [*e.g.* methyl salicylate (MeSA, **3**)], amino acid-derived products (*e.g.* simple indoles), and alkanes, alkenes, alcohols, esters, aldehydes, and ketones of various biogenetic origin.[9,20–22] Today, over 1700 volatile compounds have been identified from more than 90 plant families, constituting approximately 1% of all plant secondary metabolites currently known.[9] Some mixtures of VOCs are produced "on demand" after mechanical or biological insult, and their composition depends on the mode of damage (single or continuous wounding,[23] herbivore feeding,[24] and egg deposition[25]). On the other hand, undamaged plants also release volatiles, like in most flowers. Some VOCs emitted after insect feeding can serve not only in a direct defense role, repelling the attacking insect, but also act as an indirect defense, attracting natural enemies of the attacking insect.[26] The term "volatilome" has been recently proposed[27] to describe the VOCs emitting profile of a specific plant.

Certain monoterpenes, like carvacrol (**4**) and D-limonene (**5**), have an allelopathic role, inhibiting the cytochromic pathway of respiration, blocking the nitrogen cycle, or inhibiting growth and seed germination of neighboring plants.[28,29] The allelopathic activity of certain VOCs has led researchers to consider them as possible leads in the development of new natural product based agrochemicals.[30,31]



Plants undoubtedly benefit from chemical defense, but the expression of secondary metabolites can be detrimental in the absence of plant enemies.[32,33] Therefore, the synthesis of a specific phytochemical, a light- and soil nutrition-dependant process, bears fruit only when its reward can be reaped. When the use of resources does not benefit an organism, it will eventually disappear during evolution as a genetic trait, and during its lifetime as a phenotypic trait.[31]

Improvements in analytical techniques and molecular and biochemical methods have made VOCs one of the best-studied class of plant secondary metabolites.[10] Qualitative and quantitative analyses of VOCs emitted by plants can be efficiently studied by headspace gas chromatography (GC). Furthermore, the development of static and dynamic techniques for headspace

collection of volatiles in combination with gas chromatography–mass spectrometry (GC-MS) analysis has significantly improved our understanding of the biosynthesis and ecology of plant VOCs. Finally, advances in automated analysis of VOCs have made it possible to monitor fast changes in VOC emissions, greatly facilitating *in vivo* studies of VOC biosynthesis.[34]

The study of VOCs has reached, in some cases, the biological threshold sensitivity. By using special adsorbing/sorpting supports such as polymethydisiloxane (PDMS) for thermal desorption, the identification and the quantification of molecules is possible at limits rivaling the detection threshold of molecular sensors in living organisms.[35–38] Other methods in the study of VOCs include chemiluminescence or infrared photoacoustic (PA) spectroscopy,[39] proton transfer reaction mass spectrometry (PTR-MS),[40] supercritical extraction (SFE),[41] rapid sensing by electronic noses,[42] microwaves for high efficiency extraction[43] and sample direct injection.[44] Alternatively, the effluent from GC can be linked to electrophysiological recordings from insect antennae,[45] the main sensory organs of these organisms. Either by using the whole antenna (the electroantennogram, EAG) or recording from individual olfactory neurons (single cell recording, SCR), it can be determined which peaks eluting from the GC are of significance to a specific insect.[46] Nevertheless, the use of biological detector systems (*e.g.* in olfaction) still evidence compounds that escape physicochemical detection, at least when compound identification is required. Finally, functional genomics approaches for dissecting the metabolic pathways of plant VOCs have provided a means for high-throughput profiling of volatile metabolites of mutant and transgenic plant lines.[34] Future trends in VOC analytics will be at the level of systems biology, linking dynamic chemical signals to physiological responses, thus allowing for correlations between cellular signaling and VOC production.

3 Main biochemical pathways involved in the biosynthesis of plant volatiles

Plant volatiles are the results of many biochemical pathways leading to the production of a wide array of volatile compounds. *De novo* biosynthesis and emission of VOCs includes products of the lipoxygenase (LOX) pathway, such as oxylipins, green leaf volatiles (GLVs), as well as distinct terpenoids, including isoprene, some carotenoid derivatives, indoles and phenolics, including MeSA and aromatic VOCs.[47] Fig. 1 shows the VOCs biosynthetic tree along with some representative molecules for each branching pathway.

Higher (>C_{15}) terpenoids are generally non-volatile and are directly or indirectly involved in important cellular processes, such as membrane structure (sterols), photosynthesis (chlorophyll side chains, carotenoids), redox chemistry (quinones) and growth regulation (gibberellins, abscisic acid, brassinosteroids). Volatile terpenoids are represented by the C_5 (hemiterpenes), the C_{10} (monoterpenes) and C_{15} (sesquiterpenes) members of this family, that are the major constituents of the plant volatilome.[34] Moreover, these compounds are the bulk constituents of plant essential (volatile) oils. The degree of volatility of these terpenoids depends

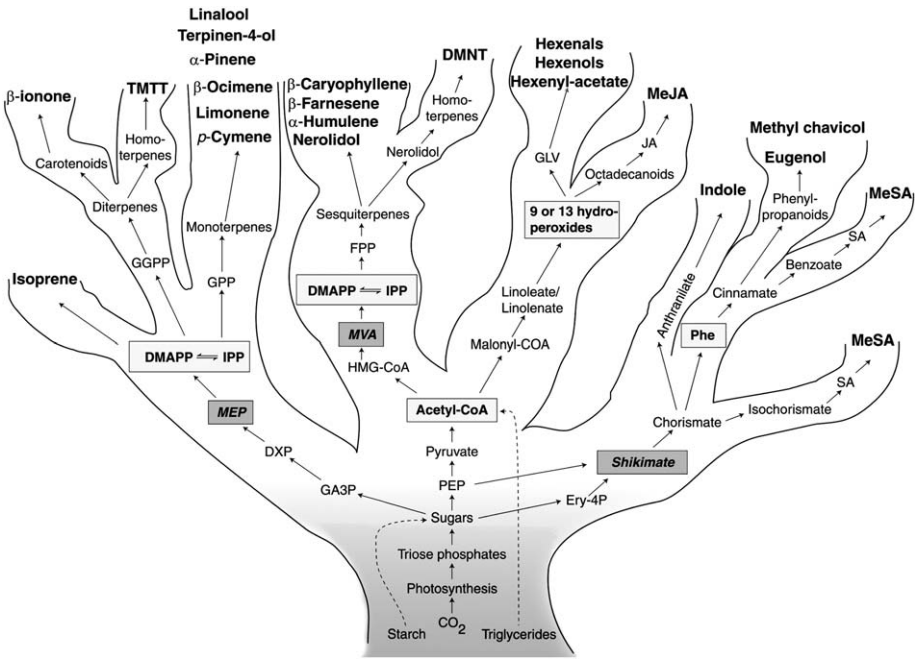


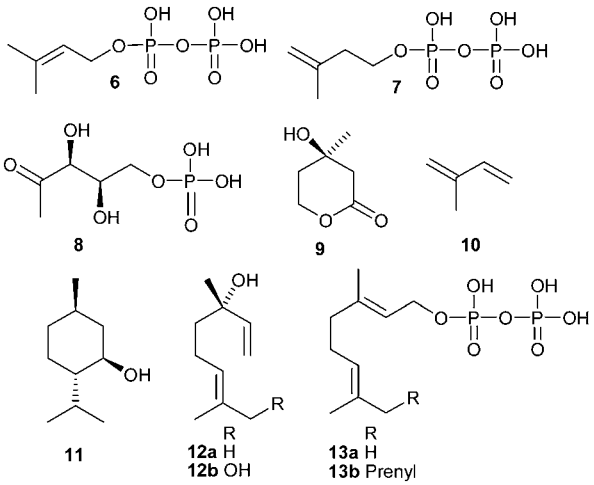
Fig. 1 Plant volatiles biosynthetic tree. VOCs are produced by different biochemical pathways. The methyl-erythritol phosphate (MEP) pathway gives rise to the formation of monoterpenes and diterpenes. The latter are precursors of the homoterpene TMTT and of the caroteoid-derived β-ionone. Isoprene is generated from DMAPP. Sesquiterpenoids are generated by FPP, deriving from the cytosolic mevalonate (MVA) pathway. The homoterpene DMTT derives from the sesquiterpene nerolidol. Oxylipins are generated from fatty acids which are cleaved into GLVs and JA derivatives. The volatile indoles are generated from anthranilate. Aromatic VOCs, such as eugenol, derive from phenylpropanoids, whereas MeSA derives from salicylic acid (SA), which is generated from benzoic acid. Alternatively, MeSA can be formed by methylation of SA deriving by isochorismate. See text for abbreviations. Adapted from 48.

on their chemistry (*i.e.* oxygenation, architecture of hydrocarbon backbone, saturation of carbon double bonds, *etc.*).

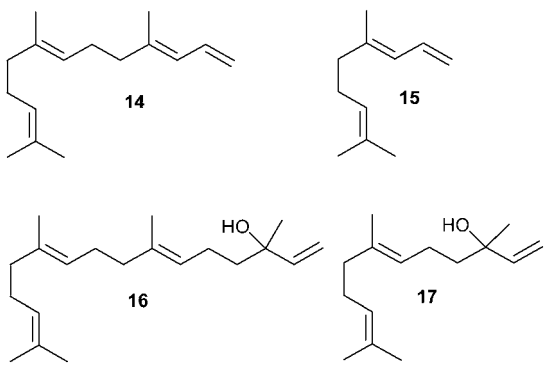
All isoprenoids are produced from the precursors dimethylallyl diphosphate (DMAPP) and its isomer isopentenyl diphosphate (IPP, **7**), which are synthesized by the deoxyxylulose-5-phosphate (DXP, **8**) (also knows as the methylerythritol phosphate, MEP) pathway in the chloroplasts and by the mevalonate (MVA, **9**) pathway in the cytoplasm (see ref. 4 for a review of the evolutionary and functional history of the two pathways for IPP and DMAPP synthesis). Some exchange and/or cooperation exists between these two pathways, that probably operate under different physiological conditions within the cell, and depend on the cell and plastid developmental state.[49] The evidence of cross-talk between the two pathways implies that they are not completely independent and share some regulatory mechanism(s).[20] In some plants, sesquiterpenes have been found to derive from the MEP pathway because of inactive MVA pathways,[50] whereas some monoterpenes have been found to have an exclusive MVA origin.[51] Sesquiterpene precursors have been shown to be derived in part from both the MEP and MVA pathways, and this metabolic crosstalk between cytosolic and chloroplastic compartments might be mediated by exchange of precursors like IPP between plastids and cytoplasm.[52]

Isoprene (**10**) is the simplest terpenoid (hemiterpene) emitted by plants, and is synthesized by the action of isoprene synthase (IspS) on DMAPP produced by the MEP pathway.[53] Labeling experiments with 1-[$^2H_1$]deoxy-D-xylulose have been performed in various higher plants and algae, demonstrating a more efficient incorporation into emitted isoprene than mevalonate.[54] Plants that emit isoprene are better able to tolerate sunlight-induced rapid leaf heating (heat flecks). They also tolerate ozone and other reactive oxygen species better than non-emitting plants.[53] Up to now, five IspS genes from different poplar (*Populus*) species or poplar hybrids, and one from *Pueraria montana* (kudzu) have been cloned.[55,56] It has been suggested that isoprene emission occurs only when a plant's needs for 'essential', higher terpenes (hormones, *e.g.* ABA and gibberellins; tocopherol; phytosterols; and photosynthetic pigments) are satisfied.[57]

In general, monoterpenes are typical leaf products whereas sesquiterpenes are typical flower fragrances, although the most common single compounds in floral scent are the monoterpenes limonene, (*E*)-β-ocimene, myrcene, linalool, α- and β-pinene.[58] Considerable amounts of monoterpenes and sesquiterpenes are also produced in leaf glandular trichomes and are emitted from herbivore-damaged foliage and roots. Monoterpenes are the smallest members of the very large class of terpenoid natural products, now encompassing over 40 000 defined structures.[59] This structural diversity is amazing, since the number of monoterpenes scaffolds is limited. In peppermint (*Mentha piperita*), the eight-step pathway to (−)-menthol (**11**) from primary metabolism has been defined by the combination of feeding studies and cell-free enzymology,[60] but the enzymology of monoterpene synthesis is still largely in its infancy, and, in general, difficult to study because of the existence of a complex network of metabolic pathways. Thus, the formation of (*S*)-linalool (**12a**) from the monoterpene precursor geranyl diphosphate (GPP, **13a**) has been studied in flowers of *Clarkia breweri*, where the floral gene linalool synthase (*LIS*) encodes an enzyme that catalyzes the reaction. Over expression of *Clarkia*'s LIS in tomato fruit caused the accumulation of (*S*)-linalool as expected, but also the unexpected formation of 8-hydroxylinalool (**12b**), a compound absent in control fruits. When the *Clarkia* LIS was over-expressed in petunia flowers, (*S*)-linalool was formed but was rapidly transformed into linalyl glycoside. In turn, when the *Clarkia* LIS was over-expressed in carnation flowers, 8-hydroxylinalool was also detected in the transgenic flowers, but it was apparently further metabolized to linalool oxides.[61]



The most typical compounds related to biotic stress are the homoterpenes 4,8,12-trimethyltrideca-1,3,7,11-tetraene (TMTT, **14**) and 4,8-dimethylnona-1,3,7-triene (DMNT, **15**).[62] TMTT might function as a signal as well as a phytoalexin that directly contributes to restricting bacterial growth in inoculated leaf tissue.[63] The biosynthesis of TMTT and DMNT has been suggested to involve the P450 enzyme-mediated oxidative degradation of the diterpene (*E*,*E*)-geranyl linalool (**16**) and the sesquiterpene (*E*)-nerolidol (**17**) as precursors, respectively.[22] In particular, DMNT could derive from the conversion of farnesyl diphosphate to the monoterpene alcohol (*E*)-nerolidol; and TMTT from the related conversion of geranylgeranyl diphosphate to the diterpene alcohol (*E*,*E*)-geranyl linalool. Feeding of deuterium-labeled (*E*,*E*)-geranyl linalool to Lima bean leaves resulted in the conversion of this precursor to TMTT, suggesting a sequential enzymatic oxidative degradation.[62]



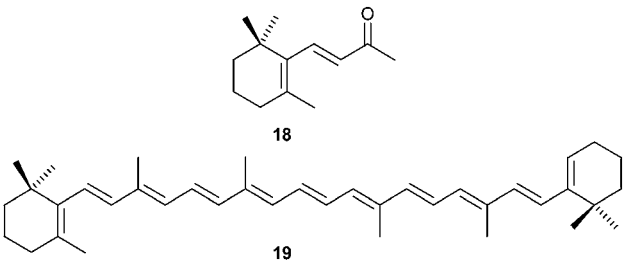
A large, structurally diverse number of terpenoids are formed by a large family of terpene synthases (TPS) using GPP (**13a**) and farnesyl diphosphate (FDP, **13b**) as substrates. Many distinct TPSs that synthesize monoterpenes and sesquiterpenes have been

characterized from various plants.[10,11,64–68] Metabolic engineering of VOCs can be achieved through the modification of existing pathways, for instance by up- or down-regulation of one or more biosynthetically steps, or by the re-direction of metabolite fluxes to a desired compound by the block of competing pathways. Alternatively, the introduction of new genes that are normally not present in the host plant can also be performed. Several examples of successful applications of these methods have been published. By over expressing a dual linalool/nerolidol synthase (FaNES1) from strawberry in chloroplasts of the model plant *Arabidopsis thaliana*, it was demonstrated that (*S*)-linalool (**12a**) significantly repels aphids.[69] Direction of FaNES1 to another compartment, the mitochondria, which contains the sesquiterpene precursor FDP, led to the formation of (*E*)-nerolidol (**17**) and its derivative, the C_{11} homoterpene DMNT (**15**). Both volatiles attracted carnivorous predatory mites, indirectly improving plant defense.[70]

While monoterpene synthases catalyze the biosynthesis of linear and cyclic compounds from the 10-carbon substrate GPP (**13a**), sesquiterpene synthases prefer the 15-carbon substrate FDP (**13b**).[68] Sesquiterpene synthases are more closely related to one another than to monoterpene synthases, and angiosperm synthases tend to phylogenetically cluster, independently from the gymnosperm synthases.[71] Many of the sesquiterpenes found in *Artemisia annua*, *Pogostemon cablin* and *Vetiveria zizanioides* are of immense perfumery and medicinal value.[72] The *Vetiveria* volatilome is characterized by the presence of monocyclic, bicyclic, tricyclic and tetracyclic sesquiterpenes that characterize the scent of the roots of this plant.[73]

Recently, by using culture-based and culture-independent approaches to analyze the microbial community of the vetiver root, Del Giudice and co-workers[74] demonstrated the presence of a broad phylogenetic spectrum of bacteria, including α–, β–, and γ–proteobacteria, high-G+C-content gram-positive bacteria, and microbes belonging to the Fibrobacteres/Acidobacteria group. The same group isolated root-associated bacteria and showed that most of them were able to grow by using vetiver sesquiterpenes as a carbon source, metabolizing and eventually releasing into the medium a large number of sesquiterpenes typically found in commercial vetiver oils. Several of these bacteria were also able to induce gene expression of a vetiver sesquiterpene synthase. These results support the intriguing hypothesis that bacteria may have a role in essential oil biosynthesis, paving the way to modify the vetiver oil by classic fermentation methods.[74] However, bacteria can also produce sesquiterpnes of wide strucutral diversity.[75] Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants have been recently reviewed.[68]

Certain VOCs, like β-ionone (**18**), are not derived directly from isoprenoid pyrophosphates, but from the degradation of tetraterpenes (carotenoids) through the action of carotenoid cleaving dioxygenases (CCDs).[76] Different isoforms of CCD probably exhibit different biochemical functions in plants, as they are differentially expressed. Although CCD4 genes have been isolated from several plants, only CsCCD4 isolated from *Crocus sativus* showed β-carotene (**19**) cleavage focused on the 9,10 (9′,10′) double bonds, yielding β-ionone.[77] The biosynthesis and function of carotenoids and their cleavage products has been recently published.[78]



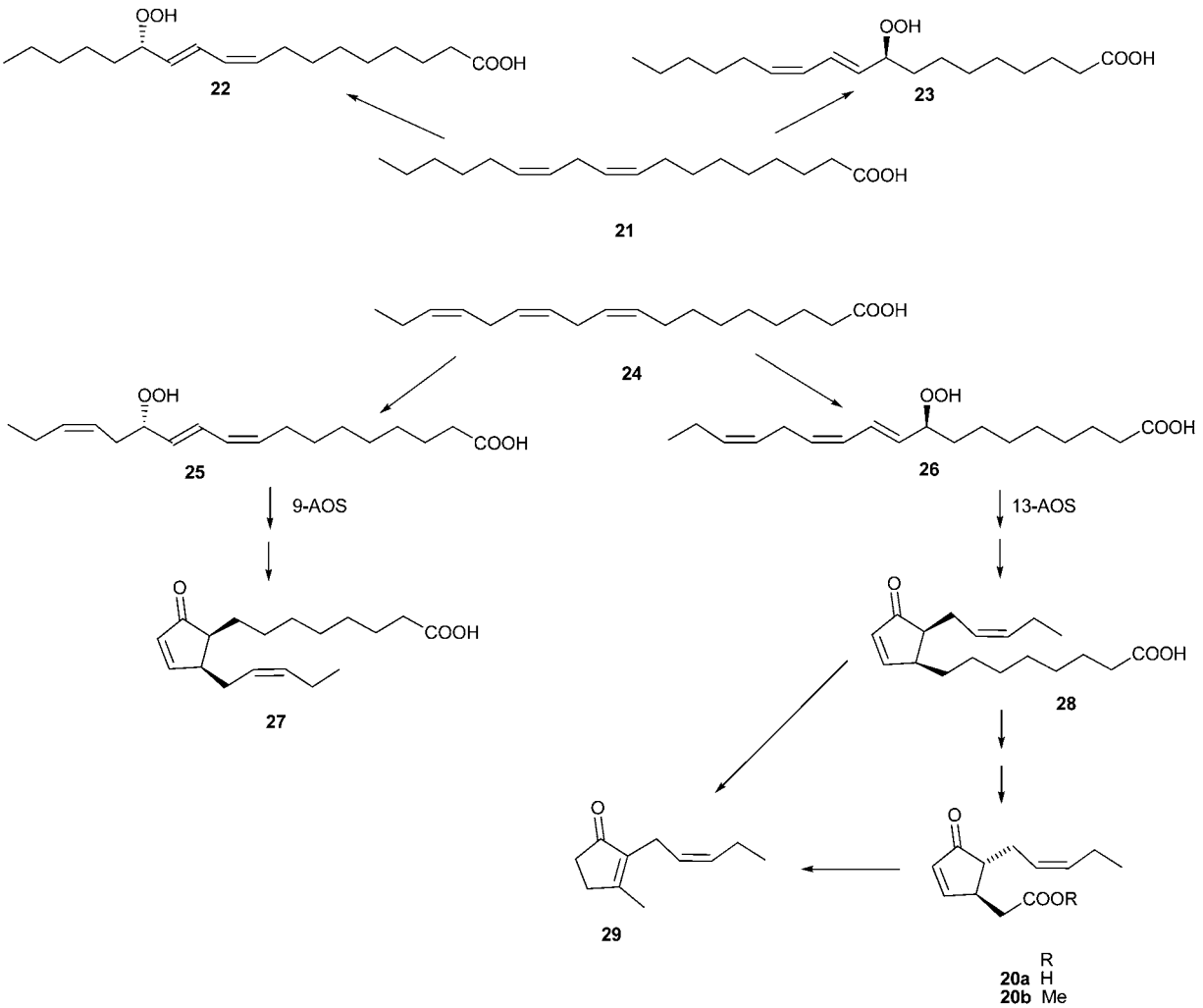
18

19

Oxylipins are another important class of VOCs. Oxylipins originate from polyunsaturated fatty acids released from chloroplast membranes by lipase activity, and represent the precursors of many oxygenated compounds, including jasmonates [jasmonic acid (JA, **20a**), jasmonic acid methyl ester (JAMe, **20b**), amino acid conjugates and further metabolites of JA, Scheme 1] and GLVs.[79,80]

Lipoxygenases (LOX) form hydroperoxides from linoleic acid (18 : 2, **21**) or α-linolenic acid (18 : 3, **24**) (Scheme 1). With linoleic acid as the substrate, (13*S*)-hydroperoxyoctadecadienoic acid (13-HPOD, **22**) and (9*S*)-hydroperoxyoctadecadienoic acid (9-HPOD, **23**) are formed, whereas with linolenic acid as the substrate, (13*S*)-hydroperoxyoctadecatrienoic acid (13-HPOT, **25**) or (9*S*)-hydroperoxyoctadecatrienoic acid (9-HPOT, **26**) are formed. Discrete 9-LOX and 13-LOX pathways have been proposed to explain the occurrence of numerous oxylipins. The activity of 9-allene oxide synthase (9-AOS) leads to the synthesis of an epoxy intermediate that yields 10-oxophytodienoic acid, whereas 13-allene oxide synthase (13-AOSs) activity forms precursors for the synthesis of *cis*-(+)-12-oxo-phytodienoic acid (**28**), eventually converted by several β-oxidations to JA (**20a**). Methylation of JA by a specific methyl transferase produces JAMe (**20b**). Constitutive over-expression of the JA-specific methyl transferase leads to a higher amount of JAMe, an unchanged JA level, and increased pathogen resistance, indicating that JAMe can be an active form of JA under specific conditions.[81] Furthermore, plant amino acid conjugates of JA have been recently demonstrated to be specifically induced by herbivores.[82] The essential oil of jasmine comprises more than a hundred components, but the most important contributions come from *cis*-jasmone (**29**). This compound has recently gained additional attention due to its production from herbivore-damaged leaves.[46] In addition to the conversion of JA into *cis*-jasmone, a novel pathway might exist, converting *cis*-(+)-12-oxo-phytodienoic acid (**28**), an early precursor of JA, into *cis*-jasmone (**29**) *via* intermediates having a *trans*-relationship between the cyclopentanone substituents, as demonstrated for many plant species and the yeast *Saccharomyces cerevisiae*.[83]

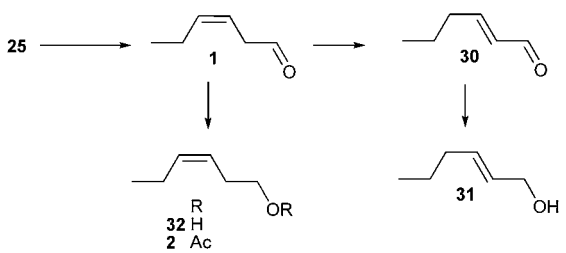
Upon mechanic and herbivore damage, GLVs are almost immediately released. GLVs are synthesized *via* the LOX pathway from C_{18} polyunsaturated fatty acids including linoleic and α-linolenic acids.[84] The C_{18} acids are cleaved to C_{12} and C_6 compounds by hydroperoxide lyases.[85] With 13-HPOT (**25**) as the precursor, the first C_6 GLV compound synthesized by the LOX/lyase pathway is (*Z*)-hex-3-enal (**1**), which is then converted to other GLVs, such as (*E*)-2-hexenal (leaf aldehyde, **30**) that leads to 2-hexenol (**31**), or to (*Z*)-3-hexenol (leaf alcohol, **32**) and (*Z*)-hex-3-enyl acetate (leaf ester, **2**).[86] The latter is formed from a reaction between (*Z*)-3-hexenol (**32**) and acetyl-CoA that is catalyzed by an acyltransferase[87] (Scheme 2).



Scheme 1 Biosynthesis of oxylipins from unsaturated fatty acids from the ω-3 and ω-6 series.

Analysis of GLVs is complicated by their chemical instability; but the use of on-line techniques, like proton transfer reaction mass spectrometry (PTR-MS), allows their easy monitoring. The biosynthesis of oxylipins has been recently reviewed.[88]

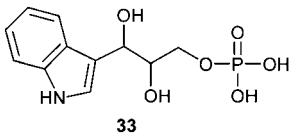
Another large class of VOCs consists of compounds containing an aromatic ring. VOCs containing nitrogen or sulfur are synthesized by cleavage reactions of modified amino acids or their precursors. For example, in corn, indole is made by the cleavage of indole-3-glycerol phosphate (IGP, **33**), an intermediate in tryptophan biosynthesis. Indole has been identified as one of the blend of VOCs emitted from corn in response to herbivore damage, and the production and release of this compound in plants has been shown to be an active process in which *de novo* synthesis is triggered in response to insect feeding.[89] Maize seedlings contain indole as an intermediate in at least two biosynthetic pathways. The BX1 enzyme catalyzes the conversion of IGP to indole, which is further transformed into the defense-related secondary metabolite DIMBOA [2,4-dihydroxy-7-methoxy-2*H*-1,4-benzoxazin-3(4*H*)-one, **34**]. Indole also serves as the penultimate intermediate for the formation of tryptophan by tryptophan synthase.[90] A gene has been characterized in corn coding for a protein having IGP lyase activity, overall catalyzing the formation of free indole from IGP, with the induction pattern of *Igl* paralleling the emission of free indole from the whole plant.[90]



Scheme 2 Biosynthesis of leaf alcohol (**32**) and related compounds.

Another important class of aromatic VOCs includes compounds derived from phenylalanine. Eugenol (**35**) is a reduced version of coniferyl alcohol (**36**), a lignin precursor.[10] Propenyl- and allyl-phenols, such as *p*-anol (**37**), methyl chavicol (estragole, **38a**) and eugenol (**35**) are important flavoring agents, and also serve as putative precursors for the biosynthesis of 9,9′-deoxygenated lignans, many of which have potential medicinal applications.[91] The biosynthesis of chavicol (**38b**) was shown to occur *via* the phenylpropanoid pathway to *p*-coumaryl alcohol

(**39a**), which can be reduced to form *p*-dihydrocoumaryl alcohol (**39b**), followed by dehydration to afford chavicol (**38b**), as well as formation of *p*-methoxycinnamyl alcohol (**39c**), whose further side-chain modification affords methyl chavicol (**38a**).[92]



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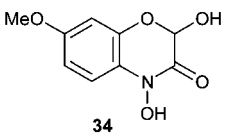

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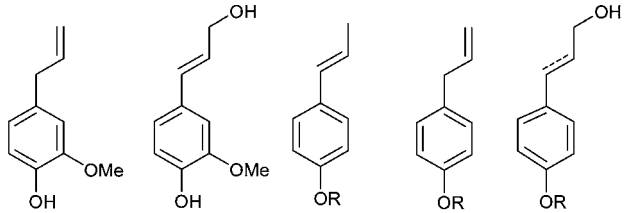
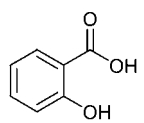


35 **36** **37**

	R			R
38a	Me	**39a**	$\Delta^{1',2'}$	H
38b	H	**39b**	-	H
		39c	-	Me

Salicylic acid (SA, **40**) is synthesized in plants by two pathways: one derived from benzoate (**41**) *via* cinnamate (**42**), and the other *via* isochorismate (**43**). Methyl salicylate (**3**) is synthesized in a reaction catalyzed by a methyltransferase whereby a methyl group is transferred from the donor molecule *S*-adenosine-methionine (SAM) to the carboxyl group of SA. Salicylic acid methyltransferase (SAMT) has been characterized in several plant species including the model plant *Arabidopsis*.[93]



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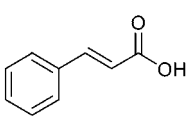

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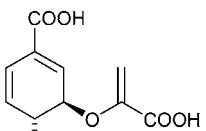


42

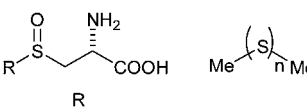
VOCs derived by oxidative cleavage and decarboxylation of various fatty acids result in the production of shorter-chain volatiles with aldehyde and ketone moieties that often serve as precursors for the biosynthesis of other VOCs.[10]

Besides detection, isolation and characterization of enzymes and genes involved in the formation of many VOCs, the structures of enzymes after crystallization are now being investigated and this information gives us hints on the catalytic mechanisms as well as probable evolutionary origins of these enzymes.[94]

Upon tissue damage, the flavors and odors of vegetables, such as *Allium cepa* L. (onion), *Allium sativum* L. (garlic), *Allium porrum* L. (leek), *Allium schoenoprasum* L. (chives), and *Allium fistulosum* L. (bunching or Welsh onion), are easily recognizable. The volatile and reactive sulphur-containing chemicals of these plants cause their best-known characteristic. The biosynthesis of the flavor precursors (+)-*S*-alk(en)yl cysteine sulphoxides (CSOs) and their γ-glutamyl peptide (γGPs) relatives has been reviewed.[95] The biosynthetic pathways proposed for the *Allium* flavor precursors are based primarily on chemical analysis and radiotracer studies, while most of the related enzyme activities have still to be established. Four non-volatile, odorless CSOs [*S*-methyl cysteine sulphoxide (**43a**), *S*-allyl cysteine sulphoxide (**43b**), *S*-*trans*-prop-1-enyl cysteine sulphoxide (**43c**) and *S*-propyl cysteine sulphoxide (**43d**)] are the precursors of the flavor and odors of the *Allium*. The enzyme alliinase cleaves these precursors to give pyruvate, ammonia, and a thiosulphinate.[95] Degradation of *S*-methyl cysteine sulphoxide produces odors described as 'fresh onions', whereas *S*-allyl cysteine sulphoxide degradation generates the typical 'garlic' smell. As well as CSOs, several γ-glutamyl peptide (γGP) derivatives of these flavor compounds have been detected within the *Alliums*.[96] γ-Glutamyl-*S*-alk(en)yl glutathiones, γ-glutamyl-*S*-alk(en)yl cysteines and γ-glutamyl-*S*-alk(en)yl cysteine sulphoxides, have been all proposed to derive from glutathione (γ-glutamyl cysteinyl glycine). Based on radiolabelling studies and analysis of *Allium* tissues, the biosynthesis of the flavor proceeds *via* *S*-alk(en)ylation of the cysteine in glutathione, followed by transpeptidation to remove the glycyl group, oxidation to the cysteine sulphoxide, and, finally, removal of the glutamyl group to yield CSOs. Alternatively, another biosynthetic route omits glutathione in favor of direct alk(en)ylation of cysteine or thioalk(en)ylation of *O*-acetyl serine followed by oxidation to a sulphoxide. The biosynthesis in different tissues and developmental states, the origin of the alk(en)yl groups, and also the relationship between CSOs and γGPs warrant further investigation.[95,97,98]

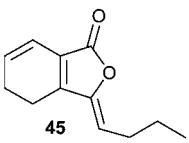
Of special relevance is the production of oligosulfides by plants pollinated by necrophoric insects. This remarkable mechanism of reproduction was investigated in the dead-horse arum (*Helicodiceros muscivorus*), a plant native to Sardinia, Corsica and the Balearics. The plant emits an odor strongly reminiscent of that of a dead animal, and it was shown that the composition of the volatiles from a rotting carcass and that from the flowers of this plant is indeed very similar, and dominated by the foul note of dimethyl mono- di-, and trisulfide (**44a-c**).[99] Oligosulfides, including compounds having a very unusual *t*-butyl group, have been reported from the roots and sap of asafoedita, a gum resin obtained from various foul-smelling ferulas from Iran and Afganistan.[100]

To date, over seventy phthalides, including dihydro-, tetrahydro-, and hexahydro- derivatives, as well as associated dimers, have been isolated from and/or implicated as being in 40 species of plants within the family Apiaceae and four plants from other families.[101,102] The best known members of this class are (*Z*)-ligustilide (**45**) from the Chinese medicinal plants *Angelica sinensis* and *Ligusticum chuanxiong*, and sedanolide (**46**) from celery.[103] Remarkably, little is known about the biosynthesis of these compounds, despite their very interesting biological profile, that includes mosquitocidal, nematocidal and antifungal properties.[104]



	R
43a	Me
43b	
43c	
43d	

	n
44a	1
44b	2
44c	3



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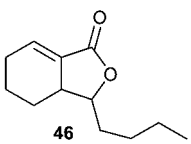

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4 Biotechnology and molecular pharming of VOCs

Plant biotechnology has evolved at a pace exceeding that of animal biotechnology, with the implementation of transgenic plants with increased resistance to disease, as well as improved functional traits. The development of transgenic plants with increased disease resistance, such as BT corn, has overshadowed other developments in plant biotechnology,[105] but rapid progress in the engineering of VOC production in plants is expected in the near future. In addition to commercial applications, these transgenic plants should increase our understanding of the biological relevance of volatile secondary metabolites.[69]

Biochemical, technical, and economic concerns with current production systems have generated enormous interest in developing new plant chemotypes as alternative production systems. However, various challenges must be met before plant systems can fully emerge as suitable, viable alternatives to current animal-based systems for the large-scale production of agrochemicals and other products.[106]

Production and genetic manipulation of natural products *via* plant cell suspension culture is renewable, environmentally friendly, and, from a processing standpoint, amenable to strict control, a considerable advantage regarding Food & Drug Administration (FDA) manufacturing standards.[107,108] Although considerable improvement have been made in terms of yields of secondary metabolite production, also for compounds that accumulate only at low levels, relatively little progress has been made in understanding and controlling the instability of some secondary metabolite production patterns.[108] However, not all compounds are ready to be engineered. The initial attempts to increase terpenoid production in transgenic plants have shown that the metabolic engineering of sesquiterpenes is a more challenging task and is not as straightforward as the generation of monoterpenes, which are formed exclusively or at least predominantly *via* the MEP pathway in the plastids.[11] Surely, genetic engineering can ameliorate some drawbacks of classical plant breeding and enhance the aroma of fruits. One advantage of this approach is that it is less complex since it introduces a single trait at a time. Another advantage is that genetic engineering allows the introduction of genes whose coding information may not be present in the cultivar of origin.[11] Metabolic engineering driven biosynthesis efforts are well suited for at least three essential natural product molecules, polyketides, flavonoids, and terpenoids, which include several well characterized and approved pharmaceutical molecules.[109]

In vitro cultures offer several possibilities to investigate secondary metabolite production. Head-space VOCs of *in vitro* cultures have been performed on several plants.[110] VOC production is tightly connected to micropropagation techniques, as well as a responses to different biotic and abiotic stresses.[111] Headspace analyses of different *in vitro* myrtle clones showed a specific and distinctive terpene emission profiles, whereas pretreatment of parsley cell cultures with methyl jasmonate (**20b**) potentiated elicitor-induced accumulation of active oxygen species and elicitation of phenylpropanoid defense responses in these cells.[112]

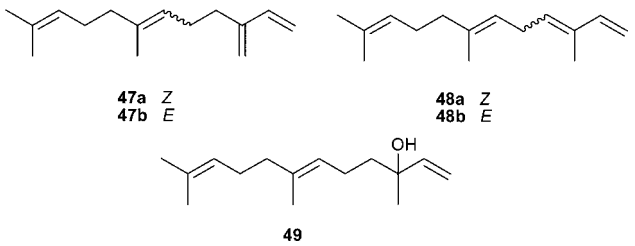
The production of substances of industrial interest from genetically modified (GM) plants is referred to as 'molecular pharming'.[113] As molecular pharming has come of age, there have been technological developments on many levels, including transformation methods, control of gene expression, protein targeting and accumulation, the use of different crops as production platforms and modifications to alter the structural and functional properties of the product.[114,115] The regulation of pharmaceutical crops is still a developing field, with the majority of experiences coming from North America. In the USA, most field trials for genetically modified organisms (GMOs) rely on risk mitigation in the form of strict confinement and regular inspections to limit any environmental exposure. These measures have become stricter for pharmaceutical crops during the past few years.[115] Both the US FDA and the European Agency for the Evaluation of Medicinal Products (EMEA) published draft documents addressing quality aspects in the processing of medicinal products made from GM Plants. However, higher compliance costs have to be anticipated for approval under respective GMO legislation and medicinal product legislation. An incomplete, or even absent, regulatory framework for GMOs producing VOCs might also increase financial risks for investors, that is the main reason why these kinds of GMOs are still in the earlier stages of R&D.[113]

5 Raison d'être of volatile compounds

Flower scents are usually emitted in an ontogenetically programmed way, whereas fruit odors are used by animals to distinguish between ripe and unripe fruits.[116] The quantity and quality of VOCs that are released from vegetative plant parts and roots can change dramatically when plants are stressed.[117] Generally speaking, inducible defenses consist of three steps: surveillance, signal transduction, and the production of defensive chemicals.[118] In the first step, the plant surveillance system detects parasite attacks by specific recognition of signals. The detected signals are then transduced through a network of signal transduction pathways, which eventually lead to the production of defense chemicals.[48,119–121] In all cases, induction of plant VOC can be triggered by both biotic and abiotic stress.[27,122]

In induced processes, rather than in the case of constitutive defenses, the recognition of the attacking insect and the subsequent signaling of the alarm are prerequisites for a fast and efficient defense. Many forms of induced defense are not restricted to local responses at the wounding site, but can be detected systemically throughout the plant. Thus, induced defenses also involve the production and accumulation of various VOCs that influence insect attraction/deterrence and inhibit insect growth and development. There are two types of plant inducible defense types: direct defenses and indirect defenses. Direct defenses include any plant trait that by itself can affect the susceptibility of host plants to insect attacks,[123] whereas indirect defenses include plant traits that by themselves are unable to affect the susceptibility of host plants, but can, nevertheless, serve as attractants to natural enemies of the attacking insect.[118] Moreover, certain volatiles may act as airborne signals that boost direct and indirect defenses in remote parts of the same plants or neighboring plants.[124,125] However, it has to be noted that herbivore-induced emission of plant VOCs is not limited to higher plants. It has been shown the arsenic hyperaccumulating fern *Pteris vittata* responds to herbivore wounding by emitting the sesquiterpenes (*Z*)-β-farnesene (**47a**),

(*E*)-β-farnesene (**47b**), (2*Z*, 6*E*)-α-farnesene (**48a**), (2*E*, 6*E*)-α-farnesene (**48b**) and (*E*)-nerolidol (**48**).[126] Takabayashi and co-workers demonstrated that a mixture of (*E*)-β-farnesene (**47b**) and (*Z*)-hex-3-enyl acetate (**2**) and other terpenoids emitted by intact young peach shoot tips attracted oriental fruit moth (*Grapholita molesta*) males, indicating the potential functional role of these molecules.[127]



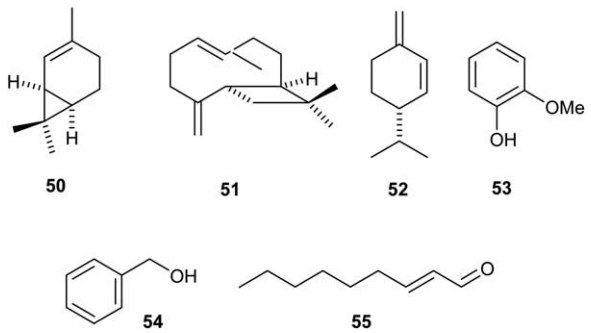
Total VOC emission from herbivore-damaged plants can be nearly 2.5-fold higher than emissions from intact plants, and this observation backs up the idea that local biotroph-induced VOCs might have substantial role in tropospheric processes.[20] The insect feeding-induced emission of volatiles has been demonstrated for several higher plant species, among others the model plant *Arabidopsis thaliana*,[70] maize (*Zea mays*),[128] Lima bean (*Phaseolus lunatus*),[66] *Nicotiana attenuata*,[129] *Medicago truncatula*[66] and spruce (*Pinus glabra*),[130] as well as for lower plants like ferns.[126] Herbivore-induced VOCs represent a phenotypically plastic response to herbivory, which result in changes in interactions between individuals in the insect–plant community.[131] Moreover, genetic variations within herbivore species affect VOCs production and there is a relationship between variations in the dispersing behavior of some insects (*e.g.* spider mite) and VOCs production.[132]

Also, insect egg deposition induces a plant volatile pattern that attracts egg parasitoids and induces the change of plant surface chemicals, thus arresting the egg parasitoids by contact cues in the vicinity of the eggs.[25] Indirect plant responses to insect egg deposition require modification of the biosynthetic activity of the terpenoid pathways especially, since changes of the quantity and/or quality of the plant's terpenoid volatiles have been detected for several plant species with eggs.[133,134]

Overall, the current picture demonstrates a high functional diversity in VOC-mediated communication within and among organisms, but it leaves us with the open question of how misunderstandings in all these communications are avoided.[124] Volatiles from primary host plants may also attract other insects, as is the case of male aphids.[135] Parasitoids also use herbivore-induced responses to assess habitat profitability and adapt patch residence time.[136] Furthermore, herbivore-induced plant volatiles emissions are inducible by other biotrophs, as well as abiotic agents.[20] Growth conditions (particularly day length) may affect the ratio of VOCs present in the emission blend, even though the response to herbivory and nutrient availability are similar.[137] Induced resistance is often associated with the ability for a faster and stronger activation of defense responses upon an attack by pathogens or insects. This physiological state is referred to as priming.[125]

The type of feeding damage clearly affects the VOCs produced, and part of the biochemical explanation is that leaf chewers in general induce only JA-signaling, while piercing-sucking herbivores and pathogens tend to induce SA-mediated resistance pathways as well.[138] Indications for a role of JA for pathogen defense in potato arose from reports that exogenous application of JA leads to local and systemic protection against subsequent pathogen attack.[139]

Plant pathogens have been demonstrated to induce the production of plant VOCs, which, because of their antimicrobial activities, probably inhibit the spread of the pathogen into plant tissues. In accordance with this view, tomato mutants deficient in the biosynthesis of the octadecanoid pathway are highly susceptible to small leaf-feeding mites and thrips, whereas JAMe treatment restores resistance.[140] Several tomato VOCs produced by leaves, such as 2-hexenal, *E*-2-nonenal, Δ-2-carene (**50**), (*E*)-β-caryophyllene (**51**), β-phellandrene (**52**), guaiacol (**53**), MeSA (**3**), benzyl alcohol (**54**), and eugenol (**35**), are effective in inhibiting the pathogen *Botrytis cinerea*. Among these constituents, 2-hexenal (**30**) and (*E*)-2-nonenal (**55**) showed the strongest inhibitory effect. Some VOCs, such as JAMe and MeSA, are plant-produced signals that are specifically activated in response to plant pathogens.[141]



In general, VOCs can be considered as infochemicals that mediate many interactions in a plant–insect community, both above and below ground.[142] Because volatile isoprenoids are reactive, and are likely to undergo rapid changes and transformations (physical, chemical and/or biological) in the soil system, a considerable proportion of rhizosphere sources of VOCs may not diffuse through soil to the atmosphere.[64] Feeding on roots can even induce changes in the volatile bouquet released from the aerial parts of a plant, although the ecological relevance of this observation remains elusive.[143] Also, the potential abundance and specific effects of VOCs in the rhizosphere environment are still substantially unknown. In maize roots (*E*)-β-caryophyllene (**51**) is necessary to attract entomopathogenic nematodes to roots damaged by the ferocious maize pest *Diabrotica virgifera virgifera*. Maize varieties that lack this signal have been shown to be far more vulnerable to maize pest.[144] In Vetiver roots, emission of a complex blend of sesquiterpene hydrocarbons and alcohols repels insects and protect the plant from microbial attacks.[73,74] Studying the effects of belowground herbivory on aboveground tritrophic signaling and *vice versa* emphasizes the important role of plants in bridging interactions between spatially distinct components of the ecosystem.[144,145]

Plant VOCs that have elicited antennal responses were also attractive to parasitoids in behavioral experiments. The summed neural activity of antennal olfactory receptors can be measured

using the gas chromatography–electroantennographic detection (GC–EAD) technique. Using plants upon which herbivores are feeding and investigating the VOCs released by GC-EAD, it is possible to identify a range of compounds that are electrophysiologically active and which may subsequently prove to be active in behavioral assays as repellents of insect pests.[120] Aerial interaction of the wild tobacco (*Nicotiana attenuata*) and sagebrush (*Artemisia tridentata tridentata*) is the best-documented example of between-plant signaling *via* above-ground VOCs in nature but, at the same time, highlights the difficulty of predicting how plant–plant signaling functions from first principles.[146] In the southwestern USA, *N. attenuata* occasionally grows in close proximity to sagebrush; and plants growing in close proximity to clipped sagebrush suffered significantly less herbivore damage than plants next to unclipped sagebrush or unexposed plants.[147]

The elevating atmospheric CO_2 concentration results in the warming of the lower atmosphere, which might lead to a higher emission of VOCs from plants, and other factors, such as temperature, light and herbivores, might conceal the effects of CO_2.[16,148] However, VOC emissions that are induced by leaf-chewing herbivores are not always influenced by elevated CO_2 concentration. Leaf photosynthetic properties may confer a valuable basis to model the seasonal variation of VOC emission capacity; especially in tropical regions where the environmental conditions vary less than in temperate regions.[149] Further consequences of reduced photosynthetic gas exchange and maintaining VOC emissions are a very high carbon loss, up to 50%, from VOC emissions related to net CO_2 uptake, and a strong increase in leaf internal isoprene concentrations.[150] It has been demonstrated that transgenic non-isoprene-emitting poplars show reduced rates of net assimilation and photosynthetic electron transport during heat stress, but not in the absence of stress. The decrease in the efficiency of VOCs has been inversely correlated with the increase in heat dissipation of absorbed light energy, measured as non-photochemical quenching (NPQ). Down-regulation of the emission of isoprene has been shown to affect thermotolerance of photosynthesis, thus inducing increased energy dissipation by NPQ pathways.[151] It has been hypothesized that VOCs like isoprene may stabilize thylakoid membranes and/or may exert antioxidant properties, increasing plant tolerance to environmental stresses. The involvement of isoprene in non-enzymatic plant defense strategy has also been suggested.[152] Isoprene appears to act on photosynthetic membranes to protect against thermal damage.[53]

Although the phytotoxic impact of ozone on plants has been well documented, the effect of O_3 on plant VOC emissions has received little attention. Chronic exposure to moderately increased concentrations of ozone on insect-induced terpene emissions indicated only very small changes in emissions, but showed induction of some terpenes, particularly the homoterpene DMNT (**15**), in response to insect feeding.[153] O_3 can affect phytophagous insect performance and behavior due to changes in the plant physiology and chemistry and the destruction of olfactory cues, disrupting insect chemical communication.[154] Laboratory studies have shown that exposing Lima bean to ozone increases the emission of TMTT (**14**) and DMNT (**15**), emissions of which are also induced by spider mite (*Tetranychus urticae*) feeding.[155] By using a free-air ozone concentration enrichment (FACE) it was found that enhanced O_3 levels activate the chemical defenses of some plants, resulting in altered VOC emission profiles, and that a combination of abiotic and biotic stress may substantially increase VOC emission.[153]

The role of VOCs produced by flowers as chemical attractants used to draw in their often highly-specific pollinators has recently been documented, by examining how these compounds are produced in flowers, how they are detected by potential pollinators, and how biotechnology can be used to alter their activity.[156–159] Since floral VOCs are part of pollination syndromes, they represent a very crucial factor to ensure sexual reproduction.[9] Moreover, the ability of flowers to attract pollinators from a distance is the reason why VOCs have been retained through natural selection and are found in floral scents.[48] Supplementary Table S1† reports additional information on the functional role of plant volatiles.

6 Plant VOCs as lead compounds for drug discovery

From a total of approximately 240 000 angiosperm plant species, only a few thousand produce sufficient amounts of VOCs (including the less volatile terpenes) to give essential oils in a reasonable yield. Despite their outstanding historical record as medicinal agents, only a small number of essential plants and their preparations are listed as medicinal by European and American health authorities (Table 1). The European pharmacopoeia (Phar. Eur. supplement 6.5) lists less than forty plant essential oils with pharmaceutical applications and the United States Pharmacopeia National Formulary (USP-NF) only lists ten. However, there are several food plants (spices in particular) with a significant VOC content (*e.g. Levisticum officinale*, *Origanum vulgare*, *O. majorana*, *Cinnamomum* spp., *Zingiber* spp., *Citrus* spp., *Elettaria cardamomum*, *Foeniculum vulgare*, *Salvia officinalis*, *Syzygium aromaticum*, *Pimpinella anisum*, and *Ocimum* spp., to name a few examples). Numerous essential oils or their constituents are used in cosmetics and in the perfume industry.[160,161,162] From an historical perspective, fragrant plants and aromatic plant oils have attracted the interest of man and since ancient times have been used as perfumes and cosmetics in virtually all civilizations.[163] Dioscorides, the father of phytotherapy, dedicated his first book of *De Materia Medica* to aromatic medicinal plants. Moreover, there is a correlation between the amount of VOCs present (*i.e.* the odorous nature of a plant) and its selection as medicinal agent.[164] The use of essential oils has a millennial history. Myrrh (resins of *Commiphora* spp. and *Balsamodendron* spp.), lotus (*Nelumbo nucifera* Gaertn.), and sandalwood (*Santalum* spp.) oils were used in ancient Egypt for purification and embalming rituals and it has been reported that coniferous resins were also used as preservatives for the embalming process.[165] During the archeological investigations of the tomb of Tutankhamon (1341–1323 BC) in 1922, numerous jars filled with solidified essential oils were discovered.[166] Another historical example of the use of VOCs in rituals and medicine is the use of agarwood (aloeswood) (*Aquilaria* spp.) which has a millennial history in China, Japan and the Middle East. Several *Aguilaria* plant species native to Indomalayan tropical forests produce an odor upon infection by the fungus *Philalophora parasitica*. This odor is composed of a highly complex blend of VOCs.[160] Its medicinal use is recorded in texts

as ancient as the Sahih Muslim, which dates to the 8th century, and the Ayurvedic medicinal texts. Although sedative effects of some of its constituents have been reported,[167] the molecular mechanism of action remains unknown. Today, agarwood-based essential oils belong to the most precious products in the scent industry.

Due to the volatile nature of fragrant VOCs, which normally occur as mixtures of different lipophilic terpenoids, their exploitation and use have always been at the interface of cosmetics and medicine. This is nicely illustrated by the history of tiger balm. During British colonial times, the trade of aromatic oils and resins which were obtained from the Dipterocarpaceae (*e.g. Shorea* spp., *Dipterocarpacea* spp.), the predominant family of Bornean rain forest canopy trees, and the collection of camphor (*e.g.* from *Cinnamomum camphora*) and benzoin resin (from *Styrax* spp.) was flourishing. Camphor (**56**), which is a prototype VOC, is produced by several trees, including *Dryobalanops aromatica* and *Ocotea usambarensis*. For industrial purposes it can be produced synthetically from turpentine oil. The use of such fragrant products led to the development of tiger balm in the 19th century, a formulated mixture of different aromatic resins that is used to topically treat headache and tension, but also for upper respiratory tract infections. Generally, rubefacients containing camphor and (−)-menthol (**11**) lead to a cooling sensation and mild irritation of skin and thus increase blood flow, but may also induce changes in pain perception (*vide infra*). Cinnamaldehyde, a VOC used in many rubefacients, evokes spontaneous pain and induces heat and mechanical hyperalgesia, cold hypoalgesia, as well as neurogenic axon reflex erythema.[168] It is known that different transient receptor potential (TRP) channels, which are molecular thermosensors that detect cold, warm and hot temperatures may be involved in the action of tiger balm constituents[169] (Fig. 2). The capsaicin receptor TRPV1 seems a good candidate to explain the skin irritancy of several essential oils, being expressed in keratinocytes from human epidermis and hair follicles. Thus, when 31 essential oils were investigated for the activation of hTRPV1 transfected in HEK293 cells, 4 of them gave positive results. In three cases, activation could be traced to a specific constituent (citronellol for rose oil and geraniol for palmarosa and thyme oils), while the nature of the potent TRPV1 agonist from tolu balsam could not be ascertained, since its major constituents (benzyl cinnamate and benzylbenzoate) were inactive.[170] These channels are not only activated by certain natural products, like capsaicin, menthol, and camphor, but also by various inflammatory signaling pathways.[171] Camphor (**56**) is noxious at high concentrations and activates various TRPs in a rather non-selective way (Table 2), but is used for its scent both in medicine and in cuisine (*e.g.* in *Ocimum kilimandscharicum*, the African camphor basil), linking food flavoring and medicine. The nervous system senses peripheral damage through nociceptive neurons that transmit a pain signal, which can be influenced by TRP channels like TRPA1, an ion channel expressed in nociceptive neurons. TRPA1 is activated by a variety of noxious stimuli, including cold temperatures, pungent natural products and distinct VOCs (Table 2). It was suggested that several of the compounds known to activate TRPA1, such as cinnamaldehyde and mustard oil components, are also able to covalently bind certain cysteine residues in the ankyrin repeats of TRPA1.[172] More recent evidence suggests that (−)-menthol and camphor, inhaled or rubbed as peripherally acting cough suppressants, are local analgesics, probably acting *via* temperature-sensitive TRPV3 channels (*vide infra*). Importantly, even though the traditional use of VOC-based medicines is well recorded, the pharmacological basis of how such medicines work at a molecular level remains to be fully elucidated. The action of VOCs on different ion channels is probably one of the most important lines of research in this field. It is important to stress that although many VOCs may have specific interactions with protein and lipid targets, they potentially also lead to nonspecific membrane effects at higher concentrations, due to their apolar and lipophilic chemical nature. Notwithstanding these limitations, there is growing evidence that TRPs, and especially those sensitive to temperature (TRPV1-V4, TRPM8 and TRPA1) are primary candidates for mediating the sensory properties of VOCs. Irritancy from certain essential oil constituents, a well-known problem for cosmetics, like linalool, is also probably mediated by TRPs. TRPs were first characterized in insects, where they are involved in vision, and it does not seem unreasonable to believe that critical plant–insect interactions involve these ion channels, although few data are available to date on this issue.

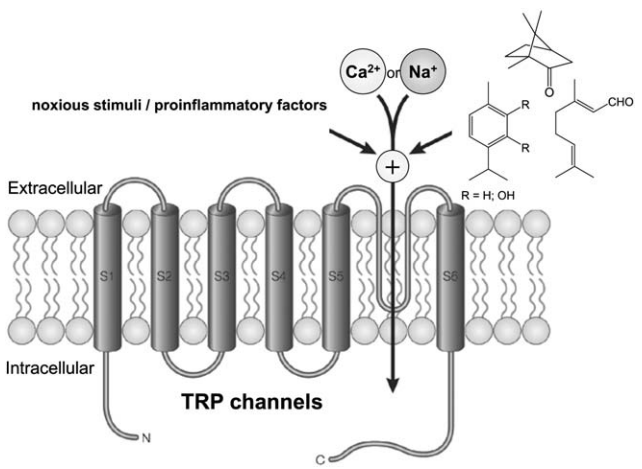


Fig. 2 Transient Receptor Potential (TRP) channels have been shown to be frequent targets of VOCs found in plant essential oils.

The medicinal use of essential oils includes topical application, inhalation, and oral ingestion. Some of the more popular uses are at the interface of molecular pharmacology and sensory-mediated psychoneuroendocrinology (*e.g.* aromatherapy). Oils containing VOCs were among the first topical and gastrointestinal antimicrobial agents used by mankind, and many VOCs have been shown to exert significant topical antimicrobial and antifungal effects.[173–175] Importantly, while the antimicrobial effect of VOCs may not be sufficiently potent (*i.e.* specific) to be useful for systemic treatment, their use as peripherally acting agents is, nonetheless, interesting. At high concentrations, most essential oils are antibacterial *in vitro*. In food, bacteriostatic and anti-*Salmonella* effects of spice essential oils have been reported.[176,177] While antibiotics have specific targets, VOCs may cause non-specific alterations in the membranes of microorganisms, leading to changes in their fatty acid composition[178] or perturbation of overall membrane integrity. Certain widespread VOCs, like (−)-menthol (**11**), thymol (**57**), and geraniol (**58**) perturb the

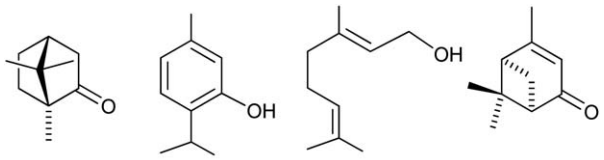
Table 2 Pharmacological targets of VOCs or essential oils

Protein Target	Compound/essential oil	Action	Reference
AChR (nicotinergic)	linalol	inhibition	254
AChR (muscarinic)	rotundifolone	activation	255
ACh esterase	α-pinene	inhibition	256
	(+)-3-carene	inhibition	256
	1,8-cineole	inhibition	257
	eugenol	inhibition	258
	limonene	inhibition	259
	Salvia spp.	inhibition	257
	tea tree oil	inhibition	260
Adenosine receptor A_1/A_2A	linalool	activation	261
ButCh esterase	*Salvia* spp.	inhibition	262
	β-pinene	inhibition	263
	(+)-3-carene	inhibition	263
CB_2 receptor	β-caryophyllene	activation	199
Dopamine receptor	*Citrus limon*	inhibition	242
Estrogen receptor	*Vitex rotundifolia*	activation	264
GABA (A)	menthol	activation	265
	methyleugenol	activation	248
	thujone	inhibition	244
	thymol	activation	246
	cis-jasmone	activation	266
	methyl jasmonate	activation	266
5-HT1A	*Citrus limon*	activation	242
5-HT3	terpinolene	inhibition	267
	α-phellandrene	inhibition	267
	β-pinene	inhibition	267
Histamine H1	*Bunium persicum*	inhibition	268
	Carum copticum	inhibition	269
	terpinen-4-ol	inhibition	270
NADPH oxidase	phytol	activation	192
NF-κB	zerumbone	inhibition	271
	Cleistocalyx operculatus	inhibition	272
NMDA receptor	α-/β-asarone	inhibition	273
	Citrus bergamia	inhibition	274
Opioid receptors	nepetalactone	activation	275
	linalool	activation	251
	1-nitro-2-phenylethane	activation	276
	Croton cajucara	activation	277
	Nepeta italica	activation	278
TRPV3	borneol	activation	279
	camphor	activation	279
	carvacrol	activation	279
	dihydrocarveol	activation	279
	menthol	activation	279
	incensole acetate	activation	280
	thymol	activation	279
	eugenol	activation	281
	citral	activation	282
TRPA1	camphor	activation	283
	cinnamaldehyde	activation	283
	menthol	activation	284
	menthol	inhibition	283
	carvacrol	activation	281
	citral	activation	282
TRPV1	camphor	activation	281
	citral	activation	282
	allicin	activation	285
TRPM7	carvacrol	inhibition	286
TRPM8	cinnamaldehyde	activation	168
	menthol	activation	168, 284
	citral	activation	282

dynamics of cellular membranes at different biophysical levels.[179] Membrane toxic effects by these lipids may also be the reason why a large number of essential oils are powerful topical and gastrointestinal antimicrobials and nearly all of them are weakly to moderately antiseptic. According to Martindale,[180] the oil of oregano is twenty-five times as effective in killing bacteria as phenol and the oil of cloves is about nine times as effective. In fact, lime and clove oils have been used in times prior to the introduction of antibiotics as local antiseptics.[173] However, very little is known about the molecular mechanisms of action

underlying bacteriostatic effects of VOCs. Overall, biological effects observed at μM concentrations may be related to the ability of VOCs to self-assemble and integrate into cellular membranes. Therefore, *in vitro* pharmacological investigations should to be interpreted with great caution. However, it is important to stress that several VOCs also exert potent pharmacological effects in the nM range, which are clearly related to specific interactions with proteins (*vide infra*). Thus, VOCs may exert biphasic or multilevel pharmacological effects, depending on the concentration at the site of action.

In traditional medicine and in various Pharmacopoeias, certain VOC containing plants or the essential oils thereof (*aetherolea*) are stated to excite peristalsis and are used for the expulsion of flatus in colic. Non-toxic essential oils that exert anti-inflammatory and antispasmodic effects, such as the ones obtained from *Foeniculum vulgare*, *O. basilicum*, or *Pimpinella anisum* are typically used as carminativa.[181] Other essential oils are said to enhance the effect of cathartic drugs. In the case of L-verbenone (**59**), a compound used as expectorant, the mechanism of action remains unknown, although due to structural similarity it may be similar to camphor, which interacts with TRP channels. It is probably due to the local irritant effect at higher doses that some VOCs are used therapeutically as expectorants and diaphoretics.[181] VOCs may act *via* the *nervus vagus*, like bitter agents, and thus cause an increase in hepatic bile and bilary excretion. However, despite traditional and even clinical evidence of the efficacy of such preparations, the exact pharmacological mechanisms of most preparations remain to be elucidated. In addition to the therapeutic applications described above, certain isolated VOCs like D-limonene (**11**)[182] and verbenone[183] have been successfully used as insect repellents. Since VOCs usually occur as complex mixtures in essential oil preparations they are likely to exert additive, synergistic or even antagonistic pharmacological effects within the same preparation. For these reasons, the molecular rationale for the use of essential oils in medicine remains still largely elusive. Nevertheless, the molecular pharmacology of VOCs has increased steadily, and important molecular mechanisms of have been elucidated, qualifying certain VOCs as potential lead structures for drug discovery. In general, because of their apolar nature, VOCs preferentially target membrane proteins and receptors sensitive to lipophilic, sometimes endogenous, compounds. The discussion of a selection of plant VOCs of current biomedical relevance as drug candidate and/or as drug lead will exemplify these concepts. In addition, Tables 2 and 3 summarize the information regarding specific targets of a series of VOCs of pharmaceutical and/or toxicological relevance.



56 **57** **58** **59**

We now describe some of the key volatiles showing promising pharmacological effects.

6.1 Zerumbone (60a)

Zerumbone, a humulane sesquiterpene accumulated in a Far-East spice (shampoo ginger, *Curcuma zerumbet*), has attracted considerable interest in the realm of cancer research because of its pleiotropic anti-cancer and chemopreventive activity, reminiscent of that of curcumin.[184,185] The remarkable biological profile of zerumbone also exemplifies the potential of VOCs from edible plants to serve as a lead structure for drug discovery. At preclinical level, zerumbone shows potent anti-inflammatory activity in animal models of both acute and chronic inflammatory offense, as well as chemopreventive activity against various carcinogenic agents. Over 20 possible molecular targets have been identified for zerumbone, with an activity profile that includes perturbation of the aberrant hedgehog (Hh)/GLI signaling pathway,[186] enhancement of tumor necrosis (TNF)-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis,[187] and interference with the NF-κB signaling pathway.[184] Zerumbone also inhibits bone resorption and osteoclast formation induced by human breast tumor cells and by multiple myeloma cells,[188] prevents human breast cancer-induced bone loss in animals, and decreases osteolysis in a dose-dependent manner in athymic nude mice bearing MDA-MB-231 breast cancer tumors.[187]

These data, while suggesting that zerumbone is worth clinical investigation for the prevention of colon cancer, osteoporosis and cancer-associated bone loss, also suggest an underlying molecular mechanism that goes beyond specific effectors, and presumably involves a critical transcriptional induction. Recent findings support this view. Thus, zerumbone has an unusual

Table 3 Toxic VOCs or essential oils

Compound/essential oil	Site of action/action	Toxicity	Reference
Coumarin	Liver	+	287
Safrole	Liver (carcinogenic)/CNS	+	288
α-thujone	CNS	++	289
Methyl chavicol (estragole)	Liver (carcinogenic)/CNS	+	290
β-asarone	Carcinogenic	+	291
Methyleugenol	Carcinogenic	+	292
Phytol	Hepatotoxic	++	196
Juniperus spp.	Carcinogenic/skin irritant	+	293, 294
Chenopodium ambrosiodes	Carcinogenic	+	295
Sassafras albidum	Liver (carcinogenic) CNS	+	295
Myristica fragrans	CNS	++	296

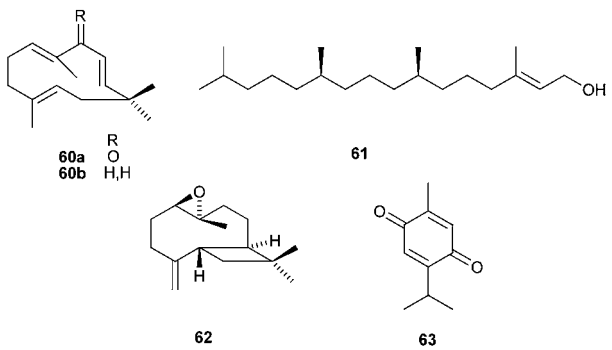
electronic structure. It is, in principle, a cross-conjugated dienone, but geometrical constraint prevents planarity of the dienone system. As a result, it behaves like a double-enone, forming bis-adducts with thiols, but, while addition to the trisubstituted enone double bond is irreversible, the one to the disubstituted double bond is not, and reversion to the corresponding enone structure is fast.[189] These observations are in line with the finding that zerumbone binds covalently certain proteins, like TRPA1, a critical player in pain and inflammation[189] and qualify this sesquiterpene as a transcriptional inductor of anti-inflammatory and ant-oxidant phase 2 enzymes *via* interaction with the reactive cysteine residues of the transcription factor Nrf2. This view is supported by the inactivity of analogues lacking the conjugated enone system (α-humulene, zerumbol) to induce glutathione *S*-transferase in rat epithelial cells.[190] Taken together, these observations suggest that zerumbone behaves like a biological analogue of the antioxidant inflammation modulator (AIM) bardoxolone methyl, a promising reversible thiol trap in advanced clinical development against pancreatic cancer and diabetes kidney disease.[191]

6.2 Phytol (61)

The diterpene alcohol phytol is a biosynthetic precursor of vitamins E and K1. It is relatively common in plants and generally occurs esterified with chlorophyll, where it confers lipid solubility. In some essential oils, this weak volatile is a major component. Phytol potently triggers oxidative burst in granulocytes. Phytol was investigated in arthritis-prone rats to evaluate its effects on inflammation.[192] In these rats, arthritis can be induced by injection of pristane, a volatile saturated terpenoid hydrocarbon biosynthetically derived from phytol. Phytol caused a strong oxidative burst in human granulocytes, but did not induce arthritis in rats; whereas pristane, which does cause arthritis, caused a lower oxidative burst in the granulocytes. Intriguingly, rats injected with phytol were protected from arthritis following a later injection of pristane. The beneficial effects of phytol were seen not only in rats bred with a form of Ncf1 that produces abnormally low amounts of ROS, but also in rats whose granulocytes produce normal oxidative bursts. The activity of phytol against arthritis was shown to involve T lymphocytes, as injection of phytol inhibited transfer of pristane-induced arthritis with these cells. In these animal experiments, phytol showed comparative effectiveness to standard therapies for arthritis, like tumor necrosis factor-α antibodies (anti-TNF-α) and methotrexate. IFN-γ appears to regulate the pathway associated with arthritis development, whereas IFN-β regulates the pathway associated with disease protection through phytol.[193] These data suggest a novel pathway of autoimmune inflammatory disease and, possibly, a novel therapeutic strategy with phytol or other oxidants. It was also shown that some dietary fats contain significant levels of phytol, which is oxidatively metabolized to phytanic acid after absorption. Phytanic acid binds the nuclear transcription factor peroxisome proliferator-activated receptor alpha (PPARα) and induces expression of genes encoding enzymes of fatty acid oxidation in peroxisomes and mitochondria.[194] Administration of dietary phytol (0.5% or 1%) to normal mice for twelve to eighteen days caused consistent PPARα-mediated responses.[195] Female mice fed 0.5% phytol and male and female mice fed 1% phytol exhibited midzonal hepatocellular necrosis, periportal hepatocellular fatty vacuolation, and corresponding increases in liver levels of the phytol metabolites phytanic acid and pristanic acid.[196] These results suggest that phytol may cause selective midzonal hepatocellular necrosis in mice, an uncommon pattern of hepatotoxic injury.

6.3 β-caryophyllene (51)

The endocannabinoid system (ECS) is an ancient lipid signaling network that in mammals modulates neuronal functions, pain perception, and inflammatory processes.[197] The cannabinoid type-2 (CB_2) receptor, which unlike the CB_1 receptor does not induce central side effects, has been shown to be a promising therapeutic target for different diseases.[198] The finding that β-caryophyllene, a ubiquitous lipophilic sesquiterpene found in many edible plants, selectively binds to the CB_2 receptor and acts as a full agonist provides an interesting novel molecular mechanism of action for a common VOC.[199] Oral administration of β-caryophyllene exerts potent anti-inflammatory effects in wild type mice but not in CB_2 receptor ($Cnr2^{-/-}$) knockout mice. Like other CB_2 ligands, β-caryophyllene also inhibits the pathways triggered by activation of the toll-like receptor complex CD14/TLR4/MD2, which typically lead to the expression of proinflammatory cytokines (IL-1β, IL-6; IL-8 and TNF-α) and promotes a TH_1 immune response. β-caryophyllene has been shown to be myorelaxant and antispasmodic in mice, analgesic[199] and further reduces colitis in experimental models.[200] Further studies will have to show whether β-caryophyllene may be therapeutically relevant to treat or prevent inflammatory diseases or diseases related to peripheral pain *via* the ECS. Remarkably, the related isomeric sesquiterpene α-humulene (**60b**) lacked cannabinoid activity, as did caryophyllene epoxide (**62**), the product of oxidative degradation of caryophyllene. These data provide support to the view that strict structure–activity relationships exists also for hydrocarbon ligands like β-caryophyllene.



60a R
60b O, H,H

61

62

63

6.4 Carvacrol (4)

Carvacrol binds to and activates a number of effects on ion channels, but was recently shown to also activate peroxisome proliferator-activated receptor gamma (PPAR-γ), causing inhibition (down-regulation) of COX-2 expression and anti-inflammatory activity.[201] This finding suggests that, apart from

TRPV channels, also PPAR-γ, a transcription factor sensitive to lipophilic ligands, should be investigated as a broadly tuned receptor for plant VOCs.

6.5 Thymoquinone (63)

This monoterpenoid quinone is a major constituent (up to over 50%) of the essential oil from black cumin (*Nigella sativa* L.), a Middle-East spice,[202] and is also present in marjoram, a culinary herb.[203] It has raised great interest as an anti-oxidant, neuroprotective, anti-inflammatory and anticancer agent. Thymoquinone is a pan-inhibitor of prostanoid synthesis, acting at both cyclooxygenase and lipoxygenase level,[204] but the molecular mechanism(s) involved in its anticancer activity remain obscure. Thymoquinone can modulate signaling involved in angiogenesis, apoptosis and cell cycle arrest, and act as a chemosensitizer when combined with anticancer drugs, with a remarkable selective toxicity for cancer cells compared to normal cells. These observations are very interesting, and suggest that thymoquinone can target critical factors involved in cell survival and progression.[205] It has been suggested that thymoquinone acts as a biological mimic of ubiquinone, interfering with the mitochondrial electron transport chain, and generating free radical in its target cells.[206] However, these effects were observed only at very high dosages, and this mechanism does not explain the selectivity of action of thymoquinone for cancer cells. A recent finding that thymoquinone increases the cellular levels of PTEN proteins better fits with the pleiotropic biological profile of this compound.[207]

The anticancer documentation on thymoquinone is only pre-clinical, and its pharmacokinetics and human toxicity are largely unknown. Nevertheless, the long-established culinary consumption of black cumin, and the promising pre-clinical potential of thymoquinone, have made the oil of black cumin a popular dietary supplement for cancer patients. Although thymoquinone shows an excellent safety profile, with a $LD_{50} > 2$ g kg^{-1} in rats, its quinone structure is presumably responsible for the depletion of glutathione observed in animal administered with high dosages of black cumin oil, as well as for very rare cases of allergic contact dermatitis reported in the toxicological literature.[208] Unsurprisingly, thymoquinone has been used as a lead structure for medicinal chemistry studies in the realm of anti-cancer drug discovery,[209] although its major medicinal potential might be in the cancer supporting care, owing to its ability to improve, in animal models, the therapeutic index of several important drugs, including cisplatin, doxorubicin, methotrexate, and isofosfamide.[210]

Paradoxically, and despite the large literature in the realm of cancer, the current clinical documentation of thymoquinone is mostly neurological, and its activity in the management of otherwise untreatable refractory epilepsy crises in children is raising considerable expectations also in this field.[211]

The molecular mechanisms involved in this activity are unknown, but black cumin shows anti-seizure activity in animal models of epilepsy, and is used in the traditional medicine of the Middle East for this indication.

6.6 D-Limonene (5)

This monoterpene diolefin is a major constituent of the essential oil of various *Citrus* fruits (orange, mandarin, grapefruit, lemon, lime) and is a major dietary terpenoid. Commercial orange juice contains *ca.* 100 mg D-limonene L^{-1}, and the average consumption of this compound in Western countries is over 16 mg day^{-1}. D-Limonene has been extensively investigated in terms of human metabolization and safety. It is rapidly absorbed in the stomach, with T_{max} of *ca.* 1 h and half-life of *ca.* 12–24 h, and it is extensively metabolized in an oxidative way, with limonene 8,9-diol (**64**), perillic acid (**65**), dihydroperillic acid (**66**) and carveol (**67**) being the major human metabolites.[212] These are excreted in the urine both in free and in conjugated (glucuronide) forms. The toxicological documentation on D-limonene is impressive, as expected by its use as a flavoring agent, as an industrial solvent and as a permeation enhancer in topical products.[213] The induction of a unique nephropathic syndrome in rats after chronic subacute administration further spurred extensive studies to evaluate its safety in humans.[213] It was demonstrated that kidney damage in these animals is associated to the accumulation in the urine of α2-globulin in hyaline droplets, an event that does not occur in humans, whose urinary protein excretion is very low compared to that of rats. Furthermore, α2-globuline is not present in human plasma, and no related protein is expressed in human kidney tissues. Spurred by its excellent safety profile in humans, and by a promising profile of activity in animal models of several diseases, clinical investigations on D-limonene have been carried out in the area of gastro-intestinal diseases and cancer.[213] D-Limonene is an excellent solvent for cholesterol, and it has been used to dissolve gallstones by direct infusion into the gallbladder.[214] In a second clinical application, D-limonene showed excellent activity in the relief of heartburn, although the mechanism underlying this property is unclear and might involve coating of the gastric surface and its protection from gastric acid exposure.[215] Finally, D-limonene has also been studied as an anticancer agent, with results that warrant further investigation, with partial responses being observed in several small studies.[213] Thus, three patients with colorectal cancer were able to suspend progression of the disease for over six months when treated with D-limonene, and a breast cancer patient maintained a response for 11 months when treated orally with 8 g m^{-2} daily of this compound. Epidemiological correlations were also observed between the consumption of Citrus peel and a reduced occurrence of certain skin cancers.[213] D-Limonene is a prenyl transferase inhibitor, and this activity might underlie its anti-cancer potential.[216]

D-Limonene has also been a favorite topic for the study of enantiomeric discrimination and its biological relevance. While humans can easily distinguish the odor properties of the two enantiomers of limonene,[217] the effect of chirality on the clinical profile of the two enantiomers is still poorly known, a surprising gap in the literature on one of the most famous member of the volatilome.

6.7 (−)-Menthol (11)

Menthol is probably the most thoroughly investigated constituent of the plant volatilome from all standpoints, due to its widespread use as a flavor, as a coolant agent and, and as a drug.[218] Its identification as the archetypal activator of the cool receptor TRPM8 further contributed to spur investigations on its molecular profile of activity.[219] From a clinical standpoint,

menthol and peppermint oil have been extensively investigated for the treatment of irritable bowel syndrome (IBS) and recurrent abdominal pain in children.[220] A meta-analysis of the clinical studies in this area concluded that enteric-coated peppermint oil capsules should be considered the treatment of choice to alleviate general symptoms and to improve quality of life in IBS patients with non-serious constipation and diarrhea.[221] Coating is important to avoid heartburn and gastric irritation, two common side-effects of menthol. Recently, menthol glucuronide, the major human metabolite of menthol, has been proposed for the colon delivery of menthol.[222] While there seems to be a certain consensus on the clinical efficacy of peppermint oil and menthol to manage IBS and related gastro-intestinal conditions, their mechanism of activity is still unclear.[221] The evidence that menthol exerts a spasmolytic activity on the intestinal smooth vasculature does not fully explain the clinical efficacy, since the duration of this effect is limited, and intestinal sensory receptors might also be involved.[223] Menthol interacts not only with TRPM8, but also with related thermoTRPs, including TRPA1, making it difficult to dissect the various responses it evoke at organ level.[223]

The menthol receptor TRPM8 was originally described as a prostate cancer marker, and is indeed over expressed in prostate cancer cells.[219] Based on this finding, menthol derivatives labeled with ^{18}F have been developed for the PET detection of prostate cancer metastases.[224]

The examples discussed above show that VOCs can interact in a specific way with a series of end-points of clinical relevance, fully qualifying as drug leads. Unsurprisingly, certain plant VOCs are currently undergoing clinical development to address conditions of great clinical relevance.

7 The pharmacology of odor

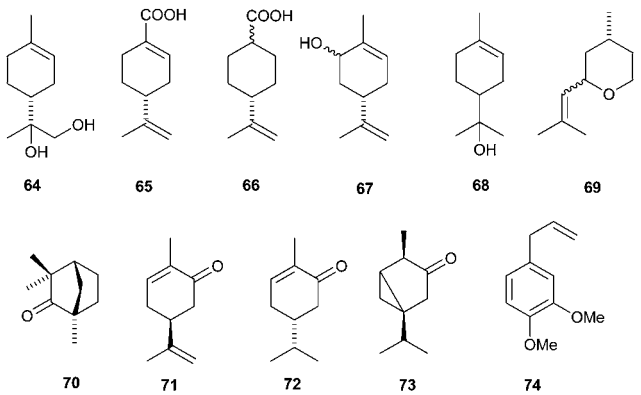
Many plant VOCs are perceived by animals and play a role in the communication between plants and animals. Despite fundamental differences in odor transduction mechanisms between insects and vertebrates, their anatomical and functional features are similar. Insects and mammals may share common principles of odor quality perception.[225] Recent progress in the study of insect olfaction has revealed that the heteromeric insect olfactory receptor complex forms a cation nonselective ion channel directly gated by odor or pheromone ligands independent of known olfactory receptor G-protein signaling pathways.[226] In mammals, odor signals typically transduce through a G-protein-dependent signal pathway in the olfactory sensory neurons that synapse ultimately in the glomeruli of the olfactory bulb, and is finally processed in higher brain structures.[227] In mammals, the olfactory epithelium secretes odorant-binding proteins (OBPs), which are lipocalins freely dissolved in the mucus layer protecting the olfactory neurons. OBPs may interact very selectively with their ligands and act as passive transporters of the predominantly hydrophobic odorant molecules across the aqueous mucus layer, or, alternatively, they may play a more active role in which the olfactory neuronal receptor recognizes the OBP–ligand complex.[228] Research on fragrant VOCs has certainly led to a better understanding of the physiology of scent perception. It was shown that honey bees are able to discriminate between the optical isomers of D-limonene, α-pinene, β-citronellol, menthol, and carvone, but fail to distinguish between the (+)- and (−)-forms of α-terpineol (**68**), camphor (**56**), rose oxide (**69**), fenchone (**70**), and 2-butanol.[225] These findings support the view that chiral-discriminating molecular odor receptors definitely exist, at least for some volatile enantiomers, and that insects and mammals may share common principles of odor quality perception, irrespective of the completely differing nature of their olfactory receptors. In humans, differentially perceived enantiomers of the same molecule are sensed differently, providing evidence that olfactory receptors are highly complex signal transducers. Thus, the carvone enantiomers are sensed distinctly by humans; with *S*-(+)-carvone (**71**) smelling like caraway and *R*-(−)-carvone (**72**) more like spearmint. Olfactory receptors (ORs) seemingly recognize different molecular features of odor molecules, cogently named "odotypes".[229] Experimental data suggest that the circuitry of the main olfactory bulb (OB) plays a critical role in olfactory discrimination. The processing of this information arises from the interaction between OB output neurons and local interneurons, and by interactions between the OB network and its centrifugal inputs, where acetylcholine acts as an enhancer.[230] Intriguingly, the elementary response in olfactory transduction is extremely small. Moreover, a ligand-bound odorant receptor has naturally a low probability of activating even one G protein molecule because the odorant dwell-time is very brief. Thus, signal amplification is crucial in olfactory transduction and appears fundamentally different from that of phototransduction.[231] Perhaps the pronounced signal amplification is one of the reasons why certain VOCs exert potent pharmacological effects (*e.g.* pain perception) when they hit a sensory receptor that is directly coupled to neuronal circuits. The molecular mechanism of odor perception is complex, and the odor quality of an odorant, while related to its constitution and configuration, is the result of the activation profile of a set, rather than a single, receptors.[232] In short, olfaction works in a combinatorial way.

8 Behavioral effects of VOCs

Maybe the most fascinating emerging field of pharmacological research on VOCs is related to the behavioral effects they can induce either as pure compounds or as mixtures. While there is little doubt that some VOCs are highly bioactive molecules capable of triggering specific physiological responses in plants and insects, the molecular interaction between plant VOCs and mammals is far less clear. There is increasing evidence that essential oil components trigger neurophysiological responses which may even result in a modulation of perception and altered behavior.[233] Different essential oils administered orally, intraperitoneally or intravenously are known to modulate behavior in animal tests. Such responses include the induction of anticonflict effects (which may in part be due to sedation or anxiolytic effects),[234] anxiety, cognition enhancing properties, and even idiosyncratic effects on both subjective and objective assessments of aspects of human behavior.[235] Sage (*Salvia officinalis*), which is a widespread spice has a longstanding anecdotal reputation as a plant that enhances memory. In a double-blind placebo controlled clinical trial it was shown that acute administration of sage could enhance memory in young adult volunteers.[236] Ambient odors of certain VOCs, such as those derived from

orange and lavender, have been reported to impact mood.[237] That such effects are likely to be due to defined molecular interactions rather than non-specific alterations of membrane properties is suggested by enantiomer-specific pharmacological actions. For example, the chirality of carvone leads to differential central effects and (*S*)-(+)-carvone (**71**) and (*R*)-(−)-carvone (**72**) appear to have depressant effect in the CNS while (*S*)-(+)-carvone shows anticonvulsant-like activity.[238] *Melissa officinalis* essential oil shows anti-agitation properties *in vivo*. Intriguingly, a placebo-controlled trial to determine the value of aromatherapy with essential oil of *M. officinalis* for agitation in people with severe dementia showed rather promising results, thus potentially providing a safe and effective treatment option for clinically significant agitation in people with severe dementia.[239] *Mentha piperita* (peppermint) and *Cananga odorata* (ylang ylang) essential oils have been shown to alter mood states in healthy volunteers.[235] How VOCs perceived *via* odor receptors are able to alter mood states remains, however, controversial[240] and needs to be elucidated. Few studies have addressed physiological responses to odor perception. For example, it was reported that essential oil scents affect autonomic neurotransmission and lipolysis in rats.[241] Moreover, it was shown that lemon oil vapors possesses anxiolytic, antidepressant-like effects *via* the suppression of dopamine activity related to enhanced serotonoergic neurons.[242] The scent of grapefruit oil and its active component, limonene, affects autonomic neurotransmission and blood pressure through central histaminergic nerves and the suprachiasmatic nucleus.[243] Such findings suggest that minor quantities of VOCs may interact with specific receptors to modulate behavior in mammals, in analogy to plant–insect interactions (*vide supra*).

VOCs are highly lipophilic compounds that may easily pass the blood brain barrier and may thus easily exert neuropharmacological effects, including toxicological effects (Table 3). While studies on the toxic effects of VOCs are relatively easily performed, the central effects induced *via* perception of odor (*e.g.* in aromatherapy) are inherently complex. For this reason, the toxicological studies performed with VOCs are much more advanced. In 2000, Höld *et al.*[244] reported a potential molecular mechanism of action of α-thujone (**73**), which is a CNS toxic agent in absinthe, the Swiss liqueur which was most popular in the 19th and early 20th centuries.[245] In fact, the adverse central effect of α-thujone is probably the best known CNS action of a VOC. It was shown that α-thujone is neurotoxic *via* negative modulation of GABA(A) receptors, which are important inhibitory chloride channels in the CNS. In the same study it was shown that α-thujone, whose concentration is sage (*Salvia officinalis* L.) is higher than in wormwood, is fast-acting but rapidly detoxified (metabolized) in mice.[244] It remains to be elucidated whether the neurotoxic effect of α-thujone is only due to its interaction with GABA(A) receptors or whether other CNS effects may be involved. Quite intriguingly, GABA(A) appears to be the target for numerous lipophilic natural products, including several VOCs (Table 2). Also the essential oils of lavender, *Melissa officinalis*, thyme, and (−)-menthol (**11**) have been reported to modulate GABA(A) action.[246,247] However, the binding interactions of these compounds have not been determined. Methyl eugenol (**74**) was reported to act agonistically on GABA(A),[248] like muscimol, the psychoactive component of *Amanita muscaria* that binds to the GABA binding site. Because the interpretation of such studies is often difficult and it cannot be excluded that VOCs are promiscuous and target different receptors and channels, the conclusions need to be interpreted with caution. Along that line, it was also shown that (*S*)-linalool (**12**), which is a common constituent in many essential oils, including lavender, exerts anxiolytic effects that are independent from GABA(A).[249] In a study by Narusuye *et al.*,[250] (*S*)-linalool (**12**) rather non-specifically suppressed voltage-gated currents not only in retinal horizontal cells and ganglion cells but also in Purkinje cells. Furthermore, bath application of (*S*)-linalool inhibited the KCl-induced Ca^{2+} (i) response of olfactory receptor cells (ORCs), suggesting that (*S*)-linalool suppresses Ca^{2+} currents in ORCs.[250] Overall, these results suggest that high concentrations of (*S*)-linalool (**12**) non-selectively suppress the voltage-gated currents in newt sensory neurons and rat cerebellar Purkinje cells. It is thus tempting to speculate that the modulation of voltage-gated currents by (*S*)-linalool may also be related to its antinociceptive effects.[251,252] If VOCs with specific pharmacological effects in the CNS could be found, they might indeed be interesting lead structures for the development of novel therapeutic agents targeting the CNS. Therefore, the widespread perception that lipid compounds, and in particular VOCs, found in essential oils, are bad drugs might have to be revised and their general incompatibility with the Lipinsky rule of 5[253] may suggest that bioactive VOCs are simply distinctly different drugs. Moreover, the high degree of *in vivo* pharmacological efficacy of VOCs and their surprisingly promising pharmacokinetic profiles (where known) makes these lipophilic compounds a promising group of hitherto neglected lead structures.



9 Concluding remarks

As science moves from single problems to whole concepts and to the study of more complex systems, the plant volatilome should be considered as an emerging entity. A growing body of evidence indicates that VOCs are important signaling molecules, and the deciphering of this chemical information will be of enormous relevance for the early detection of plant responses to biotic and abiotic stress, facilitating the search for new sustainable methods for pest and environmental control. Moreover, a better understanding of the emerging molecular interactions of VOCs and protein targets may bridge ecological chemistry and drug discovery.

Due to structural simplicity and lipophilicity, VOCs have been largely overlooked by medicinal chemists and pharmacologists alike. However, research on the volatilome shows that VOCs are very potent signaling molecules that have evolved to serve multiple functions, including pharmacological interactions with mammals. Therefore, the view of VOCs being simple biological spectators has been largely revisited in the last few decades. Membrane proteins, like those involved in signal transduction, are characterized by lipophilic pockets and domains necessary for anchoring to the lipid bilayer. Owing to their lipophilicity and a generally covalent (rings) or conformational (branching) shape constraint, plant VOCs have the potential to perturb signal transduction in a specific way, as shown by the identification of several VOCs-sensitive targets in plant and animal transduction pathways. The production VOCs by plants is generally diversity-oriented, with the generation of complex mixtures of compounds. This strategy suggests a broadly-tuned defense system that has the potential to regulate not only plant–insect, but also plant–mammal interactions. Consequently, the bioactive volatilome is now emerging as a novel potential source of interesting lead structures for drug discovery.

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