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MINI-REVIEW

Potential applications of glucosyltransferases in terpene glucoside production: impacts on the use of aroma and fragrance

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Abstract The detection of glucoconjugated forms of monoterpene alcohols in rose petals in the late 1960s opened the new field of nonvolatile aroma precursors in flavor research. It is now well established that odorless glycosides represent a significant pool of aroma precursors in plants where they act as preformed but inactivated defense or attractive chemicals. Technical improvements in the separation and identification of plant secondary metabolites have provided a multitude of chemical structures, but functional characterization of glycosyltransferases that catalyze their formation lags behind. As technical efforts and costs for DNA sequencing dramatically dropped during the last decade, the number of plant genome sequences increased significantly, thus providing opportunities to functionally characterize the glycosyltransferase gene families in plants. These studies yielded the first glycosyltransferase genes that encode efficient biocatalysts for the production of monoterpene glucosides. They have applications in the food, feed, chemical, cosmetic, and pharmaceutical industries as slow release aroma chemicals.

Keywords Flavor · Fragrance · Terpenes · Glycosyltransferases · Terpene glucosides

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Introduction

The isolation of geranyl, neryl, and citronellyl glucosides from rose petals in 1969 opened a new area of flavor research even though the increase in essential oil content during storage of, e.g., peppermint, was reported decades before (Francis and Allcock 1969). Research in the field of monoterpene glycosides remained limited and made insufficient progress showing that these results were not properly appreciated by the scientists involved in essential oil analysis. Besides, the low concentration of the plant secondary metabolites and restricted analytical capacities added to the low success in this area (Williams et al. 1982). However, improvements in separation and detection techniques as well as the development of novel high sensitive analytical instruments have significantly facilitated the identification of low abundant glycosides (Allwood and Goodacre 2010; Wang et al. 2000). A plethora of terpene glycosides from plants are known, now but the functional characterization of glycosyltransferase (GT) enzymes that catalyze their formation is lagging behind partly due to several technical constraints including availability of substrates and standards. Furthermore, their detection by mass spectrometry is challenging by virtue of their chemical composition.

Recently, the 55th plant genome has been sequenced and is publicly available (Michael and Jackson 2013). These genome databases contain GT gene families comprising 15 (*Physcomitrella patens*) to 377 (*Eucalyptus grandis*) GT family 1 members (http://supfam.cs.bris.ac.uk/SUPERFAMILY/). The GT families provide the unique opportunity to systematically identify genes and their encoded proteins that are involved in terpene glycoside formation. Metabolite profiling analyses in combination with gene expression experiments and biochemical characterization of recombinant proteins resulted in the identification of the first

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monoterpene GTs (Bönisch et al. 2014a, b; Yauk et al. 2014). This paper reviews the recent progress in research on terpene GTs.

Glucosyltransferases

The Carbohydrate-Active EnZymes (CAZy) database presents a sequence-based classification of GTs (EC 2.4.x.y) into over 90 families (Cantarel et al. 2009; Lombard et al. 2014; Lao et al. 2014). GTs are found in all kingdoms of life and are required for a wide range of metabolic processes as they catalyze the transfer of sugar moieties from activated donor molecules, usually uridine diphosphate (UDP) α-D-glucose to specific acceptor molecules, such as sugars, lipids, proteins, nucleic acids, antibiotics, and other small molecules, with either retention or inversion of configuration at the anomeric center (Lairson et al. 2008; Wang 2009). GTs that use sugar nucleotides are called Leloir enzymes. The product of glycosyl transfer may be an O-, N-, S-, or C-glycoside. Glucosyltransferases catalyze the formation of D-glucosides whereas glycosyltransferases transfer any type of sugar. Originally, GTs have been classified according to their reaction catalyzed and their substrate specificity; however, the genome sequencing projects have revealed a large number of GT genes with still unknown function. Therefore, a novel classification system based on amino acid sequence similarities has been proposed to accommodate the increasing number of GT genes (Coutinho et al. 2003; http://www.cazy.org/GlycosylTransferases.html). These GT families are classified hierarchically from their 3D structures (fold GT-A, GT-B, or predicted GT-C) to their mechanism (inverting or retaining GTs). The same three-dimensional fold is expected to occur within each of the families. Leloir glycosyltransferases have been found to possess only two different folds, termed the GT-A and GT-B folds (Unligil and Rini 2000). The GT-A fold consists of two dissimilar domains, one involved in nucleotide binding and the other binding the acceptor. The GT-B fold consists of two similar Rossmann fold subdomains (Fig. 1). The proportion of GT genes among protein coding genes is similar across a wide range of sequenced genomes (Yonekura-Sakakibara and Hanada 2011; Lairson et al. 2008). However, the proportions of GT genes in each CAZy GT family differ among organisms. The GT1 and GT31 families contain the majority of the GTs of an organism. Genes of the GT2, 8, and 47 families appear to be specific to plants, while those of GT29 are human-specific. The specificities reflect the unique aspects of the metabolism of each organism as the GT2, 8, and 47 families comprise enzymes involved in plant cell wall formation (Yonekura-Sakakibara and Hanada 2011).

CAZyfamily 1 GTs play important roles in the stabilization, improvement of water solubility, and deactivation/detoxification of natural products. They function in the regulation of metabolic homeostasis, detoxification of xenobiotics, and the biosynthesis, storage, and transport properties of secondary metabolites. In plants, they are generally localized in the cytosol where they are involved in the glycosylation of flavonoids, phenylpropanoids, terpenoids, steroids, steroidal alkaloids, and in the regulation of plant hormones (Bowles et al. 2006; Lim et al. 2003). GT1 enzymes contain a highly conserved sequence comprising 44 amino acid residues which is involved in binding to the UDP sugar as studies of crystal

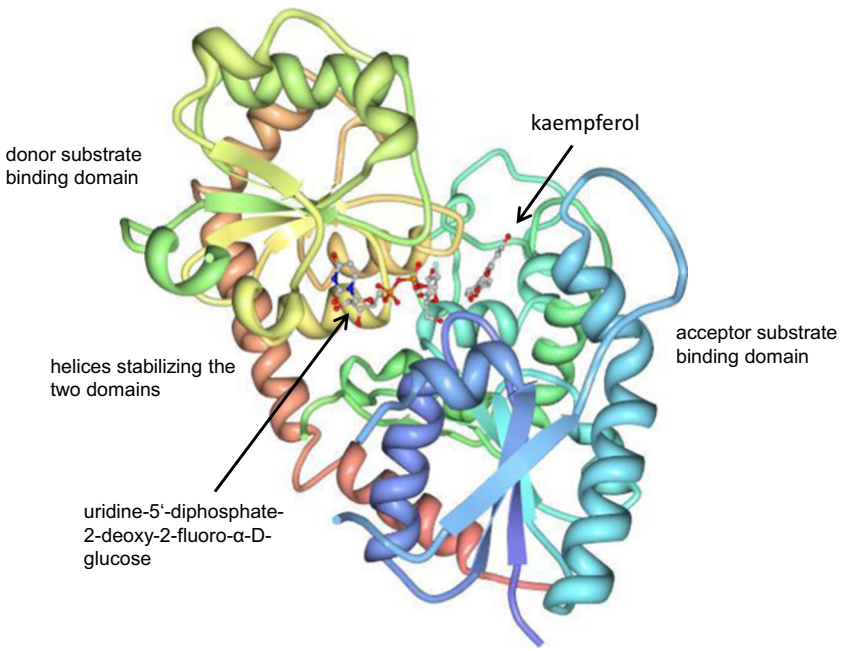

Fig. 1 3D structure of a CAZy family 1 member. VvGT1 from *Vitis vinifera* has been shown to preferentially glucosylate anthocyanidins and flavonols, albeit with lower efficiency than anthocyanidins (Offen et al. 2006). It is an inverting GT of the GT-B type; pdb accession number 2C1Z with the ligands kaempferol (acceptor) and uridine-5′-diphosphate-2-deoxy-2-fluoro-α-D-glucose (substrate analogon) is shown

structures of a limited number of GTs have confirmed (Offen et al. 2006). Interestingly, despite low sequence similarities, all GT1 proteins contain a GT-B fold, consisting of two ß/α/ß Rossmann-like domains whereas the C- and N-terminal domains interact with the activated sugar and acceptor molecule, respectively. The 3D arrangement of both domains to each other is stabilized by a backbone which is built by two helices (Fig. 1; shown in red; Jánváry et al. 2009).

Numerous GTs with verified and/or mostly putative functions have been identified in various plant species (Yonekura-Sakakibara 2009). In a recent study out of four GTs involved in formation of steroidal glycoalkaloids in tomato and potato, three were found to be clustered on chromosome 7 (Itkin et al. 2013). Besides, recent progress in genome sequencing projects reveals continuously a multitude of additional GT genes. However, the functions of many of the GTs remain obscure. Even in *Arabidopsis thaliana*, a very well-studied species, the proportion of GTs functionally characterized *in planta* is less than 20 % (Bowles 2002). Considerable progress has been made in understanding the function and substrate preference of GTs by biochemical assays, reverse genetics and comparative genomic, transcriptomic and metabolomic analyses, but more straightforward methods are required to speed the functional identification of GTs. Although screening of recombinant GT enzymes with randomly chosen substrates provides valuable information about GT promiscuity, it will not necessarily reveal the *in planta* substrates because not all of the substrates might be available in sufficient quantity for enzyme assays. For example, more than 200 different volatile aglycones alone have been detected in grape extracts from *Vitis vinifera*, and the total number of all possible acceptors including the nonvolatile ones will be even higher.

Only recently, a novel method based on aglycone libraries has been reported for the identification of in vivo substrates of GT enzymes (Bönisch et al. 2014a). In brief, glycosides were isolated from plant tissues that showed the highest expression levels of the target gene. An aglycone library of the tissue was obtained by enzymatic hydrolysis of the total glycosidic extract followed by extraction of the released alcohols, acids, amines, and thiols. This physiologic library that contained potential natural substrates of the studied GT was screened with the recombinant GT enzyme and either radiochemically labeled or unlabeled UDP glucose. The formed glucosides were separated by thin layer chromatography and visualized by radiodetection, whereas identification was achieved by liquid chromatography-tandem mass spectrometry (Fig. 2; Bönisch et al. 2014a). The method has been successfully applied in the identification of the first monoterpene GTs from grapevine (*Vitis vinifera*) that are involved in the glucosylation of volatile, small molecule secondary metabolites. The method will facilitate the functional characterization of novel GTs in the future.

Aroma and fragrance

A chemical compound (odorant) has a smell or odor when it is sufficiently volatile to be transported to the olfactory system in the upper part of the nose and interacts with the olfactory receptors (Dunkel et al. 2014; Marks et al. 2012; Rowan

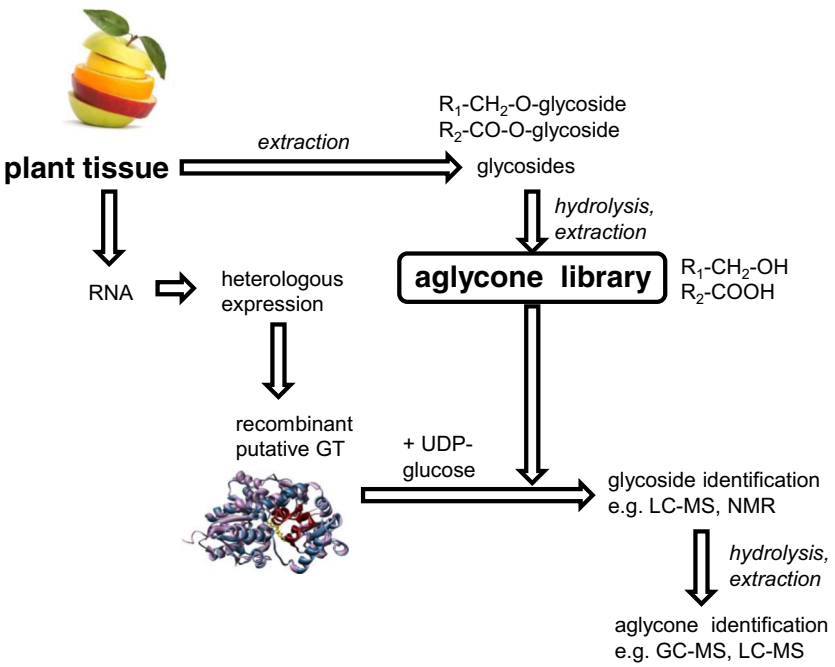

Fig. 2 Targeted functional screening of small molecule glucosyltransferases by means of aglycone libraries prepared from different plant tissues

2011). Generally, molecules meeting this specification have molecular weights of less than 300 Daltons. Flavors affect both the sense of taste and smell, whereas fragrances and aromas affect only smell. Flavors and aromas tend to be naturally occurring and are used to describe all sensory properties of food whereas fragrances tend to be synthetic and are utilized in the context of cosmetics, especially in perfumery. Volatile compounds are biochemically produced by plant enzymes in fruits, flowers, leaves, roots, or more specifically differentiated organs such as glandular trichomes, oil ducts, or cavities, are formed as by-products during fermentation processes, or are chemically generated during food processing (Schwab et al. 2008). Aroma compounds such as short-chain esters, alcohols, aldehydes, ketones, lactones, thiols, amines, phenolics, and terpenoids play a significant role in the production of flavorants and flavorings, which are used in the food industry to flavor, improve, and generally increase the appeal of their products (Berger 2009; Serra et al. 2005).

Terpenes are a large and very diverse group of organic compounds derived from the two basic C5 precursors dimethyl allyl pyrophosphate and isopentenyl pyrophosphate (Schwab et al. 2008; Bouvier et al. 2005). They are produced by plants, though also many insects emit terpenes (Gershenzon and Dudareva 2007). When olefinic terpenes are chemically modified such as by oxidation or rearrangement of the carbon skeleton, the resulting compounds are generally referred to as terpenoids or isoprenoids (Bohlmann and Keeling 2008). Terpenes and terpenoids are the primary constituents of the essential oils of many types of plants and are widely used as natural flavor additives for food, as fragrances in perfumery, and in medicine and aromatherapy (Caputi and Aprea 2011). While the volatile hemiterpenoids (C5), monoterpenoids (C10), and sesquiterpenoids (C15) mainly contribute to the odor of a product, higher terpenoids are perceived as tastants such as the steviosides, which are sweet tasting glycosides of the diterpenoid steviol (Genus 2003)

Flavor and fragrance are dynamic and elusive. The appealing odor of fresh products can be lost in a day. It is important to harness flavors and fragrances and incorporate them into products to make them appear fresh, wholesome, and attractive to consumers for as long as possible. Just like some other compounds, odorants will react and escape from products. Once a product is on the store shelf, oxidation, hydrolysis, and other processes may cause it to lose its desired attributes and even develop off-flavors (Tikunov et al. 2013). Thus, methods are required to stabilize flavor and fragrance molecules. One way to limit aroma degradation or loss during processing and storage is to encapsulate volatile ingredients prior to use in foods or beverages (Feng et al. 2009). Flavor encapsulation methods are spray drying, fluidized bed coating, melt extrusion, complex coacervation, aqueous diffusion, and novel fat-coating. Alternatively, aroma chemicals can be stabilized by adsorption to carriers such as cyclodextrins or by chemical derivatization to esters (Herrmann 2007). In contrast, glycosylation of flavor and fragrance molecules is a unique type of aroma stabilization as it is copied from nature (Wen et al. 2014; Vilanova et al. 2012; Rocha et al. 2004).

Application of glycosyltransferase genes

GTs have been widely used in the synthesis of the glycoconjugates of polyketides, flavonoids, nonribosomal peptides, and further antibiotics (Xiao et al. 2014; Song et al. 2013; Singh et al. 2012; Thibodeaux et al. 2007; Lim et al. 2004). Suitable enzymes are isolated from natural sources or produced by heterologous expression (Gachon et al. 2005). In addition, whole cell biotransformation systems utilizing either endogenous glycosyl donors or containing cloned and expressed systems for synthesis of glycosyl donors have been developed (Lim et al. 2004; Caputi et al. 2008). In cell-free approaches, the large-scale application of GTs for glycoconjugate synthesis has required access to large quantities of the glycosyl donors. Nucleotide recycling systems which allow the resynthesis of glycosyl donors, e.g., UDP glucose from the released nucleotide, e.g., by sucrose synthase, have been developed (Bülter and Elling 1999). The recycling approach has the further benefit of reducing the amount of nucleotide formed as second product, thereby minimizing GT inhibition, a commonly observed feature of the nucleotide by-product (Masada et al. 2007).

In vivo and in vitro combinatorial biosynthesis technologies using substrate tolerant GTs have been used for the synthesis of natural product antibiotics with altered glycosylation patterns that may have efficacious bioactivities (Song et al. 2013). Understanding the biochemical processes that are involved in the biosynthesis of sugar moieties of natural products and the glycosylation reactions coupled with attempts to engineer GTs will play a key role in the biological synthesis of novel glycosylated products (Singh et al. 2012; Kubo et al. 2004).

GT enzymes which act on hormones play an important role in regulating the hormonal activity in plants, mammals, and insects. Gain-of-function and loss-of-function mutant analyses have revealed that they play a crucial role in hormone homeostasis and in adaptation to various abiotic stresses (Dong and Hwang 2014). Most conjugates of plant hormones such as auxin, brassinosteroids, cytokinin, gibberellin, abscisic acid, jasmonates, and salicylate do not show physiological activity, but rather are involved in transport, signaling, storage, and degradation of the phytohormones (Ostrowski and Jakubowska 2014). Thus, GTs might also find applications in plant breeding.

Enzymatic glycosylation of terpenoids is a useful tool for synthesis due to the high selectivity and the mildness of the

reaction conditions in comparison with chemical reactions (Rivas et al. 2013; Toshima and Tatsuta 1993). Several types of biocatalysts have been used in the enzymatic glycosylation of monoterpenoids such as geraniol which plays an important role in the flavor and fragrance industry. Geraniol has a rose-like scent and is released from its odorless and nonvolatile glucoside by slow enzymatic or acidic hydrolysis (Clark 1998). This controlled release of a fragrance has much potential for application. Geranyl glucoside was enzymatically produced by reverse hydrolysis in a bioreactor from geraniol and glucose using almond ß-glucosidase as a biocatalyst (de Roode et al. 2001). Similarly, geranyl, neryl, and citronellyl glucosides have been produced by recombinant *Pichia etchellsii* ß-glucosidase II (Bachhawat et al. 2004). The major drawback of these enzymatic glycosylation reactions is the unfavorable equilibrium state of the reverse hydrolysis reaction that inescapably results in a low product yield (Desmet et al. 2012). Hence, the use of GTs for the biocatalytic production of monoterpenoid glucosides was tested using a microbial-based whole cell biotransformation system capable of regenerating the cofactor, UDP-glucose. A high cell density 3-L fermentation system was shown to produce 240 mg of geranyl glucoside during 6 h of the production phase (Caputi et al. 2008). The bioprocess can be readily scaled-up by using microbial whole cell biocatalysis systems that regenerate sugar donors and enable good yields of glycosides without the requirement for cofactor addition (Arend et al. 2001).

Monoterpenoid GT genes have been rarely reported until now. In contrast, diterpenoid and triterpenoid-specific GTs have been well described, for example for steviosides and glycoalkaloids such as tomatine (Itkin et al. 2013; Yang et al. 2014). Recombinant GT proteins encoded by genes from *Eucalyptus perriniana*, *Rauvolfia serpentine*, *Sorghum bicolor*, and *Medicago truncatula* are multisubstrate enzymes that glucosylate monoterpenols, although with much lower efficiency than their putative *in planta* substrates (Jones et al. 1999; Hefner et al. 2002; Hansen et al. 2003; Nagashima et al. 2004; He et al. 2006). It has also been shown that 27 Arabidopsis GTs glycosylate a diversity of monoterpenes, sesquiterpenes, and diterpenes such as geraniol, linalool, terpineol, and citronellol, but kinetic data have not been presented (Caputi et al. 2008). In contrast, UGT85A24 from *Gardenia jasminoides* and UGT8 from *Catharanthus roseus* seem to catalyze the glucosylation of the iridoids 7-deoxyloganin and 7-deoxyloganetic acid, respectively (Nagatoshi et al. 2011; Asada et al. 2013). Iridoids are odorless, oxygen containing, cyclic monoterpenes derived from 10-oxogeranial and are often intermediates in the biosynthesis of alkaloids (Höfer et al. 2013).

Only recently, the first monoterpenol-specific GTs were reported from grapevine (*Vitis vinifera*) and kiwi (*Actinidia delicosa*; Bönisch et al. 2014a,b; Yauk et al. 2014). Transcript accumulation correlated with the production of monoterpenyl ß-D-glucosides in grape and kiwi fruit during ripening. The recombinant proteins glucosylate a range of short chain alcohols, including terpenoids, but prefer geraniol as substrate. Kinetic data confirmed the efficient transformation of monoterpene alcohols (Table 1), and site-specific mutagenesis identified amino acids essential for enzymatic activity and substrate tolerance (Bönisch et al. 2014b). Finally, biotransformation experiments demonstrated the applicability of the novel GTs in biocatalytic processes for the production of monoterpenyl glucosides.

Table 1 Kinetics of VvGT14, 15a-c from *Vitis vinifera* and AdGT4 from *Actinidia delicosa*

Enzyme	Substrate	K_M [μM]	k_{cat} [s^{-1}]	k_{cat}/K_M [s^{-1} mM^{-1}]
VvGT14				
	Citronellol (rac)	9±0.3	0.02	2.5
	Geraniol	9±1.2	0.02	2.6
	Nerol	10±0.7	0.02	2.0
	8-Hydroxylinalool	48±2.0	0.002	0.03
	Terpineol	33±4.4	0.003	0.1
	Linalool (rac)	47±0.1	0.0003	0.01
	UDP-glucose	16±0.03	0.03	1.6
VvGT15a				
	S-Citronellol	29±3.0	0.02	0.9
	Geraniol	63±2.4	0.12	1.9
	Nerol	48±1.7	0.04	0.7
	8-Hydroxylinalool	32±1.4	0.003	0.1
	UDP-glucose	49±12.0	0.18	3.7
VvGT15b				
	S-Citronellol	55±1.3	0.03	0.6
	Geraniol	81±1.0	0.10	1.2
	Nerol	40±3.7	0.03	0.8
	8-Hydroxylinalool	33±1.8	0.003	0.1
	UDP-glucose	43±1.0	0.17	4.1
VvGT15c				
	S-Citronellol	20±1.6	0.03	1.8
	Geraniol	43±0.7	0.17	3.9
	Nerol	28±0.9	0.06	2.2
	8-Hydroxylinalool	17±0.2	0.004	0.3
	UDP-glucose	51±1.6	0.26	5.0
AdGT4				
	Geraniol	76±11.1	11.05	14.5
	Octan-3-ol	67±16.2	0.75	1.1
	Hexanol	117±28.1	2.72	2.3
	(Z)-hex-3-enol	57±20.0	0.49	0.9
	UDP-glucose	45±15.2	6.93	15.6

Adapted from Bönisch et al. (2014a); Yauk et al. (2014)

Terpene glycosides

Analysis

A majority of the volatile terpenoids occur also as glycoconjugates in plants (Winterhalter and Skouroumounis 1997). Since the first detection of geranyl glucoside in rose petals, various methods have been developed and applied to isolate, identify, and quantify the odorless terpenoid derivatives (Mateo and Jiménez 2000). The isolation of terpene glycosides from plant extracts by selective retention of the metabolites on a nonpolar solid-phase adsorbent (reversed phase RP18 or polystyrene XAD8) is the most frequently used technique. By washing the loaded adsorbent with water following the adsorption step, free sugars and other polar constituents (polysaccharides, amino acids, acids, proteins, and inorganic ions) can be removed while the less polar glycosides are retained. Finally, elution with a nonpolar or semipolar solvents such as pentane or diethyl ether, respectively, removes fatty acids and other nonpolar compounds whereas elution with a polar organic solvent such as methanol or acetonitrile yields a glycosidic fraction (Mateo and Jiménez 2000). Reversed phase HPLC systems in combination with MS has emerged as the most suitable method for the detection and identification of terpene glycosides as it offers selectivity and specificity in both the chromatographic separation and detection steps (Gray et al. 2010; Yang et al. 2011). Surprisingly, only a very limited number of publications report on the direct quantification of monoterpenyl glucosides using the LC-MS approach, probably due to the lack of authentic standards that are mandatory for the proper validation of the analytical process (Bönisch et al. 2014a, b). Thus, in most of the publications dealing with the analysis of monoterpenol glycosides, indirect methods are used that focus on the quantification of the released aglycones by GC-MS after enzymatic hydrolysis. However, nuclear magnetic resonance spectroscopy (NMR) is still the method of choice to identify novel chemical structures (Berkov et al. 2014; Vera Saltos et al. 2014).

Physiological activities

Terpene glycosides, in particular iridoid glycoconjugates that are derived from 10-oxogeranial, are associated with a wide range of health benefits (Geu-Flores et al. 2012). Extensive studies on pharmacological activities such as neuroprotective, anti-inflammatory, immunomodulator, hepatoprotective, and cardioprotective effects of the isolated iridoid glycosides revealed that many of them are the bioactive principles of the plants for which these plants are used in traditional medicines (Dinda et al. 2011). Anticancer, antioxidant, antimicrobial, hypoglycaemic, hypolipidemic, choleretic, antispasmodic, and purgative properties were also reported (Tundis et al. 2008). Similarly, acylated α-terpinyl glucosides preferentially inhibit leucine transport upon which new antiprostate cancer therapies may be based (Wang et al. 2014), and two unusual monoterpene glycosides, cyclopside 1 and 2 from *Acacia cyclops*, show cytotoxic effect in vitro against the human breast cancer (MCF-7) and ovarian cancer (OVAR) cell lines (Jelassi et al. 2014). Besides, monoterpene glycosides from the root of *Paeonia suffruticosa* were found to function as anti-inflammatory agents as they are active against cyclooxygenase 1 and 2 enzymes (Zhu and Fang 2014).

The iridoid glycosides are produced by plants primarily as a defense against herbivores or against infection by microorganisms (Biere et al. 2004). Insects also use iridoids obtained through its diet as a defense against avian predators (Burse et al. 2009). To humans and other mammals, iridoids are often characterized by a deterrent bitter taste (Behrens et al. 2009).

The specific role of glycoconjugated terpenes in plants is the subject of still on-going discussions. Terpene glycosides have been considered as accumulation and storage forms of aroma substances and as transport forms of aroma volatiles (Winterhalter and Skouroumounis 1997) and may play an important role in the mechanism of flower fragrance formation. In the absence of specific storage organs and tissues, the glycoconjugated terpenes may protect the plant from any toxic effect mediated by the free lipophilic aglycone (Stahl Biskup et al. 199393).

Applications

Fragrance and flavor chemicals have been widely applied in food, medicine, tobacco, textile, leather, papermaking, cosmetics, and other products of human consumption. Besides aromatic compounds such as vanillin, phenylethanol, and benzyl acetate as well as esters of lower fatty acids, monoterpenes such as linalool, menthol, and geraniol are among the economically most important aroma chemicals which reach annual consumption rates of more than 5000 tonnes (Schwab et al. 2008).

However, fragrance and flavor substances such as monoterpenes are volatile and predominantly poorly water-soluble which limits their number of industrial applications. In contrast, terpene glycosides show increased water solubility and are more stable than their corresponding aglycones. Although monoterpene glycosides are odorless, they are of interest for flavor and fragrance industry due to the possibility of controlled release of the bound aroma compound. Thus, monoterpene glycosides have been proposed for improving smoking quality of cigarettes (Bai et al. 2012), inhibiting unpleasant odor of sanitary and pet-care products (Yamada et al. 1998), enhancement of plant fragrance emitted by cut flowers (Iwamoto et al. 1994; Kimura and Hattori 1998), as oral deodorant to reduce feces odor (Arakawa and Yasuda 1998), to impregnate garmets (Kimura et al. 1997a), as

ingredients of long-lasting deodorants (Kimura et al. 1997b), to mask perspiration-related odor in beddings (Kimura et al. 1997c), as ingredients of massage oil (Donho and Kimura 1996) and bath preparations (Donho et al. 1996) for sustained release of perfumes, and use in air freshener (Tanaka and Kotsuna 1993).

The applicability of using odorless glycosidically bound volatiles as fragrance materials was demonstrated by incubating skin microflora with various kinds of glycosides. Most glycosidically bound volatiles, particularly ß-D-glucosides, were metabolized by the skin microbiome thereby releasing the aglycones as fragrance materials (Ikemoto et al. 2002, 2003). Similarly, monoterpene glycosides may be hydrolyzed by glucosidases excreted by the skin microbiome (Fig. 3).

In addition to their function as slow release aroma chemicals, glycosides might also act as slow release antimicrobial and antifungal compounds similar to esculin (esculetin glucoside; Duncan et al. 2004). It was demonstrated that ß-glucosidases are produced by dermatophytes as well as members of the dermal microbiota, and that this activity is sufficient to hydrolyze esculin to esuletin with concomitant antifungal activity (Mercer et al. 2013). The antimicrobial activity of monoterpenes is well known and documented (Schmidt et al. 2012).

Decyl glucoside is a mild nonionic surfactant used in cosmetics and in products for individuals with a sensitive skin. As the starting material of its synthesis is plant-derived and the product is biodegradable, many natural personal care companies use this detergent (Rather and Mishra 2013). Since monoterpene glucosides have a related chemical structure and can be biotechnologically produced from natural products, they might be used as alternative surfactants particularly in applications where the additional function of the terpene glucoside as slow release aroma compounds and antimicrobials is beneficial.

Conclusion

Flavors in food and fragrances in cosmetics must be present in an optimum concentration not only at the first moment of consumption or release, but also for a prolonged time period. This often cannot be accomplished with volatile flavors and fragrances evaporating from food and cosmetic products, respectively, not even for food during storage in the freezer. Flavor precursors present in a nonvolatile stable form, which

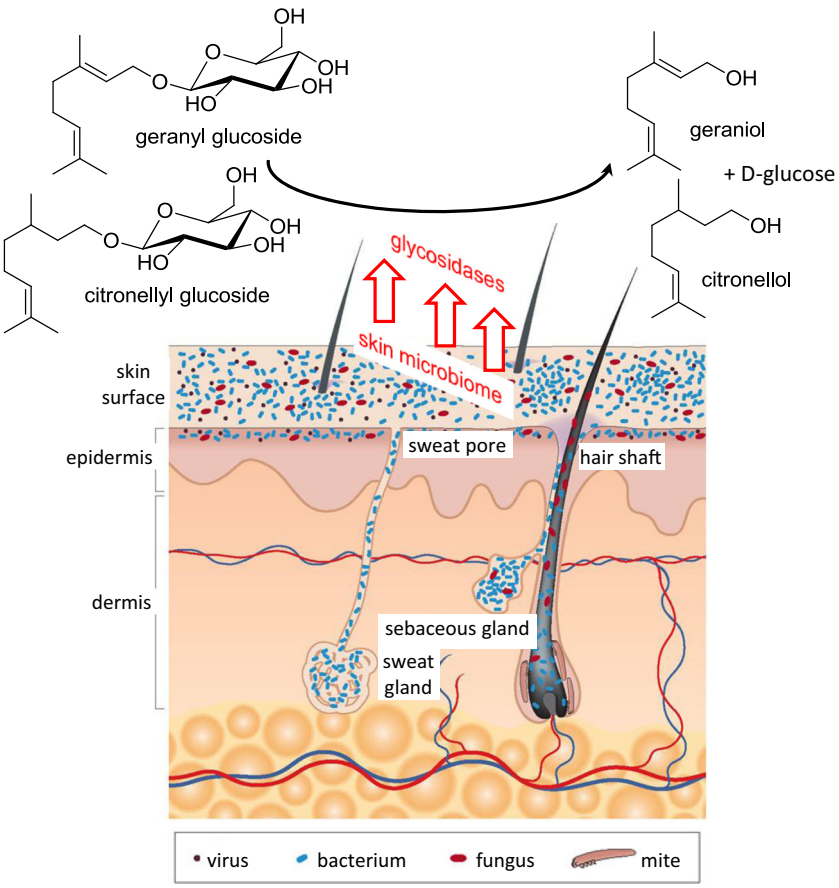

Fig. 3 Monoterpene glucosides may function as "slow release" aroma chemicals triggered by glycosidase that are excreted by the skin microbiome, adapted from Grice and Segre (2011)

allows the release of the aroma chemical upon a trigger, are highly favorable. Glycosides are an optimum choice, having very low vapor pressures, are stable and water-soluble, can be prepared in a natural, biocatalytic manner, and release only a nontoxic anchor-molecule. Nature makes glycosides using glycosyltransferases, but until now, they have been rarely used in synthetic chemistry for the production of glycosidically bound aroma and fragrance compounds. The identification of highly reactive monoterpene GTs from grapevine and kiwi and the availability of engineered host organisms that efficiently regenerate the cosubstrate UDP glucose now enables the biotechnological production of terpene glucosides in an economical way.

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References

Allwood JW, Goodacre R (2010) An introduction to liquid chromatography-mass spectrometry instrumentation applied in plant metabolomic analyses. Phytochem Anal 21:33–47

Arakawa T, Yasuda H (1998) Oral deodorants for feces containing glycosides of fragrant compounds; From Jpn Kokai Tokkyo Koho, JP 10028724 A 19980203

Arend J, Warzecha H, Hefner T, Stöckigt J (2001) Utilizing genetically engineered bacteria to produce plant-specific glucosides. Biotechnol Bioeng 76:126–131

Asada K, Salim V, Masada-Atsumi S, Edmunds E, Nagatoshi M, Terasaka K, Mizukami H, De Luca V (2013) A 7-deoxyloganetic acid glucosyltransferase contributes a key step in secologanin biosynthesis in Madagascar periwinkle. Plant Cell 25:4123–4134

Bachhawat P, Mishra S, Bhatia Y, Bisaria VS (2004) Enzymatic synthesis of oligosaccharides alkyl and terpenyl glucosides by recombinant *Escherichia coli*-expressed *Pichia etchellsii* beta-glucosidase II. Appl Biochem Biotechnol 118:269–282

Bai X, Mou D, Dong W, Peng G, Gong R, Wang L, Zhang Y (2012) Method for compensating flavor of low tar cigarettes; From Faming Zhuanli Shenqing, CN 102423114 A 20120425

Behrens M, Brockhoff A, Batram C, Kuhn C, Appendino G, Meyerhof W (2009) The human bitter taste receptor hTAS2R50 is activated by the two natural bitter terpenoids andrographolide and amarogentin. J Agric Food Chem 57:9860–9866

Berger RG (2009) Biotechnology of flavours–the next generation. Biotechnol Lett 31:1651–1659

Berkov S, Mutafova B, Christen P (2014) Molecular biodiversity and recent analytical developments: A marriage of convenience. Biotechnol Adv 32:1102–1110

Biere A, Marak HB, van Damme JM (2004) Plant chemical defense against herbivores and pathogens: generalized defense or trade-offs? Oecologia 140:430–441

Bohlmann J, Keeling C (2008) Terpenoid biomaterials. Plant J 54:656–669

Bönisch F, Frotscher J, Stanitzek S, Rühl E, Wüst M, Bitz O, Schwab W (2014a) Activity-based profiling of a physiologic aglycone library reveals sugar acceptor promiscuity of family 1 UDP-glucosyltransferases from grape. Plant Physiol 166:23–39

Bönisch F, Frotscher J, Stanitzek S, Rühl E, Wüst M, Bitz O, Schwab W (2014b) A UDP-glucose:monoterpenolglucosyltransferase adds to the chemical diversity of the grapevine metabolome. Plant Physiol 165:561–581

Bouvier F, Rahier A, Camara B (2005) Biogenesis molecular regulation and function of plant isoprenoids. Prog Lipid Res 44:357–429

Bowles D (2002) A multigene family of glycosyltransferases in a model plant *Arabidopsis thaliana*. Biochem Soc Trans 30:301–306

Bowles D, Lim EK, Poppenberger B, Vaistij FE (2006) Glycosyltransferases of lipophilic small molecules. Annu Rev Plant Biol 57:567–597

Bülter T, Elling L (1999) Enzymic synthesis of nucleotide sugars. Glycoconj J 16:147–159

Burse A, Frick S, Discher S, Tolzin-Banasch K, Kirsch R, Strauss A, Kunert M, Boland W (2009) Always being well prepared for defense: the production of deterrents by juvenile *Chrysomelina* beetles (Chrysomelidae). Phytochemistry 70:1899–1909

Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B (2009) The Carbohydrate-Active EnZymes database (CAZy): an expert resource for Glycogenomics. Nucleic Acids Res 37:D233–8

Caputi L, Aprea E (2011) Use of terpenoids as natural flavouring compounds in food industry. Recent Pat Food Nutr Agric 3:9–16

Caputi L, Lim EK, Bowles DJ (2008) Discovery of new biocatalysts for the glycosylation of terpenoid scaffolds. Eur J Chem 14:656–6662

Clark GS (1998) Geraniol. Perfum Flavor 23:19–25

Coutinho PM, Deleury E, Davies GJ, Henrissat B (2003) An evolving hierarchical family classification for glycosyltransferases. J Mol Biol 328:307–317

De Roode BM, Oliehoek L, van der Padt A, Franssen MC, Boom RM (2001) Downstream processing of enzymatically produced geranyl glucoside. Biotechnol Prog 17:881–886

Desmet T, Soetaert W, Bojarová P, Křen V, Dijkhuizen L, Eastwick-Field V, Schiller A (2012) Enzymatic glycosylation of small molecules: challenging substrates require tailored catalysts. Eur J Chem 18:10786–10801

Dong T, Hwang I (2014) Contribution of ABA UDP-glucosyltransferases in coordination of ABA biosynthesis and catabolism for ABA homeostasis. Plant Signal Behav 29(9 pii):e28888

Donho M, Kimura T (1996) Perfume precursor-containing compositions for massage; From Jpn Kokai Tokkyo Koho, JP 08188525 A 19960723

Donho M, Kimura T, Sekya M (1996) Bath preparations containing perfume precursors and the precursor-hydrolyzing enzymes; From Jpn Kokai Tokkyo Koho, JP 08188529 A 19960723

Duncan SH, Leitch EC, Stanley KN, Richardson AJ, Laven RA (2004) Effects of esculin and esculetin on the survival of *Escherichia coli* O157 in human faecal slurries continuous-flow simulations of the rumen and colon and in calves. Br J Nutr 91:749–755

Dinda B, Debnath S, Banik R (2011) Naturally occurring iridoids and secoiridoids. An updated review, part 4. Chem Pharm Bull 49:803-833

Dunkel A, Steinhaus M, Kotthoff M, Nowak B, Krautwurst D, Schieberle P, Hofmann T (2014) Nature's chemical signatures in human olfaction: a foodborne perspective for future biotechnology. Angew Chem Int Ed Engl 53:7124–7143

Feng T, Xiao Z, Tian H (2009) Recent patents in flavor microencapsulation. Recent Pat Food Nutr Agric 1:193–202

Francis MJ, Allcock C (1969) Monoterpene beta-D-glucosides in roses: isolation and formation from DL-2-^{14}C mevalonate. Biochem J 113:38

Gachon CM, Langlois-Meurinne M, Saindrenan P (2005) Plant secondary metabolism glycosyltransferases: the emerging functional analysis. Trends Plant Sci 10:542–549

Genus JMC (2003) Stevioside. Phytochemistry 64:913–921

Gershenzon J, Dudareva N (2007) The function of terpene natural products in the natural world. Nat Chem Biol 3:408–414

Geu-Flores F, Sherden NH, Courdavault V, Burlat V, Glenn WS, Wu C, Nims E, Cui Y, O'Connor SE (2012) An alternative route to cyclic terpenes by reductive cyclization in iridoid biosynthesis. Nature 492:138–142

Gray MJ, Chang D, Zhang Y, Liu J, Bensoussan A (2010) Development of liquid chromatography/mass spectrometry methods for the quantitative analysis of herbal medicine in biological fluids: a review. Biomed Chromatogr 24:91–103

Grice EA, Segre JA (2011) The skin microbiome. Nat Rev Microbiol 9: 244–253

Hansen KS, Kristensen C, Tattersall DB, Jones PR, Olsen CE, Bak S, Møller BL (2003) The in vitro substrate regiospecificity of recombinant UGT85B1 the cyanohydrin glucosyltransferase from *Sorghum bicolor*. Phytochemistry 64:143–151

He XZ, Wang X, Dixon RA (2006) Mutational analysis of the Medicago glycosyltransferase UGT71G1 reveals residues that control regioselectivity for (iso)flavonoid glycosylation. J Biol Chem 281:34441–34447

Hefner T, Arend J, Warzecha H, Siems K, Stöckigt J (2002) Arbutin synthase a novel member of the NRD1ß glycosyltransferase family is a unique multifunctional enzyme converting various natural products and xenobiotics. Bioorg Med Chem 10:1731–4171

Herrmann A (2007) Controlled release of volatile compounds under mild reaction conditions: from nature to consumer products. Angew Chem 119:5938–5967

Höfer R, Dong L, André F, Ginglinger JF, Lugan R, Gavira C, Grec S, Lang G, Memelink J, Van der Krol S, Bouwmeester H, Werck-Reichhart D (2013) Geraniol hydroxylase and hydroxygeraniol oxidase activities of the CYP76 family of cytochrome P450 enzymes and potential for engineering the early steps of the (seco)iridoid pathway. Metab Eng 20:221–232

Ikemoto T, Okabe B-I, Mimura K, Kitahara T (2002) Formation of fragrant materials from odourless glycosidically-bound volatiles on skin microflora (Part 1). Flavour Fragr J 17:452–455

Ikemoto T, Mimura K, Kitahara T (2003) Formation of fragrant materials from odourless glycosidically-bound volatiles on skin microflora (Part 2). Flavour Fragr J 18:45–47

Itkin M, Heinig U, Tzfadia O, Bhide AJ, Shinde B, Cardenas PD, Bocobza SE, Unger T, Malitsky S, Finkers R, Tikunov Y, Bovy A, Chikate Y, Singh P, Rogachev I, Beekwilder J, Giri AP, Aharoni A (2013) Biosynthesis of antinutritional alkaloids in Solanaceous crops is mediated by clustered genes. Science 341:175–179

Iwamoto M, Gocho S, Fujita A, Waku M, Pponda T (1994) Fragrance enhancers for plants; From Jpn Kokai Tokkyo Koho, JP 06336401 A 19941206

Jánváry L, Hoffmann T, Pfeiffer J, Hausmann L, Töpfer R, Fischer TC, Schwab W (2009) A double mutation in the anthocyanin 5-O-glucosyltransferase gene disrupts enzymatic activity in *Vitis vinifera* L. J Agric Food Chem 57:3512–3518

Jelassi A, Zardi-Bergaoui A, Ben Nejma A, Belaiba M, Bouajila J, Ben Jannet H (2014) Two new unusual monoterpene acid glycosides from *Acacia cyclops* with potential cytotoxic activity. Bioorg Med Chem Lett 24:3777–3781

Jones PR, Moller BL, Hoj PB (1999) The UDP-glucose:p-hydroxymandelonitrile-O-glucosyltransferase that catalyzes the last step in synthesis of the cyanogenic glucoside dhurrin in *Sorghum bicolor* Isolation cloning heterologous expression and substrate specificity. J Biol Chem 274:35483–35491

Kimura T, Hattori N (1998) Enhancement of plant fragrance enhancers containing ß-D-galactosides and the plants with enhanced fragrance; From Jpn Kokai Tokkyo Koho, JP 10060472 A 19980303

Kimura T, Ito H, Sekiya M, Donho M, Nakajima H (1997a) Odor-absorbing garments; From Jpn Kokai Tokkyo Koho, JP 09158041 A 19970617

Kimura T, Ito H, Sekiya M, Donho M, Nakajima H (1997b) Deodorants containing perfume precursors; From Jpn Kokai Tokkyo Koho, JP 09154930 A 19970617

Kimura T, Ito H, Sekiya M, Donho M, Nakajima H (1997c) Beddings containing perfume precursors to mask perspiration-related odor; From Jpn Kokai Tokkyo Koho, JP 09154677 A 19970617

Kubo A, Arai Y, Nagashima S, Yoshikawa T (2004) Alteration of sugar donor specificities of plant glycosyltransferases by a single point mutation. Arch Biochem Biophys 429:198–203

Lairson LL, Henrissat B, Davies GJ, Withers SG (2008) Glycosyltransferases: structures functions and mechanisms. Annu Rev Biochem 77:521–555

Lao J, Oikawa A, Bromley JR, McInerney P, Suttangkakul A, Smith-Moritz AM, Plahar H, Chiu TY, González Fernández-Niño SM, Ebert B, Yang F, Christiansen KM, Hansen SF, Stonebloom S, Adams PD, Ronald PC, Hillson NJ, Hadi MZ, Vega-Sánchez ME, Loqué D, Scheller HV, Heazlewood JL (2014) The plant glycosyltransferase clone collection for functional genomics. Plant J 79:517–529

Lim EK, Baldauf S, Li Y, Elias L, Worrall D, Spencer SP, Jackson RG, Taguchi G, Ross J, Bowles DJ (2003) Evolution of substrate recognition across a multigene family of glycosyltransferases in Arabidopsis. Glycobiology 13:139–145

Lim EK, Ashford DA, Hou B, Jackson RG, Bowles DJ (2004) Arabidopsis glycosyltransferases as biocatalysts in fermentation for regioselective synthesis of diverse quercetin glucosides. Biotech Bioeng 87:623–631

Lombard V, GolacondaRamulu H, Drula E, Coutinho PM, Henrissat B (2014) The carbohydrate-active enzymes database (CAZy) in 2013. Nucleic Acids Res 42:D490–5

Marks LE, Veldhuizen MG, Shepard TG, Shavit AY (2012) Detecting gustatory-olfactory flavor mixtures: models of probability summation. Chem Senses 37:263–277

Masada S, Kawase Y, Nagatoshi M, Oguchi Y, Terasaka K, Mizukami H (2007) An efficient chemoenzymatic production of small molecule glucosides with in situ UDP-glucose recycling. FEBS Lett 581: 2562–2566

Mateo JJ, Jiménez M (2000) Monoterpenes in grape juice and wines. J Chromatogr A 881:557–567

Mercer DK, Robertson J, Wright K, Miller L, Smith S, Stewart CSO, Neil DA (2013) A prodrug approach to the use of coumarins as potential therapeutics for superficial mycoses. PLoS One 8(11):e80760

Michael TP, Jackson S (2013) The First 50 Plant Genomes. Plant Genome 6:2

Nagashima S, Tomo S, Orihara Y, Yoshikawa T (2004) Cloning and characterization of glucosyltransferase cDNA from *Eucalyptus perriniana* cultured cells. Plant Biotechnol 21:343–348

Nagatoshi M, Terasaka K, Nagatsu A, Mizukami H (2011) Iridoid-specific glucosyltransferase from *Gardenia jasminoides*. J Biol Chem 286:32866–32874

Offen W, Martinez-Fleites C, Yang M, Kiat-Lim E, Davis BG, Tarling CA, Ford CM, Bowles DJ, Davies GJ (2006) Structure of a flavonoid glucosyltransferase reveals the basis for plant natural product modification. EMBO J 25:1396–1405

Ostrowski M, Jakubowska A (2014) UDP-glycosyltransferases of plant hormones. Adv Cell Biol 2:271–288

Rather MY, Mishra S (2013) ß-Glycosidases: An alternative enzyme based method for synthesis of alkyl-glycosides. Sustainable Chemical Processes 1:7

Rivas F, Parra A, Martinez A, Garcia-Granados A (2013) Enzymatic glycosylation of terpenoids. Phytochem Rev 12:327–339

Rocha SM, Coutinho P, Delgadillo I, Dias Cardoso A, Coimbra MA (2004) Effect of enzymatic aroma release on the volatile compounds of white wines presenting different aroma potentials. J Sci Food Agric 85:199–205

Rowan DD (2011) Volatile Metabolites. Metabolites 1:41–63

Schmidt E, Wanner J, Hiiferl M, Jirovetz L, Buchbauer G, Gochev V, Girova T, Stoyanova A, Geissler M (2012) Chemical composition olfactory analysis and antibacterial activity of *Thymus vulgaris* chemotypes geraniol, 4-thujanol/terpinen-4-ol, thymol and linalool cultivated in southern France. Nat Prod Commun 7:1095–1098

Schwab W, Davidovich-Rikanati R, Lewinsohn E (2008) Biosynthesis of plant-derived flavor compounds. Plant J 54:712–732

Serra S, Fuganti C, Brenna E (2005) Biocatalytic preparation of natural flavours and fragrances. Trends Biotechnol 23:193–198

Singh S, Phillips GN Jr, Thorson JS (2012) The structural biology of enzymes involved in natural product glycosylation. Nat Prod Rep 29:1201–1237

Song MC, Kim E, Ban YH, Yoo YJ, Kim EJ, Park SR, Pandey RP, Sohng JK, Yoon YJ (2013) Achievements and impacts of glycosylation reactions involved in natural product biosynthesis in prokaryotes. Appl Microbiol Biotechnol 97:5691–5704

Stahl Biskup E, Intert F, Holthuijzen J, Stengele M, Schulz G (1993) Glycosidically bound volatiles – a review 1986–91. Flav Frag J 8: 61–80

Tanaka M, Kotsuna K (1993) Fragrant compositions containing a single fragrant substance; From Jpn Kokai Tokkyo Koho , JP 05239491 A 19930917

Thibodeaux CJ, Melançon CE, Liu HW (2007) Unusual sugar biosynthesis and natural product glycodiversification. Nature 446:1008–1016

Tikunov YM, Molthoff J, de Vos RCH, Beekwilder J, van Houwelingen A, van der Hoooft JJJ, Vries MN, Labrie CW, Verkerke W, van de Gees H, Zamora MV, Presa S, Rambia JL, Granell A, Hall RD, Bovy AG (2013) NON-SMOKY GLYCOSYLTRANSFERASE1 prevents the release of smoky aroma from tomato fruit. Plant Cell 25:3067–3078

Toshima K, Tatsuta K (1993) Recent progress in O-glycosylation methods and its application to natural products synthesis. Chem Rev 93:1503–1531

Tundis R, Loizzo MR, Menichini F, Statti GA, Menichini F (2008) Biological and pharmacological activities of iridoids: recent developments. Mini Rev Med Chem 8:399–420

Unligil UM, Rini JM (2000) Glycosyltransferase structure and mechanism. Curr Opin Struct Biol 10:510–517

Vera Saltos MB, Naranjo Puente BF, Malafronte N, Braca A (2014) A new monoterpene glycoside from *Siparuna thecaphora*. Nat Prod Res 28:57–60

Vilanova M, Genisheva Z, Bescansa L, Masa A, Oliveira JM (2012) Changes in free and bound fractions of aroma compounds of four *Vitis vinifera* cultivars at the last ripening stages. Phytochemistry 74: 196–205

Wang X (2009) Structure mechanism and engineering of plant natural product glycosyltransferases. FEBS Lett 583:3303–3309

Wang D, Yoshimura T, Kubota K, Kobayashi A (2000) Analysis of glycosidically bound aroma precursors in tea leaves 1. Qualitative and quantitative analyses of glycosides with aglycons as aroma compounds. J Agric Food Chem 48:5411–5418

Wang Q, Grkovic T, Font J, Bonham S, Pouwer RH, Bailey CG, Moran AM, Ryan RM, Rasko JE, Jormakka M, Quinn RJ, Holst J (2014) Monoterpene glycoside ESK246 from *Pittosporum* targets LAT3 amino acid transport and prostate cancer cell growth. ACS Chem Biol 9:1369–1376

Wen YQ, He F, Zhu BQ, Lan YB, Pan QH, Li CY, Reeves MJ, Wang J (2014) Free and glycosidically bound aroma compounds in cherry (*Prunus avium* L). Food Chem 152:29–36

Williams PJ, Strauss CR, Wilson B, Massy-Westropp RA (1982) Studies on the hydrolysis of *Vitis vinifera* monoterpene precursor compounds and model monoterpene ß-D-glucosides rationalizing the monoterpene composition of grapes. J Agric Food Chem 30:1219–1223

Winterhalter P, Skouroumounis GK (1997) Glycoconjugated aroma compounds: occurrence role and biotechnological transformation. Adv Biochem Eng Biotechnol 55:73–105

Xiao J, Muzashvili TS, Georgiev MI (2014) Advances in the biotechnological glycosylation of valuable flavonoids. Biotechnol Adv 32: 1145–1156

Yamada T, Jinsawa M, Sakata K, Usui T, Watanabe S, Yamagishi M, Ooishi K (1998) Unpleasant odor-inhibiting compositions containing fragrance inclusion compounds and sanitary and pet-care products using them; From Jpn Kokai Tokkyo Koho, JP 10263062 A 19981006

Yang T, Stoopen G, Yalpani N, Vervoort J, de Vos R, Voster A, Verstappen FW, Bouwmeester HJ, Jongsma MA (2011) Metabolic engineering of geranic acid in maize to achieve fungal resistance is compromised by novel glycosylation patterns. Metab Eng 13:414–425

Yang YH, Huang SZ, Han YL, Yuan HY, Gu CS, Zhao YH (2014) Base substitution mutations in uridinediphosphate-dependent glycosyltransferase 76G1 gene of *Stevia rebaudiana* causes the low levels of rebaudioside A: mutations in UGT76G1, a key gene of steviol glycosides synthesis. Plant Physiol Biochem 80:220–225

Yauk YK, Ged C, Wang MY, Matich AJ, Tessarotto L, Cooney JM, Chervin C, Atkinson RG (2014) Manipulation of flavour and aroma compound sequestration and release using a glycosyltransferase with specificity for terpene alcohols. Plant J doi: 10.1111/tpj12634 [Epub ahead of print]

Yonekura-Sakakibara K (2009) Functional genomics of family 1 glycosyltransferases in *Arabidopsis*. Plant Biotechnol 26:267–274

Yonekura-Sakakibara K, Hanada K (2011) An evolutionary view of functional diversity in family 1 glycosyltransferases. Plant J 66: 182–193

Zhu X, Fang ZH (2014) New monoterpene glycosides from the root cortex of *Paeonia suffruticosa* and their potential anti-inflammatory activity. Nat Prod Res 28:301–305