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Abstract A significant number of terpenoid compounds are glycosides with the sugars linked to the active groups. Sometimes, the glycosidic residue is crucial for their activity, but in other cases glycosylation only improves pharmacokinetic parameters. Enzymatic glycosylation of terpenoids is a useful tool due to the high selectivity and the mildness of the reaction conditions, in comparison with chemical methods. Several types of biocatalysts have been used in the enzymatic glycosylation of terpenoids. These include the use of glycosyltransferases, trans-glycosidases, and whole-cell biotransformation systems capable of regenerating the cofactor, such as fungi, bacteria, plant-cell cultures, etc. Many biosynthesized terpenoid glycosides display medicinal and pharmacological properties and can be used as pro-drug substances. These terpenoid glycosides have also been employed as food additives (e.g. low-caloric sweetener compounds) and cosmetics, and even have applications as controlled-release fragrances.

Keywords Biotransformation · Filamentous fungi · Glycosidase · Glycosyltransferase · Plant-cell culture

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Abbreviations

ATCC	American type culture collection
CICC	China center of industrial culture collection
DHA	Dehydroabietic acid
Gal	Galactopyranose
GalT	β-1,4-Galactosyltransferase
GHs	Glycoside hydrolases
Glc	Glucopyranose
GTs	Glycosyltransferases
HeLa	Human epithelial carcinoma cell line
HIV	Human immunodeficiency virus
K562	Human erythromyeloblastoid leukemia cell line
Man	Mannopyranose
NRRL	Agricultural research service culture collection (ARS)
POH	Perillyl alcohol
Rha	Rhamnopyranose
TGs	Trans-glycosidases
UDP	Uridine diphosphate

Introduction

Terpenoids constitute one of the largest groups of natural products, with more than 40,000 structures, many of plant origin (Bohlmann and Keeling 2008). Many terpenoids are essential for plant growth, development, and general metabolism, and these are

found in almost all plant species. The structures of terpenoid compounds usually contain oxygen atoms, such as alcohols, aldehydes, ketones, and carboxylic acids. Because of their many different structures, the terpenoid group includes compounds with many different physical and chemical properties, and often diverse biosynthetic pathways.

Terpenoids display a great number of important biological activities, including cancer chemopreventive, antimicrobial, antifungal, antiviral, anti-hyperglycemic, anti-inflammatory, and anti-parasitic effects (Paduch et al. 2007; Yoo and Park 2012; Kuttan et al. 2011; De las Heras and Hortelano 2009; Grassmann 2005; Akihisa et al. 2003; Sun et al. 2003; Cantrell et al. 2001). These compounds are utilized in many different applications, from medical and pharmaceutical uses to flavouring and scenting food and cosmetics.

A significant number of terpenoid compounds are glycosides with the sugars linked to the active groups (Dembitsky 2006; Brandle and Telmer 2007). In many cases, the glycoside group is essential for the activity of terpenoids, although in others, glycosylation only improves their pharmacokinetic parameters (Kren and Martinkova 2001). While terpenoid glycosides exist as natural products, their levels in plant extracts are often limited, leading to an increasing interest in their synthesis (Gauthier et al. 2009).

Glycosylation is a significant method for the structural modification of compounds with biological activities. This process allows the conversion of lipophilic compounds into hydrophilic ones, thus modifying their pharmacokinetic properties, e.g. their circulation, elimination, and concentrations in body fluids. Glycosides are responsible for functions related to accumulation, storage, and transport of hydrophobic substances. In comparison with their free aglycones, they show a greater solubility in water and, generally, a lesser reactivity, facilitating their storage in the plant's vacuole and protecting the organism from aglycone toxicity (De Roode et al. 2003). Attaching a glycosidic moiety to the molecule increases its hydrophilicity.

Enzymatic glycosylation of complex biologically active substances is a useful tool due to their selectivity and the mildness of the reaction conditions, in comparison with chemical methods, where generally harsh conditions or the use of toxic catalysts are undesirable (Weignerova and Kren 2010; Gantt et al. 2011a; Kren and Thiem 1997). These enzymatic methods also constitute a good alternative in the chemistry of food additives where the use of synthetic chemistry is sometimes unacceptable.

A number of alternative glycosylation tools can be applied in biotransformation strategies. One of them is the use of glycosyltransferases (GTs), which typically employ nucleotide-activated sugars as glycosyl donor (Chang et al. 2011; Lim 2005; Bowles et al. 2005). The stringent specificity of several GTs remains a limiting factor in natural-product glycosylation, but using GT engineering and evolution platforms, the promiscuity of a natural-product GT can be expanded via direct evolution (Williams et al. 2007). Moreover, the use of specific activated aromatic glycosides to drive the equilibria of GT-catalysed reactions has been studied (Gantt et al. 2011b), which represents an improvement in the small-molecule glycodiversification processes. Another alternative is the use of trans-glycosidases (TGs), which are able to synthesise saccharides by transferring a glycosyl group from one carbohydrate chain to another (Desmet and Soetaert 2011). Whole-cell biotransformation systems can also be used, these being capable of regenerating the cofactor, such as fungi, bacteria, plant-cell cultures, etc. (Kren and Thiem 1997).

Glycoside hydrolases (GHs), also called glycosidases, catalyse the transfer of the glycosyl group between oxygen nucleophiles. Such a transfer reaction results in hydrolysis of the β(1-4)-glycosidic bond between the monosaccharide and the terpene aglycone of a glycoside. However, although glycosidases typically degrade their substrate in quantitative yields, they can also be used for glycoconjugate synthesis changing the reaction conditions. Such changes include a high substrate concentration, lower water activity, and an introduction of organic solvent into the reaction environment. The enzymatic glycosylation reaction performed by glycosidases can be kinetically controlled (trans-glycosylation) or thermodynamically controlled (direct glycosylation, reversed hydrolysis). In the trans-glycosylation reaction, higher yields can be reached in a shorter period of time than in the direct glycosylation. Direct glycosylation would be preferred if a high yield can be achieved (Desmet and Soetaert 2011).

Biocatalysis is a powerful tool not only for the regioselective and enantioselective synthesis of bioactive compounds but also for the generation of new,

active, and less toxic metabolites (e.g. glycosides) for the bioactive natural products, since it produces significant quantities of metabolites that would be difficult to obtain either from biological systems or from chemical synthesis (Rasor and Voss 2001).

Concern about the environment and sustainability has resulted in the development of cleaner processes, and much attention is now paid to reusability and recycling. In this context, biotransformation processes that use biological systems to induce chemical changes in synthetic or natural compounds, offer an attractive alternative compared to traditional chemical methods. Biotransformation takes place under mild conditions without generating toxic wastes.

Most published research papers on enzymatic glycosylation of terpenoids focus on certain compounds with a specific terpene skeleton, while only a few of them address the study of different compounds with a varied number of terpene scaffolds (Caputi et al. 2008). This review concentrates on biotransformation of terpenoids to produce glycosylated derivatives with diverse properties.

Monoterpenoids

Glycosylation allows the conversion of water-insoluble and unstable organic compounds into the corresponding water-soluble and stable ones to improve their bio- and pharmacological properties, and thus glycosides of volatile compounds such as monoterpenoid compounds have revealed different biological activities (Dembitsky 2006). The enzymatic synthesis of these compounds is preferred over chemical pathways because few nonspecific by-products are formed and synthesis involves fewer steps.

Geraniol (**1**) plays an important role in the fragrance and flavour industry because its odour typifies the fragrance of roses. Geraniol (**1**) is released by the plant, from the non-odorous and non-volatile glucoside of geraniol (**2**), through a slow hydrolysis of this glucoside. This "controlled-release" of a fragrance has industrial potential. Geranyl glucoside (**2**) was produced enzymatically in a bioreactor from geraniol (**1**) using almond β-glucosidase as a biocatalyst (De Roode et al. 2001) (Fig. 1). The major drawback of this enzymatic glucosylation reaction is the unfavourable equilibrium position that results in a low product yield.

Frequently, β-glycosidases perform hydrolysis reactions of glycoside compounds. Nevertheless, like many other hydrolytic enzymes, changes in the reaction environment lead to the display of biosynthetic activities by these enzymes. Such changes include a high substrate concentration, a decrease in water activity, and an introduction of organic solvent in the reaction environment. It is proposed that synthesis occurs by the reversal of hydrolysis (long incubation periods) or by a trans-glycosylation approach (shorter time periods). The biosynthetic activity of yeast *Pichia etchellsii* β-glucosidase II expressed in recombinant *Escherichia coli* has been used to synthesize several monoterpene glucosides, such as geranyl (**2**) neryl (**3**), and citronellyl (**4**) glucosides (Bachhawat et al. 2004) (Fig. 2).

The monoterpene intermediate (−)-(4*R*)-isopiperitenone (**5**) is found at the branching point leading either to menthol or piperitone in *Mentha piperita* L. (Lamiaceae). There have been attempts to produce major components of peppermint oils through biotransformations of biosynthetic intermediates. The feeding of (−)-(4*R*)-isopiperitenone (**5**) to the cell culture of this plant resulted in 7-hydroxylation (**6**) and subsequent glucosylation (**7**) of the hydroxylated terpene (Park and Kim 1998) (Fig. 3).

Cultured cells of *Eucalyptus perriniana* were able to convert the compounds found in spices, such as thymol (**8**), carvacrol (**9**), and eugenol (**10**), into the corresponding β-glucosides (**11**, **13**, and **15**) and β-gentiobiosides (**12**, **14**, and **16**), which accumulate in the cells (Shimoda et al. 2006) (Fig. 4). The yield of glycosides of thymol (**8**) at 6 h was about 1.5 times

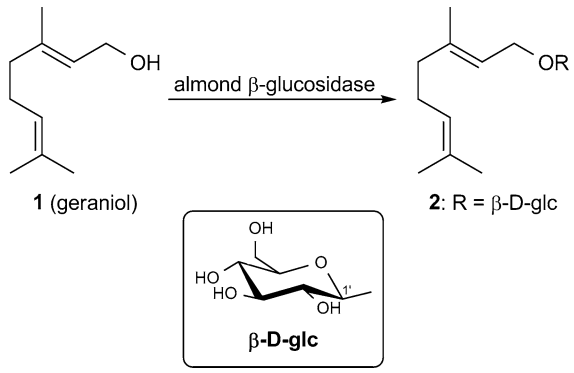


Fig. 1 Glucosylation of geraniol (**1**) with almond β-glucosidase

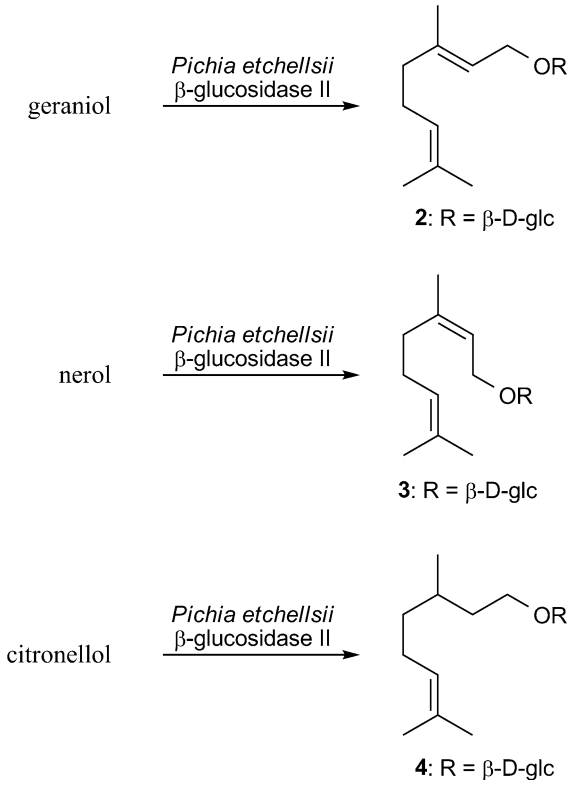


Fig. 2 Formation of geranyl (**2**), neryl (**3**), and citronellyl (**4**) glucosides using β-glucosidase II (*P. etchellsii*)

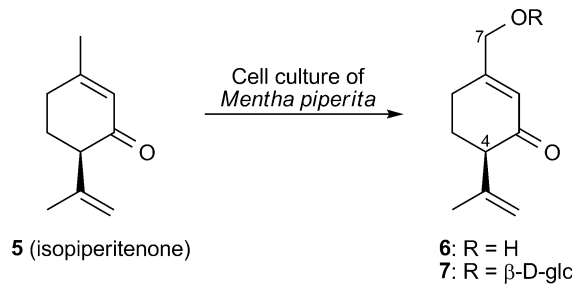


Fig. 3 Biotransformation of (-)-(*4R*)-isopiperitenone (**5**) by a cell culture of *M. piperita*

higher than that of carvacrol (**9**) and about 4 times higher than that of eugenol (**10**). This suggests that glucosyltransferases in the cultured cells of *E. perriniana* have the highest specificity for thymol (**8**) among these substrates. Physiologically, the glycosides of thymol (**8**), carvacrol (**9**), and eugenol (**10**), which are aroma compounds of naturally occurring herbs and spices, can be used in pharmacology as well as for food additives and cosmetics.

The tropolone derivative β-thujaplicin (hinokitiol, **17**) occurs in the heartwood of cupressaceous plants and is used as a medicine, a food additive, and a preservative, and in cosmetics as hair tonic. The cultured plant cells of *Nicotiana tabacum* glycosylated β-thujaplicin (**17**) to two glucosides, 4-isopropyltropolone 2-O-β-D-glucoside (**18**, 6 %) and 6-isopropyltropolone 2-O-β-D-glucoside (**19**, 12 %), and two gentiobiosides, 4-isopropyltropolone 2-O-β-D-gentiobioside (**20**, 2 %) and 6-isopropyltropolone 2-O-β-D-gentiobioside (**21**, 5 %). The use of immobilized cells from *N. tabacum* in sodium alginate gel and a medium without iron ions much improved the yield of these metabolites (Kwon et al. 2008) (Fig. 5).

The monoterpene perillyl alcohol (POH, **22**), an intermediate in the plant terpenoid biosynthetic pathway, has well-established tumour chemopreventive and chemotherapeutic potential. The primary hydroxyl group of POH is essential for its antitumor and anti-angiogenic activities. The enzymatic synthesis of perillyl glucoside (**23**) was performed with D-(+)-glucose using almond β-glucosidase, in a low-water system. The antitumor cell proliferation activity against mouse Lewis lung carcinoma cells was retained in the perillyl glucoside (Xanthakis et al. 2009, 2010) (Fig. 6).

Sesquiterpenoids

(*S*)-(+)-curcuphenol (**24**) and (*S*)-(+)-curcudiol (**25**) are bioactive sesquiterpene phenols isolated from the marine sponges *Didiscus flavus* and *Epipolasis* sp. Curcuphenols display, among other biological activities, potent antifungal activity against *Candida albicans* as well as antitumor activity against several human cancer cell lines. Microbial transformation for 6 days of **24** with *Rhizopus arrhizus* (ATCC 11145) afforded (*S*)-curcuphenol-1α-D-glucopyranoside (**26**). The α-orientation of the new glycosidic linkage was deduced from the small coupling constant of the hydrogen atom situated at C-1 of the sugar group (H-1′). Similarly, *Rhodotorula glutinus* (ATCC 15125) gave (*S*)-curcudiol-1α-D-glucopyranoside (**27**) when incubated for 8 days with **25** (El Sayed et al. 2002) (Fig. 7).

Cyclonerodiol (**28**) is a plant-growth regulatory sesquiterpene, which has been isolated from several fungi. Biotransformation of cyclonerodiol (**28**),

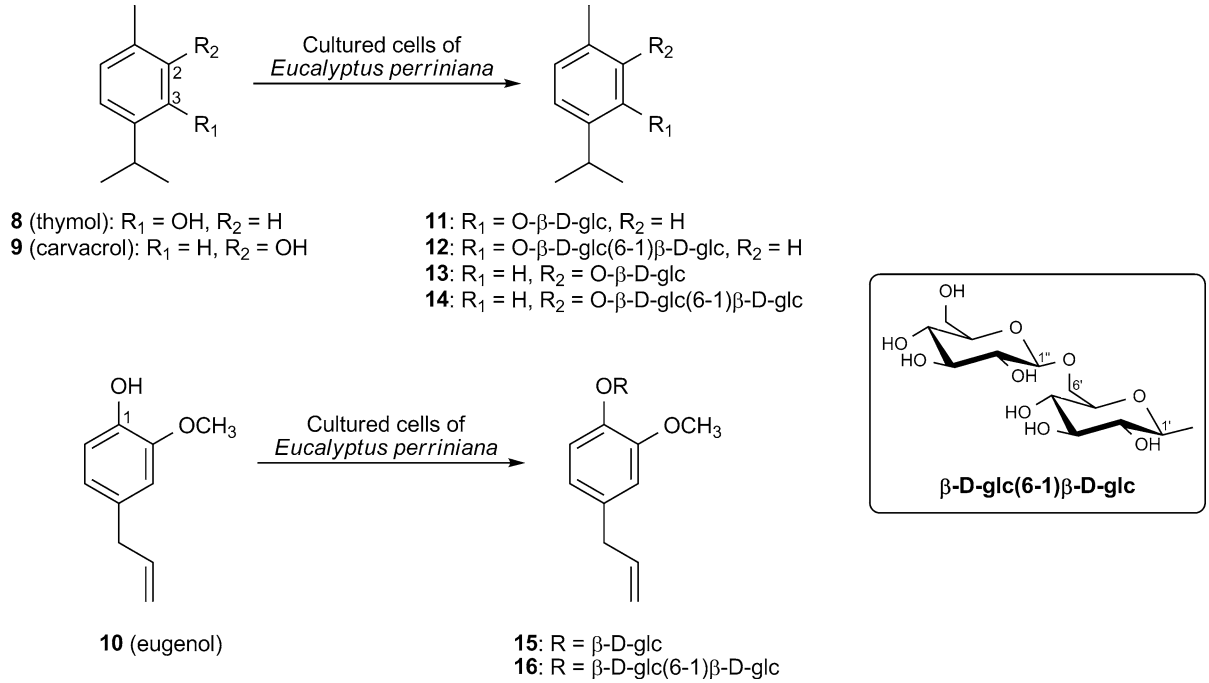


Fig. 4 Formation of glucosides and gentiobiosides of thymol (**8**), carvacrol (**9**), and eugenol (**10**) by a cell culture of *E. perriniana*

isolated from the marine fungus *Myrothecium* sp., was carried out with the marine ascomycete *Penicillium* sp. by using a two-stage fermentation protocol to yield a mannopyranosyl glycoside, 7-O-(β-D-mannopyranosyl)cyclonerodiol (**29**). The stereochemistry of the anomeric position of the sugar moiety was assigned as β-configuration on the basis of the ^{13}C NMR spectroscopic data (Li et al. 2007) (Fig. 8).

Artemisinic acid (**30**) is a cadinane-type sesquiterpene compound that has been proposed to be one of the intermediates in the biosynthetic pathway of artemisinin, a sesquiterpene lactone that possesses very good anti-hepatitis and anti-cancer effects in addition to anti-malarial activity. Artemisinic acid (**30**) was used as a substrate in cultured cells of *Averrhoa carambola* in order to expand the range of artemisinin analogues. A new sesquiterpene glycoside, artemisinic acid 3-β-O-β-D-glucopyranoside (**31**) and other two biotransformation products, 3β-hydroxyartemisinic acid (**32**) and 3β-hydroxyartemisinic acid β-D-glucopyranosyl ester (**33**), were achieved in the biotransformation of artemisinic acid (**30**) by the cultured cells of *A. carambola*. A biosynthetic pathway was proposed in which artemisinic acid (**30**) was first regio- and stereoselectively hydroxylated at C-3 to yield compound **32** and then glycosylated to yield compounds **31** and **33**. Additionally, the anti-tumour activity of metabolites **31** and **33** was evaluated against K562 and HeLa cell lines, showing that compound **33** exhibited stronger anti-tumour activity than compound **31**. The HeLa cell line was more sensitive to the glycosylated compounds than the K562 cell line (Yang et al. 2012) (Fig. 9).

Diterpenoids

Antimicrobial, anti-ulcer, and cardiovascular activities are the most representative for compounds with an abietane diterpenoid skeleton, while others such us allergenic, antiallergic, filmogenic, surfactant, antifeedant activities, have also been reported. Two synthetic abietane diterpenes, isotriptophenolide (**34**) and triptophenolide (**35**) were incubated with the filamentous fungi *Cunninghamella echinulata* and *C. elegans* to obtain novel analogues of these diterpenes. Both species of *Cunninghamella* glucosylated isotriptophenolide (**34**) at C-12 to yield 12-O-β-D-glucopyranosyl-isotriptophenolide (**36**). When triptophenolide (**35**) was incubated with *C. elegans* and *C. echinulata*, among

other metabolites, 14-O-β-D-glucopyranosyl-triptophenolide (**37**) was produced. The major metabolism pathway of isotriptophenolide (**34**) was β-glucosyl conjugation, whereas in triptophenolide (**35**) only 5 % of the starting material was glycosylated (Milanova et al. 1995, 1996) (Fig. 10).

Tripterygium wilfordii has been used in traditional Chinese medicine to treat various diseases including rheumatoid arthritis, nephritis, systemic lupus erythematosus, and skin disorders, as well as in male-fertility control. Triptonide (**38**) is a diterpenoid triepoxide with an abietane skeleton and one of the major active ingredients of *T. wilfordii*, although its biological activity is limited by its strong toxicity. Triptonide (**38**) was chosen for a structural modification by a biotransformation approach to find more effective and less toxic compounds. Triptonide (**38**) was incubated with suspension cells of *Platycodon grandiflorum*, resulting in, among other metabolites, epitriptolide-14-O-β-D-glucoside (**39**) (Ning et al. 2003) (Fig. 10).

Dehydroabietic acid (DHA, **40**) is an abundant resin acid in conifers, constituting a natural wood protectant. It is also one of the constituents found in by-products of the kraft chemical pulping industry. DHA (**40**) was biotransformed by *N. tabacum* (tobacco) and *Catharanthus roseus* (Madagascar periwinkle) cell cultures in various concentrations. The aim of this research was to use whole-cell catalysts as tools for modifying selected resin acids in order to obtain

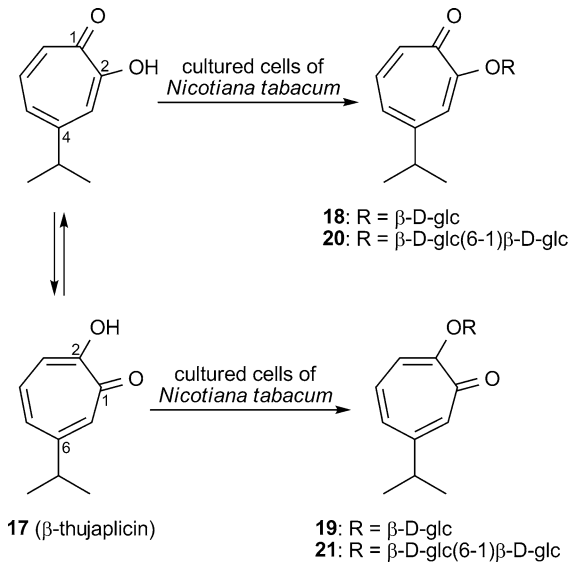


Fig. 5 Glycosylation of β-thujaplicin (**17**) by *N. tabacum*

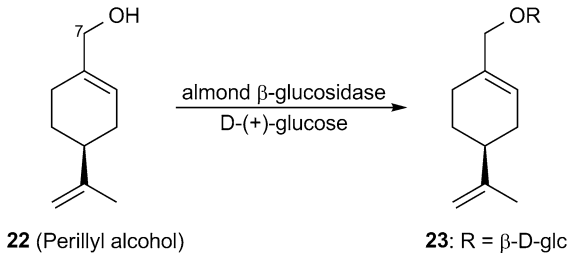


Fig. 6 Enzymatic glycosylation of perillyl alcohol (**22**) with almond β-glucosidase

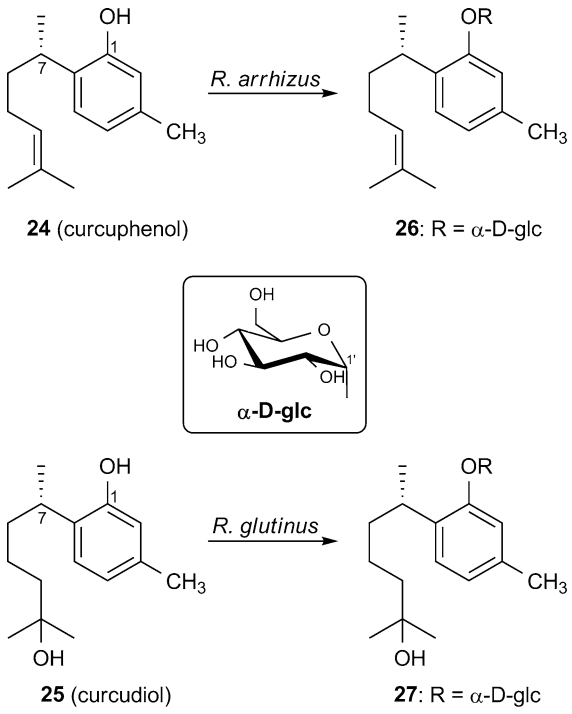


Fig. 7 Biotransformation of (*S*)-(+)-curcuphenol (**24**), with *R. arrhizus*, and (*S*)-(+)-curcudiol (**25**), with *R. glutinus*

value-added functional derivatives. Incubation of DHA (**40**) with *N. tabacum* cell suspension cultures produced DHA-18-O-glucoside (**41**), indicating that DHA was glycosylated at the carboxyl moiety. *C. roseus* cells first converted DHA into a 17-hydroxylated derivative (**42**), and later transformed this metabolite (**42**) into two isomeric 17-O-glucosylated derivatives, possibly consisting of α- and β-configurations of the same structure (**43** and **44**) (Häkkinen et al. 2012) (Fig. 11).

Steviol glycosides, which present intense sweetness, are found in high concentrations in the leaves of the Paraguayan perennial herb *Stevia rebaudiana*.

These glycosides possess valuable biological properties. Stevioside (**45**), a diterpenoid glycoside, is used, either alone or in combination, for sweetening drinks and other foods. Because of a slight bitter residual aftertaste and low solubility, the modification of the sugar moieties of stevioside (**45**) has been investigated to develop new derivatives with improved organoleptic properties and increased solubility. β-1,4-Galactosyltransferase (GalT) from bovine colostrum was used to galactosylate stevioside (**45**) and steviolbioside (**46**) at the hydroxyl group (C-4), of the external glucose of the disaccharide moiety attached at C-13, of the aglycone skeleton. The corresponding galactosyl derivatives **47** and **48** were achieved in good yields and with high regioselectivity. The solubility in water of the galactosyl derivative **47** was higher than that of stevioside (**45**), and also the relative sweetness (Danieli et al. 1997) (Fig. 12). Other studies have been made of enzymatic transglycosylation reactions of steviol glycosides (Tanaka 1997; Abelyan et al. 2004; Kochikyan et al. 2006; Jaitak et al. 2009).

The diterpenoid compound *ent*-16β-hydroxybeyeran-19-oic acid (**49**) has potential antihypertensive activity and can be synthesized from isosteviol. In an effort to obtain novel and more-effective compounds, **49** was incubated with *Bacillus megaterium* ATCC 14581. This microorganism hydroxylated and glucosylated compound **49** to yield *ent*-7α,16β-dihydroxybeyeran-19-oic acid (**50**), *ent*-16β-hydroxybeyeran-19-oic acid α-D-glucopyranosyl ester (**51**), and *ent*-7α,16β-dihydroxybeyeran-19-oic acid α-D-glucopyranosyl ester (**52**). This is the first report of the formation of glycosides by *B. megaterium*, showing that this

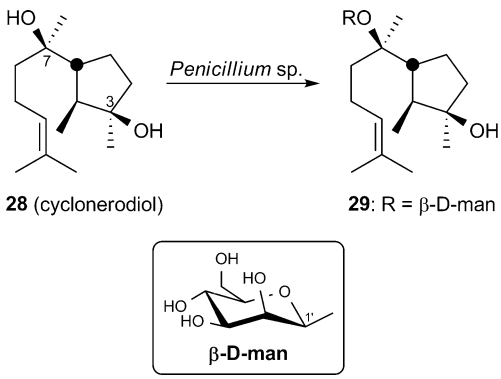


Fig. 8 Formation of a mannopyranosyl glycoside of cyclonerodiol (**28**) by *Penicillium* sp.

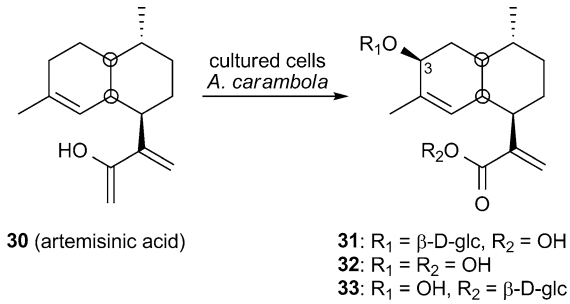


Fig. 9 Biotransformation of artemisinic acid (**30**) by the cultured cells of *A. carambola*

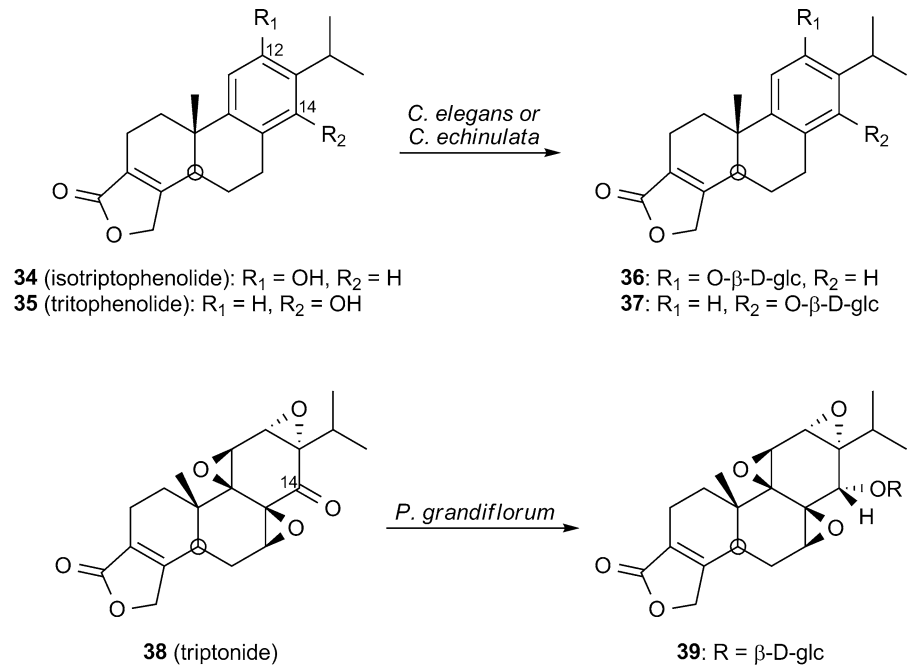
microorganism can catalyse not only hydroxylation but also conjugation (Yang et al. 2004) (Fig. 13).

Triterpenoids

Many biologically active compounds are glycosides, and clear correlations between the presence of specific sugar residues and the biological activity of these molecules have been shown in many cases (Kren and Martinkova 2001).

The lupane-type pentacyclic triterpene betulinic acid (**53**, 3β-hydroxy-lup-20(29)-en-28-oic acid), widely distributed in nature, exhibits a variety of biological activities, including inhibition of human immunodeficiency virus (HIV), replication in H9 lymphocyte cells, blockage of HIV-1 entry into cells, and cytotoxicity against a variety of cultured human tumour cells (e.g. melanoma). Microbial transformation of this compound (**53**) with resting-cell suspensions of *Cunninghamella* sp. NRRL 5695 resulted in the production of a 28-O-β-D-glucopyranosyl derivative (**54**). The in vitro cytotoxicity assay of the new metabolite (**54**) revealed no activity against several human melanoma cell lines (Chatterjee et al. 1999) (Fig. 14).

The water extracts of the dried roots and leaves of *Panax ginseng* have been used as an effective "tonic" in the traditional Chinese medicine for at least some 2000 years. The major active principles of *P. ginseng* extracts are ginsenosides, glycosylated derivatives with a triterpene dammarane aglycone structure. Ginsenoside Rg_1 (**55**, 6,20-di-O-glucopyranosyl-20(*S*)-protopanaxatriol) is one of the most representative ginsenosides from *P. ginseng*, which belong to the protopanaxatriol family. This ginsenoside (**55**) was galactosylated by the action of β-(1,4)-



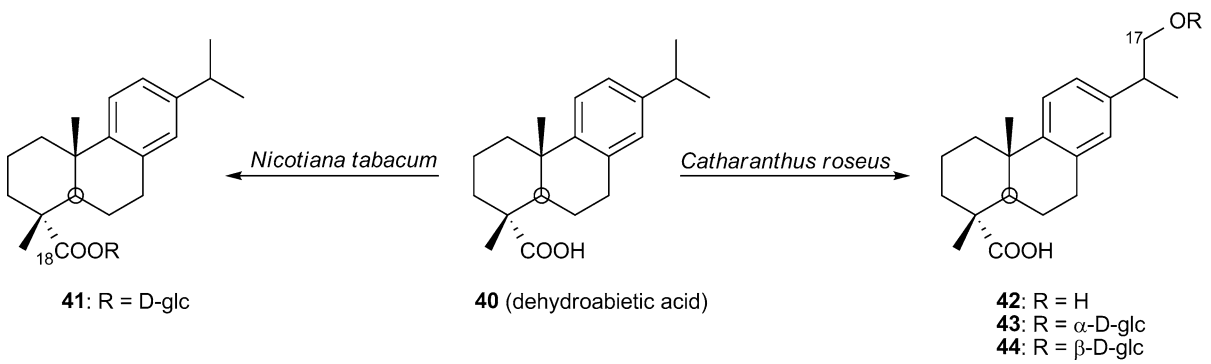


Fig. 10 Biotransformation of isotriptophenolide (**34**) and triptophenolide (**35**) by *C. echinulata* and *C. elegans*. Glycosylation of triptonide (**38**) with a suspension cells of *P. grandiflorum*

Fig. 11 Biotransformation of dehydroabietic acid (**40**) by *N. tabacum* and *C. roseus* cell cultures

galactosyltransferase (GalT) from bovine colostrum, using UDP-galactose as an activated sugar donor. This enzyme (GalT) showed the well-known specificity for the formation of a β-linkage with the hydroxyl group at C-4 of the glucose acceptor, but it was not able to discriminate between the two glucose moieties of **55**, giving a mixture of mono- (**56** and **57**) and digalactosylated (**58**) derivatives. This enzyme (GalT) was also able to accept UDP-glucose as an activated sugar donor, giving rise to cellobiosyl derivatives of Rg_1 (**59** and **60**) (Danieli et al. 2001) (Fig. 15). The two glucopyranosyl moieties of **55** were not similarly recognized by this enzyme, and this different reactivity was confirmed by comparing the galactosylation rates of other natural Rg_1-analogues such as F_1, Rh_1, and Re, as well as the synthetic derivative 6′-O-acetyl-Rg_1.

Asiaticoside (**61**) is a saponin component, isolated from the perennial herb *Centella asiatica*, with an ursane-type triterpene structure that carries a trisaccharide unit containing a α-L-rhamnopyranoside linked to the disaccharide gentiobiose. The selective modification of this carbohydrate unit was attempted by a combined use of glycosidases and GTs. The enzymes produced by *Fusarium oxysporum* were selected for the synthesis of derhamno-asiaticoside (**62**, DeRha-Asia) and also of derhamno-deglucoasiaticoside (**63**, DeRha-DeGlc-Asia), by in situ glucose-inhibition of contaminating β-D-glucosidases.

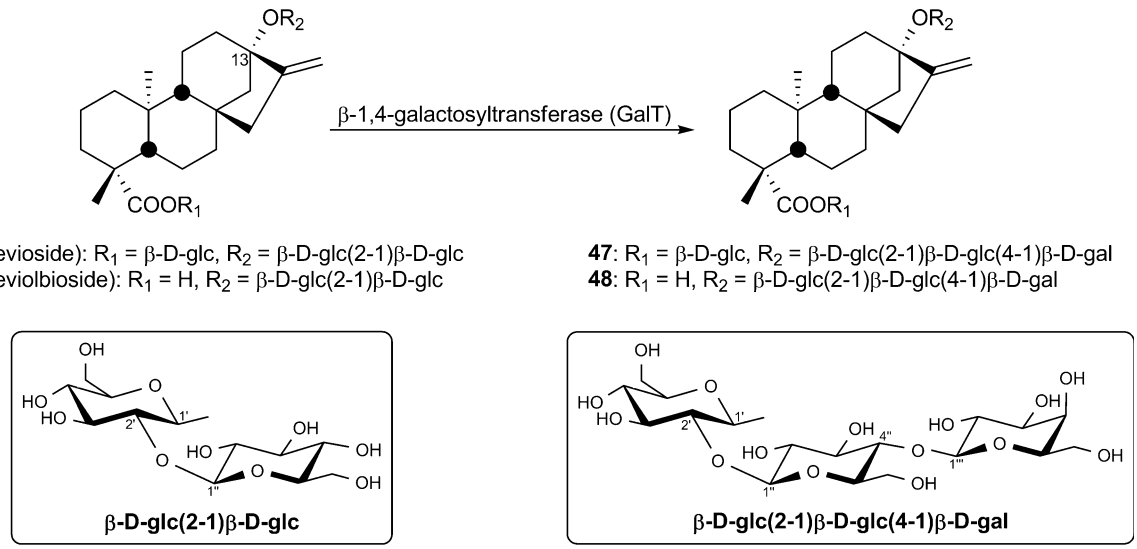


Fig. 12 Enzymatic galactosylation of stevioside (**45**), and steviolbioside (**46**) with β-1,4-galactosyltransferase (GalT) from bovine colostrum

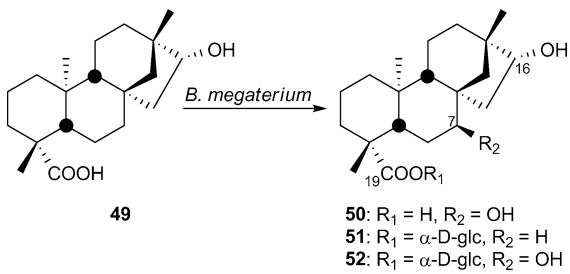


Fig. 13 Biotransformation of *ent*-16β-hydroxybeyeran-19-oic acid by *B. megaterium* (**49**)

The β-1,4-galactosyltransferase (β-GalT) from bovine milk was subsequently used for the galactosylation of the two asiaticoside derivatives **62** and **63**, as these compounds possess a free hydroxyl group at C-4 on their external glucopyranosyl moiety. The corresponding galactosylated derivatives **64** and **65** were isolated (Monti et al. 2005) (Fig. 16).

Oleanan-type pentacyclic triterpenes possess a wide range of pharmacological applications, such as anti-tumour, anti-HIV, anti-microbial, anti-diabetic, and anti-inflammatory activities. In view of the complex skeleton, microbial transformation has been employed as an efficient method to explore more effective and/or less toxic derivatives. Oleanolic acid (**66**), one of most extensively studied pentacyclic triterpenoids, is widely present in food and medicinal plants. A *xylariaceous* fungus isolated from fruits of *Sapindus saponaria* showed the ability to transform oleanolic acid (**66**) into other compounds. The analysis of these products using liquid chromatography and mass spectrometry showed that these biotransformations are mainly monohydroxylation and glycosylation. The position of the glucose unit in the oleanolic acid (**66**) was not clearly determined, but this sugar was probably added to the existing hydroxyl group at C-3 (Amaral et al. 2008). The biotransformation of

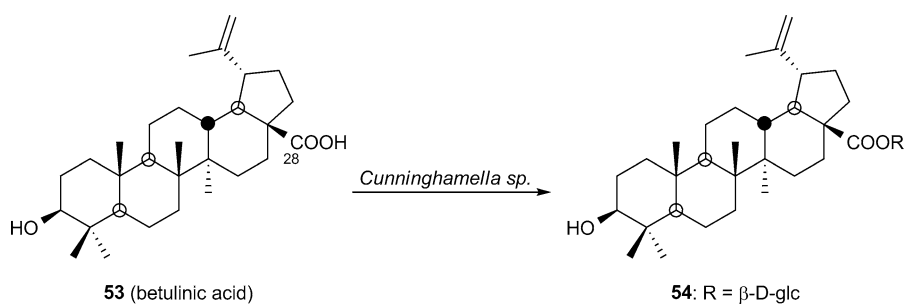


Fig. 14 Enzymatic glycosylation of betulinic acid (**53**) by *Cunninghamella* sp. NRRL 5695

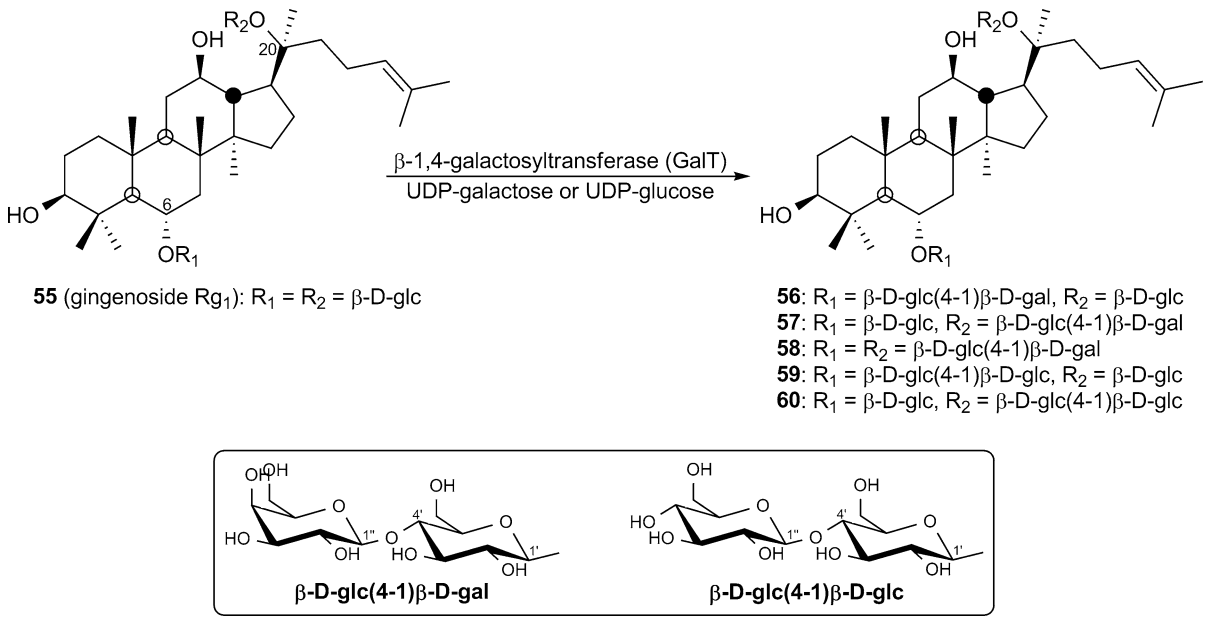


Fig. 15 Enzymatic glycosylations of ginsenoside Rg_1 (**55**) with β-(1,4)-galactosyltransferase (GalT) from bovine colostrum

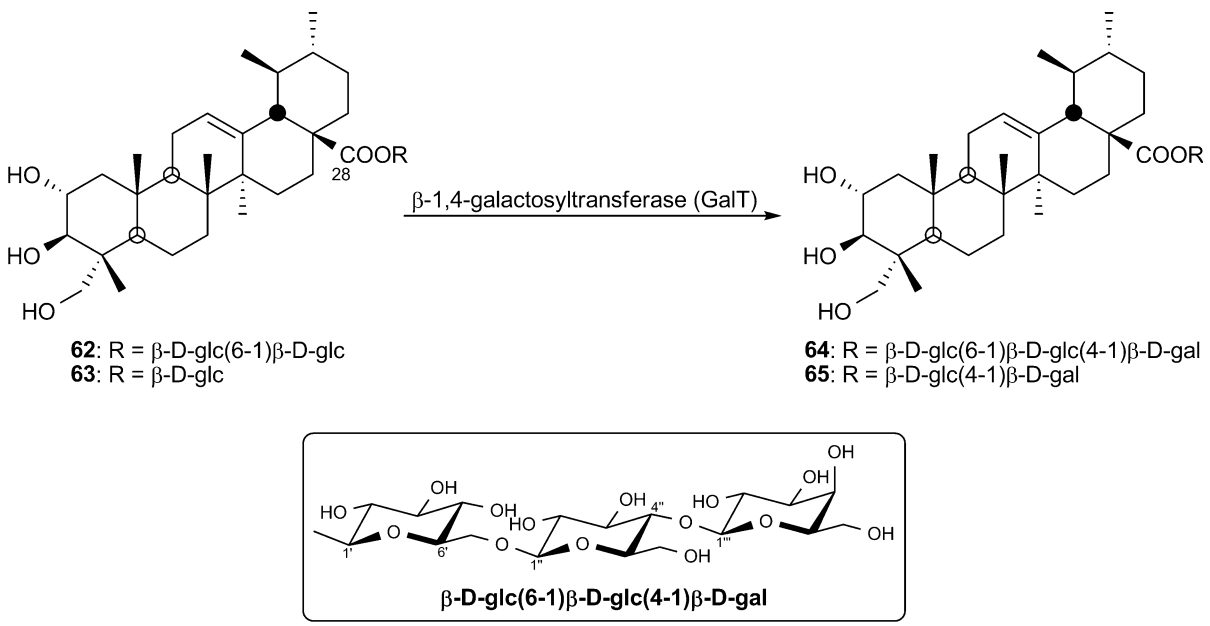


Fig. 16 Galactosylation of asiaticoside derivatives (**62** and **63**) with β-1,4-galactosyl transferase (β-GalT) from bovine milk

oleanolic acid (**66**) by *Alternaria longipes* and *Penicillium adametzi* was performed to find new chemical entities, in order to improve its activities and solubility. This biocatalytic process gave rise to, among other metabolites, several glycosylation products: oleanolic acid 28-O-β-D-glucopyranosyl ester (**67**), oleanolic acid 3-O-β-D-glucopyranoside (**68**), 3-O-(β-D-glucopyranosyl)-oleanolic acid 28-O-β-D-glucopyranoside (**69**), and 21β-hydroxyloleanolic acid 28-O-β-D-glucopyranoside (**70**). Compounds **68** and **70** had stronger cytotoxic activities in vitro against HeLa cell lines than did the substrate (**66**) (Liu et al. 2011).

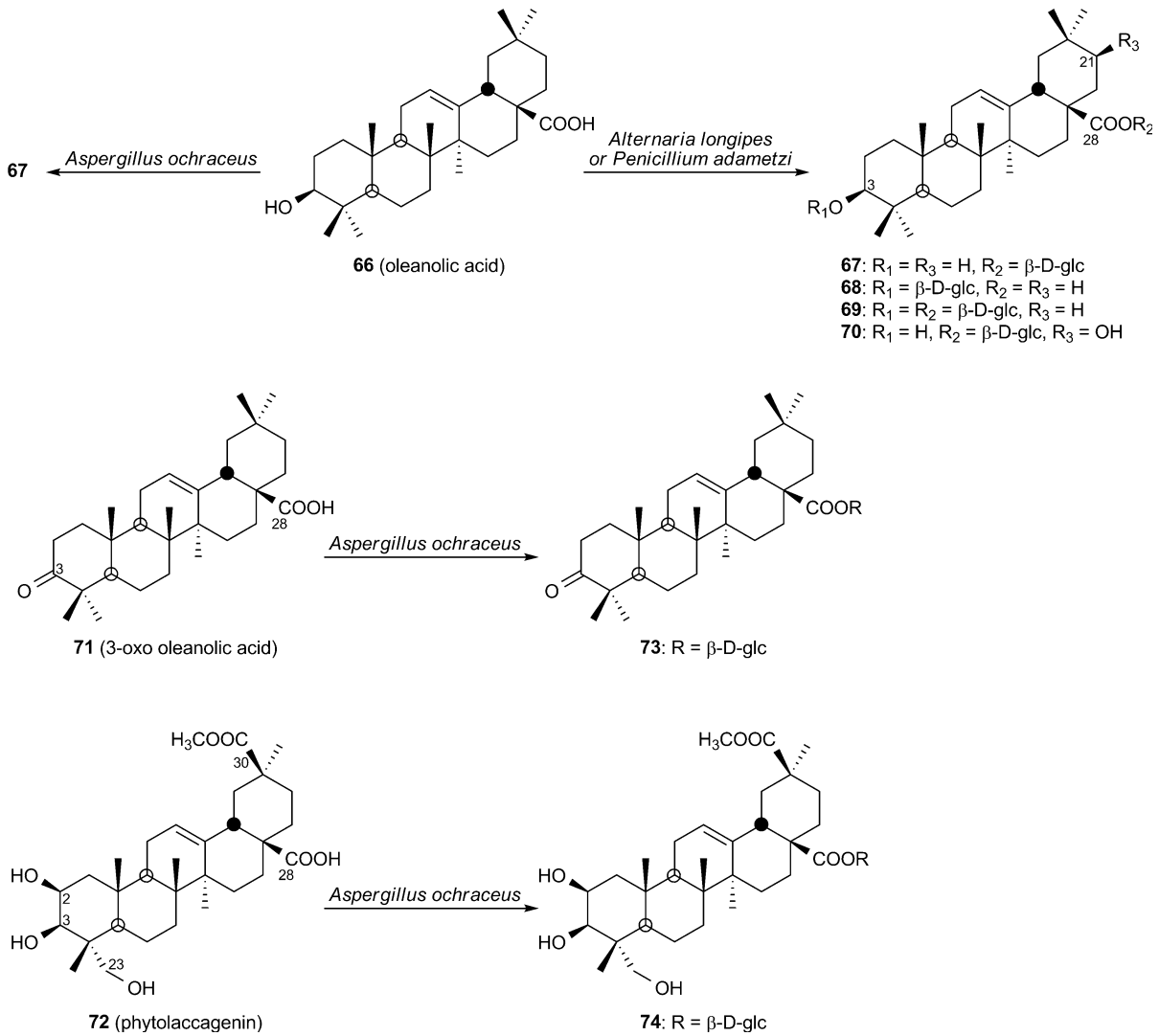


Fig. 17 Biotransformation of oleanolic acid (**66**) by *A. longipes* and *P. adametzi*. Enzymatic glucosylation of oleanolic acid (**66**), 3-oxo-oleanolic acid (**67**), and phytolaccagenin (**68**) by *A. ochraceus*

Oleanolic acid (**66**), 3-oxo-oleanolic acid (**71**), and phytolaccagenin (**72**) were incubated with the fungus *Aspergillus ochraceus* CICC 40330. In all cases, a more polar metabolite, a 28-O-β-D-glucopyranosyl derivative (**67**, **73**, and **74**), was isolated. This highly efficient and regio-selective glucosylation of the carboxyl group at C-28 from *A. ochraceus* will have great advantages over other chemical methods (Zhu et al. 2011) (Fig. 17).

Conclusions

Terpenoid glycosides have one major advantage over other derivatives, as they carry a physiologically acceptable glycosyl moiety that can be cleaved in most tissues by the glycosidases present and further metabolized in a natural way without generating any harmful metabolites. The pharmacological applicability of the terpenoid glycosides is improved by better solubility, facilitated transport and improved selective binding to the target tissues. In many cases, terpenoid glycosylation lowers toxicity without substantially compromising the respective activity. Therefore, glycosylation is an excellent method for pro-drug preparation.

Enzymes and whole-cell biotransformation systems are able to modify xenobiotic terpenoid compounds.

These biological systems carry out the microbial transformation processes following a typical detoxification pattern. The mechanism of this detoxification pattern can be divided into two phases: (a) functionalization (phase I), mainly the introduction of a hydroxyl group or other functional groups into the terpenoid molecule; and (b) conjugation (phase II), generally to form conjugates with a monosaccharide and the terpenoid compound (glycosides).

Enzymatic glycosylation methods in comparison with chemical methods are especially useful, not only because of the high selectivity of the enzymatic processes, but also by the mildness of the reaction conditions and compliance with food and drug regulations. The main disadvantages of chemical glycosylation include the need for protection and deprotection steps of the substrates and products, and the use of toxic catalysts and solvents. However, reasonable yields can be achieved using these methods.

With respect to the enzymatic glycosylation, GTs comprise a wide range of specificities, although their use is hampered by the high price of their nucleotide-activated donors. Consequently, they have been used mainly for the synthesis of glycosides with therapeutic properties. Trans-glycosidases (TGs) are biocatalysts of great interest but their applications are limited by the small number of specificities available and the use of sugar donors that are sometimes expensive. TGs are basically retaining glycosidases that are able to avoid water as an acceptor in the deglycosylation step. The major drawback of the glycosidases is the unfavourable equilibrium position that results in a low product yield. However, improvement of the product yield is possible by "fine-tuning" the reaction conditions, such as working at high substrate concentrations, working at low water activities, or selectively removing the product.

One of the most important research lines for the enzymatic glycosylation of terpenoids is the development of processes that increasingly require green chemistry, such as fine chemicals, flavours, fragrances, and food additives.

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