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REVIEW

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Recent biotechnological progress in enzymatic synthesis of glycosides

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Abstract Glycosylation is one of the most important post-modification processes of small molecules and enables the parent molecule to have increased solubility, stability, and bioactivity. Enzyme-based glycosylation has achieved significant progress due to advances in protein engineering, DNA recombinant techniques, exploitation of biosynthetic gene clusters of natural products, and computer-based modeling programs. Our report summarizes glycosylation data that have been published within the past five years to provide an overall review of current progress. We also present the future trends and perspectives for glycosylation.

Keywords Glycosylation · Enzyme · Protein engineering · Natural product · Glycosyltransferase · Glycosidase

Introduction

Biocatalysts for enzymatic synthesis of small glycosides

Glycosides include several important classes of glycosylated bioactive compound derivatives of hormones, alkaloids, flavonoids, antibiotics, and sweeteners. Attachment of a sugar moiety to the respective aglycon leads to a dramatic change in activity of parent molecules that is either crucial for their physiochemical properties or that enhances pharmacokinetic parameters. In particular, glycosides are more water soluble than the corresponding aglycons and increasing hydrophilicity results in increased activity and ease of passage through the cell membrane.

Glycosidases

Glycosidases (EC 3.2.1.-) are a group of biologically important carbohydrate-processing enzymes that catalyze the hydrolysis of glycosidic bonds to release an oligosaccharide and a monosaccharide. These enzymes are widely distributed in organisms such as archaea, bacteria, fungi, animals, and plants. Glycosidases play essential roles in many processes such as carbohydrate-degraded amylase, cellulose, or glucoamylase; in pathogenetic mechanisms such as with neuraminidases, or as anti-bacterial agents in lysozymes [31, 53].

Glycosidases can be classified by their action on substrates such as endo- or exo-enzymes. However, the sequence-based classification is the most popular and applicable method in which about 132 families have been identified using sequence and folding similarities as well as three-dimensional structure (www.CAZy.com). This approach is advantageous, as it provides the relationship between structure and function of the enzyme, reveals the evolutionary relationships between enzymes, and predicts the mechanism of action of newly isolated glycosidases. For example, a new cellulase in *Armillaria gemina* has been identified as endo β-1,4-glucanase or a member of glycosidase 61 and has been investigated for converting lignocellulosic biomass to biofuels and chemicals [57].

Glycosidases catalyze three types of reactions including hydrolysis, reverse hydrolysis and trans-glycosylation [5, 147], and glycosides are the products of the latter two reactions. Reserved hydrolysis is a thermodynamically-controlled

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reaction in which the equilibrium is shifted towards synthesis of a glycoside from a carbohydrate and an alcohol by addition of co-solvent to reduce water activity, enhancing the substrate concentrations and the products of reaction being taken out simultaneously, if possible [49, 127]. The rate of the reaction increases if water is removed from the system. Trans-glycosylation is a kinetically controlled approach in which the enzyme transfers a glycosyl residue from the donor to acceptor, and a (mono)saccharide is the leaving group.

Mechanism of glycosidases

Glycosidases catalyze via two mechanisms in which the configuration of the anomeric carbon of the product is inverted ($\alpha \rightarrow \beta$ or $\beta \rightarrow \alpha$) or is retained ($\alpha \rightarrow \alpha$ or $\beta \rightarrow \beta$), namely, an inversion and retention mechanism, respectively. The typical retaining glycosidases require acid/base and nucleophilic residues and catalyze the reaction via a double-displacement mechanism. But, inverting enzymes only require a catalytic acid and base residues, and the reaction proceeds though single-displacement. Both mechanisms involve oxacarbenium-ion-like transition states and a pair of carboxylic acids at the active site [132] (Fig. 1). Several glycosidase families do not follow these classic mechanisms, as they lack typical catalytic carboxylate base/nucleophile residues [27, 48]. A variety of alternative mechanisms function such as substrate-assisted catalysis, which is a network of several residues or exogenous nucleophiles [167].

Glycosyltransferases

Glycosylation is usually carried out by glycosyltransferases (GTs) (EC 2.4.1.-), which transfer glycosidic residues from NDP-sugar to the respective aglycon. Aglycons are highly diverse and include carbohydrates, lipids, steroids, polyphenols, and proteins. The GTs are classified as GT-A and GT-B using the Rossmann fold, a classic structural motif of the nucleotide-binding domain as a basic unit to identify structure. Despite containing a similar active-site domain, two of these folds have no significant sequence identity [90]. The GT-A includes a central β-sheet surrounded by α-helices and form a type of β/α/β combination, similar to the Rossmann fold. The spsA (nucleotide-diphospho-sugar transferase) enzyme from *Bacillus subtilis* is a typical example of this class [16]. The GT-B fold consists of two separate Rossmann-like fold domains. A linker connects two such domains and a catalytic site is located between the two less tightly associated domains. In this enzyme class, the N-terminal domain contains a binding site for the acceptor, while the nucleotide binding site is believed to be located in the C-terminal part [30, 181]. In addition, a third enzyme fold named the GT-C superfamily has been proposed based on a BLAST iterative sequence analysis. This class is characterized by a modified DxD signature in the first extracellular loop and includes integral membrane glycosyltransferases [90, 95].

Glycosyltransferase mechanism

The GT-A and the GT-B are also classified as inverting and retaining enzymes with mechanisms similar to those of glycosidases. Almost all predicted GT-C enzymes are in the inverted glycosyltransferase family [90]. These enzymes are usually metal ion-dependent, with metals such as magnesium (Mg^{2+}) or manganese (Mn^{+2}) found in the active site and acting as a Lewis acid by binding to the (di) phosphate leaving group. Inverting GTs catalyze via a single displacement mechanism with nucleophilic attack by the acceptor at the anomeric carbon (C1) of the sugar (Fig. 2).

Several typical methods for biological synthesis of glycosides

High-throughput screening for directed evolution of GTs

A cell-based assay for ST (sialyltransferases) activity was developed using fluorescence-activated cell sorting. Product formation is detected by direct correlation to cell fluorescence using a designed fluorescently-labeled sugar and selectively trapping the sialylated fluorescent product in the cell. Based on this method, Aharoni et al. [1] reported screening a library of $>10^6$ ST mutants and found a variant with extremely high activity (400-fold increase) to a variety of labeled sugars, including thiosugars, resulting in metabolically stable glycosides. A modification of this method was introduced using a two-color screening protocol to minimize the probability of false-positive mutants and to successfully apply β-1,3-galactosyltransferase CgtB for synthesis of important therapeutic glycosphingolipids or asialo G(M1) oligosaccharides as variants. This method is promising for screening almost all GT activities and promotes engineering of glycosyltransferases [183].

In vivo glyco-randomization of small molecules

Escherichia coli and *Streptomyces* are often used as hosts for metabolic engineering of a biosynthetic pathway and as recombinant plasmids harboring genes of interest for glycosylation of small molecules and drugs. Two prototype *E. coli* strains, containing a versatile GT (OleD-ASP, TDP16) and endogenous sugar gene cassettes, were prepared for producing the respective glycosides. Furthermore, diversification of the host-based sugar biosynthetic pathway using promiscuous anomeric kinase (GalK

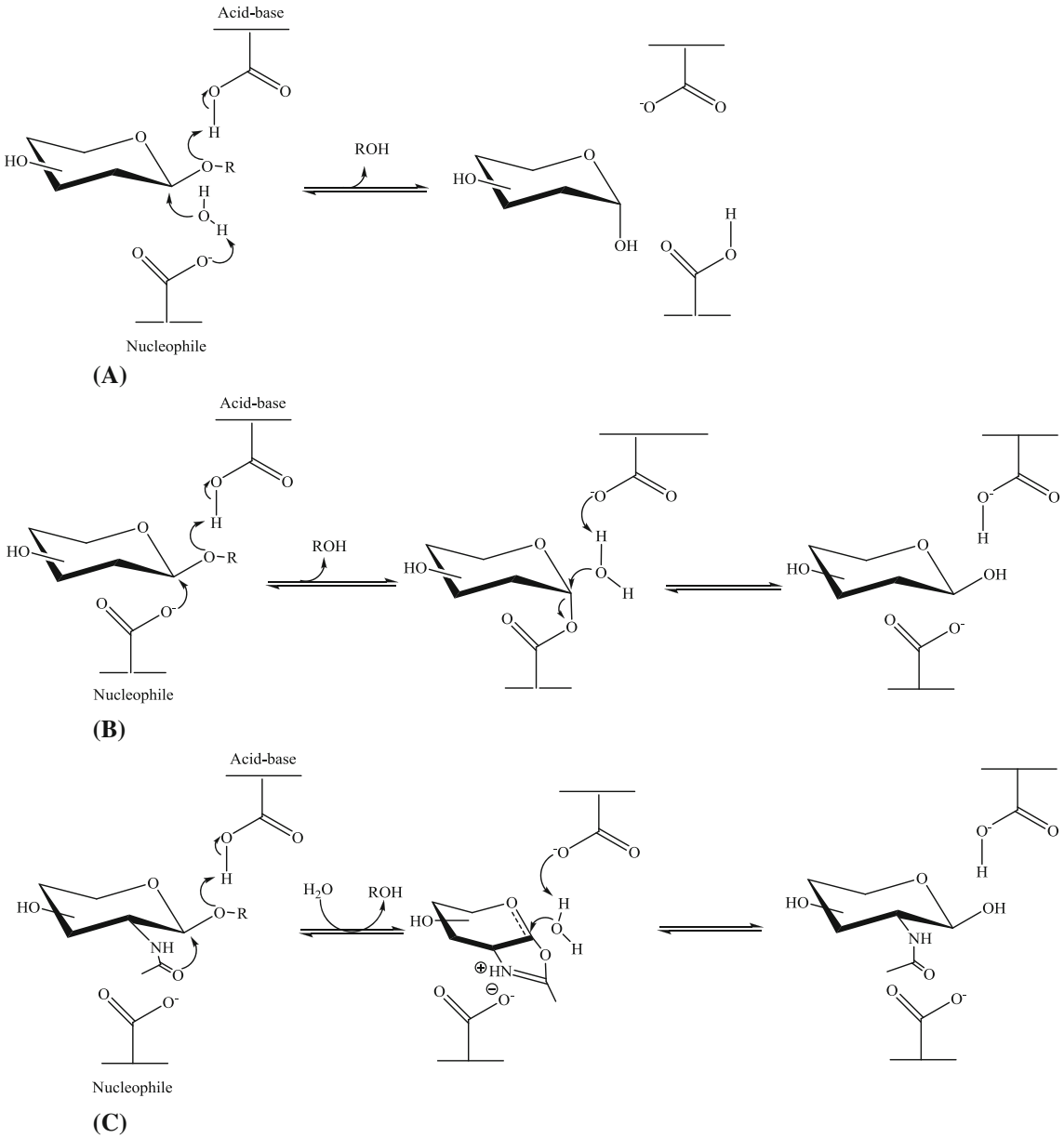


Fig. 1 Mechanisms of different types of glycosidases. **a** Inverting (β → α), **b** retaining (β → β) and **c** modified retaining (double-displacement) or substrate-assisted catalysis, for example, β-*N*-acetylhexosaminidases [8]

M173L/Y371H), engineered flexible nucleotidyltransferase (RmlA L89T), and GT–TDP16 resulted in a prototype *E. coli* host that can produce a series of novel glycosides by feeding exogenous aglycon such as flavonoids, aminocoumarin, or polyene [176]. Another typical example of using *Streptomyces* as a host was reported by Yoon et al. A *S. venezuelae* mutant carrying a plasmid containing different deoxysugar gene cassettes, a flexible GT–AknS and AvrE, efficiently supported biosynthesis of TDP-4-epi-L-daunosamine, and was used for improved production of epirubicin. Furthermore, seven novel rhodomycin D molecules were isolated from a series of products by inserting a variety of deoxysugar gene cassettes into the host. These exemplify the power of combinatorial biosynthesis to generate new small molecules as well as drug glycosides [43]. Similar studies can be found for macropolyketide [44, 45] and anthracycline-like polyketides [109].

Development of highly organic tolerant glycosidases

An enzyme has been isolated from a hydrophilic organic solvent-tolerant organism, *Arthrobacter nicotianae XM6*. As all flavonoids can be dissolved in DMSO up to a DMSO concentration of 25 %, tolerant enzymes exhibit high catalyst activity for glycosylation of flavones, glucosides, and puerarin both in vitro and in vivo to produce puerarin di- and

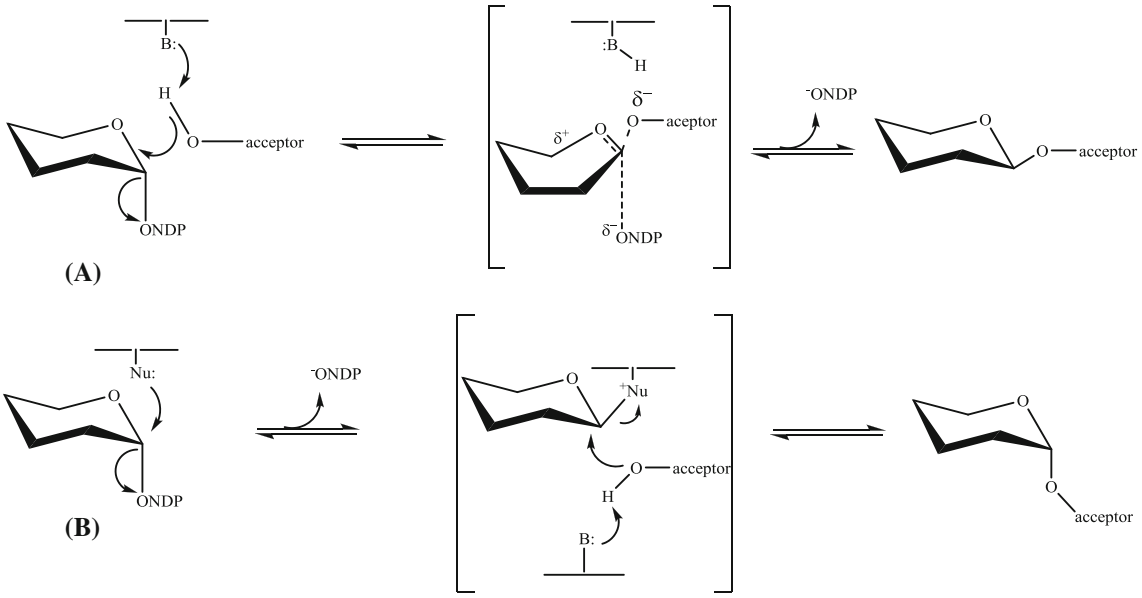


Fig. 2 Reaction mechanism of glycosyltransferases. **a** Invertion. Inverting GTs-1: general base (B)-catalyzed S_N2 attack at C1 of the NDP-sugar donor by the acceptor forms an oxocarbenium-type transition state leading to the inversion of stereochemistry at the anomeric carbon. **b** Retention. Retaining GTs require the double-displacement mechanism in which the S_N2 attack of an active site nucleophile (*Nu*) at C1 of the NDP-sugar to form a covalent sugar–enzyme intermediate, followed by an S_N2 attack of the acceptor at C1 to form the glycosidic linkage with retention of anomeric configuration [100]

tri-glucosides [180]. A similar approach to enzyme development can be used as a catalyst for quercetin [172] or alkyl [129].

In vitro glycol randomization and high-throughput screening

Some GTs catalyze not only the glycosylation of substrates but also perform a reversible reaction to produce NDP-sugars and aglycons. In an effort to drive the equilibrium GT-catalyzed reaction, Thorson et al., synthesized a series of simple glycosides as donors for promiscuous glycosyltransferase OleD mutants. Using high throughput screening, they found the most active glycoside donor and respective OleD mutant, and the reaction was indicated by a colorimetric assay. By combining a reversible reaction to produce an NDP-sugar (OleD mutant 1) and a glycosidic donor for glycosylation of other substrates (in the presence of OleD mutant 2), they succeeded in synthesizing the un-natural glycosides [35].

Synthesis of glycosides by engineered glycosidase via transglycosylation

Enzymatic glycosylation of a nucleoside analog using glycosidase has a long history and has gained much interest. For example, *E. coli* β-galactosidase, using galactose as a sugar donor to galactosylate nucleosides and acrylic nucleoside analogues, follows a two step reaction. First, the glycosidic bond of galactose is cleared by the enzyme, and an enzyme-galactose complex forms. Second, the hydroxyl group of the acceptor molecule breaks the complex resulting in the formation of new glycosides and leaves the enzyme active site. This mechanism leads to retention of the glycosidic configuration, so the product is always in the β-configuration [7]. Trans-glycosylation activity of *Serratia proteamaculans* chitinase D improved significantly through a point mutation. Various mutants such as M226A, Y228A, F125A, S116G, F64W, G119S, R284A, and W247A showed almost double the concentration of transglycosylation products such as chitopentaose and chitohexaose in comparison to that of the parent molecule using chitotetraose as the substrate [99]. Similarly, *Thermotoga neapolitana* β-glucosidase activity towards arbutin glycosylation was upgraded by site-directed mutagenesis with the N291T and N219T/F412S variants compared with that of the wild-type enzyme. The N291T shows replaced regioselectivity as well as an increase in the trans-glycosylation product [23]. These data reveal that Asn-291 is highly involved in the catalytic mechanism by controlling the transglycosylation reaction.

Recent progress in biological synthesis of small molecule glycosides

Flavonoids and stilbenoid glycosides

Flavonoids are ubiquitous secondary metabolites in vascular plants. Most flavonoids exist in a glycosylated form

in which they show more stability, bioactivity, and solubility than those of their aglycons [88]. Glycosylation of flavonoids occurs at the hydroxyl group or directly on the carbon atom of the skeleton. Enzymes are responsible for the glycosylation of flavonoids including GTs isolated from plants (*Arabidopsis and Withania*) or bacteria (*Streptomyces, Bacillus, and Streptococcus*) or transglycosylation by glycosidases [21, 103, 143, 144, 153]. Different types of activated sugars such as NDP-glucose, NDP-xylose, NDP-glucuronic acid, NDP-galactose, NDP-rhamnose, or NDP-6-deoxy-allose are recruited as donors for glyco-diversification of glycosylated flavonoid derivatives. These compounds are well-known anti-cancer, anti-bacterial, anti-inflammatory, antioxidant, and neuroprotective inhibiting agents that have been studied extensively. Structure–activity relationship studies have discovered many interesting and valuable properties of flavonoids. This is exemplified by the inhibitory effects of 14 selected flavonoids with various structures on the activity of purified bovine liver cytochrome b5 reductase. The data revealed that a number of hydroxyl groups in ring B and the carbonyl group at C-4 accompanied with the presence of a double bond enhance activity of the compounds, but substituting a hydroxyl group at C-3 might reduce its inhibitory effect [11]. Two glycosides such as isoquercitrin and hyperin show better protective activity against glycation-associated diseases than that of their aglycon derivatives [73]. Due to structural properties, flavonoids are soluble in strong organic solvents such as acetone, methanol, or DMSO, but they have limited water solubility, which limits travel of flavonoids through cell membranes as well as oral biotransformation. In addition, flavonoid glycosides are more stable and bioavailable [87]. Commercial glycosylated flavonoids are available, but they remain very expensive. The main reason is the low-yield of particular products and the time involved with plant-based chemical extraction. Enzymatic production has advantages such as high regio-specificity and simple optimization for culture and has become the primary approach to produce flavonoid glycosides [72, 115]. In addition, applications of advanced protein engineering and DNA manipulation techniques such as combinatorial biosynthesis and metabolic engineering allow not only enhanced yields of value-added compounds but the generation of new high-value products. Our group has successfully synthesized several types of glycosides such as flavonol glycosides [115, 143, 144, 162], flavone glucosides [163], and phloretin glucosides [116], and their bioactivities are being tested (data not shown). Recent information on synthesis of flavonoids and glycosylation is provided in Table 1 and Figs. 3, 4.

Stilbenoids are hydroxylated derivatives of stilbene, and their biosynthetic pathway is similar to that of chalcones [134, 148, 164]. These compounds are widely distributed in plants and bacteria, and they play critical roles as antioxidants [13], inhibit the growth of human colorectal cancer cells [117], activate the immune system [60] or inhibit viral neuraminidases [110]. But, stilbenoid compounds such as resveratrol are less water soluble, limiting their application in the food processing and pharmaceutical industries. Glycosylation of stilbenoids is a way to change the properties of the aglycon such as solubility, permeation, and bio-availability due to the presence of a sugar moiety. This is exemplified by resveratrol glucuronoides and sulfates as major metabolites of human cell metabolism [168, 173]. Trans-resveratrol-3-*O*-D-glycoside (piceid) exhibits higher scavenging activity against hydroxyl radicals than that of resveratrol in vitro [151] (Fig. 5).

Resveratrol is glycosylated by glucosidase via trans-glycosylation or GTs. Plou et al., reported synthesis of a series of α-glucosyl derivatives of resveratrol (3,5,4′-trihydroxystilbene) by a trans-glycosylation reaction mediated by the cyclodextrin glucanotransferase (CGTase) using starch as the glucosyl donor. Three groups of glucosylated products were obtained at position 3-OH, 4′-OH, and at both 3-OH and 4′-OH [159]. In addition, two trans-resveratrol-*O*-β-glucoside products including 4′-*O*-β-glucoside and 3-*O*-β-glucoside were regiospecifically synthesized by a glucosyltransferase (PaGT3) of *Phytolacca americana* (PaGT3) and expressed in *E. coli*. Molecular modeling analysis and mutagenesis of this protein revealed that the His-20 residue might perform a catalytically essential function [113]. Furthermore, those authors also found that the PaGT2 enzyme is a PaGT3 isoenzyme and both were successfully used to catalyzed stereo- and regio-selective monoglucosylation of 3,5,3′,4′-tetrahydroxy-*trans*-stilbene to yield 3,5,3′,4′-tetrahydroxy-*trans*-stilbene-4′-*O*-β-D-glucopyranoside [55]. Alternatively, glycosylated *trans*-resveratrol is produced by plant-culture cells of *Catharanthus roseus, Strophanthus gratus*, and *Ipomoea batatas* [54].

Ginsenosides

Ginsenosides consist of a dammarane core structure with sugar moieties attached by a glycosidic bond at C-3 and C-6 of the skeleton. Several typical sugars occur in ginsenosides such as glucose, arabinose, and galactose (Fig. 6). These compounds are the main components in ginseng, and more than 180 types of naturally ginsenosides are known [25]). The ginseng root is a very valuable herb (*Panax gingseng*, Chinese medicine) due to its ginsenoside content, which possesses many physiological and pharmacological activities such as anti-cancer, anti-inflammatory and anti-diabetic effects [19, 22]. Ginsenosides are divided into two major groups including 20(*S*)-protopanaxadiol and 20(*S*)-protopanaxatriol. Several high-content

Table 1 Enzymatic synthesis of flavonoid- and stilbenoid glycosides

Enzyme	Gene size (bp)	Strains	Acceptor/donor	Products	MW (kDa)	pH	Tem (°C)	Yield of product (mg/L) or conversion rate (%)	Synthetic methods	References
Dextransucrase		*Leuconostocmesenteroides* B-512FMCM	Kaempferol/sucrose	Kaempferol-3-*O*-β-D-nigeroside, kaempferol-3-*O*-β-D-isomaltoside		5.2	28	21.8 mg	In vitro	[71]
Dextransucrase	8,511	*Leuconostoc mesenteroides* B-1299CB4	Ampelopsi/sucrose	Ampelopsin-4′-*O*-α-D-glucopyranoside	313.3	5.2	28	3,400 mg	In vitro	[179]
Dextransucrase (LLDexT, 512FDexT, SMDexT)	4,503	*Leuconostoc lactis* EG001, *Leuconostoc mesenteroides*B-512F DexT, *Streptococcus* mutans DexT (SMDexT)	Puerarin/sucrose	α-D-glucosyl (1 → 6)-puerarin, α-D-isomaltosyl (1 → 6)-puerarin	165	5.0–5.2	28–30	14,500 mg	In vitro	[78, 83]
α-Amylase	1,389	*Trichoderma viride* JCM22452	(+)-catechin and (−)-epigallocatechingallate (EGCG)/dextrin	s (+)-catechin 5-*O*-α-D-glucopyranoside, (+)-catechin 5-α-D-maltoside, (+)-catechin 4′-*O*-α-D-maltoside, EGCG 5-*O*-α-D-glucopyranoside, EGCG 7-*O*-α-D-maltoside		5.0	45–55		In vitro	[107]
UGT78D1	1,362	*Arabidopsis thaliana*	Quercetin, kaempferol/TDP-rhamnose	Kaempferol 3-*O*-rhamnoside (1), quercetin 3-*O*-rhamnoside (2)			30	150 mg for (2) and 200 mg for (1)	In vivo	[69]
UGT78D2	1,383	*Arabidopsis thaliana*	Quercetin/UDP-*N*-acetylglucosamine	Quercetin 3-*O*-*N*-acetylglucosamine			30	380 mg	In vivo	[70]
UGT78D1		*Arabidopsis thaliana*	Quercetin/dTDP-6-deoxytalose	Quercetin-3-*O*-6-deoxytaloside			30	98 mg	In vivo	[184]
YijC	1,179	*Bacillus lincheniformis*	Phloretin/UDP-glucose	Phloretin 4′,4-*O*-diglucoside (1), phloretin 4,6′-*O*-diglucoside (2) and phloretin 2′,4′,4-*O*-triglucoside (3)	43.67	7.5	25	23.3 %	In vitro & in vivo	[116]
OleD	1,248	*Streptomyces antibioticus*	Daidzein, flavopiridol, resveratrol, 10-hydroxycamptothecin, 2-methoxyestradiol/UDP-glucose	Flavopiridol-(1), 2-methoxyestradiol-(2), resveratrol-(3), daidzein-(4), 10-hydroxycamptothecin (5) glucosides		8.0	25	4.9 mg of (1); 1.2–2.2 mg of (3); 6 mg of (5); 0.1–4 mg of (2); 1–5 mg of (4) in died form	In vitro	[189]
UGT78D1 (*), UGT89C1 (**)	1,308 for (*); 1,362 for (**)	*Arabidopsis thaliana*	Quercetin/UDP-glucose, UDP-rhamnose	Quercetin 3-*O*-glucoside-7-*O*-rhamnoside (**1**), quercetin 3,7-*O*-bisrhamnoside (**2**)			30	67 mg/L of (**1**); 67.4 mg/L of (**2**)	In vivo	[72]
*Ar*GT3		*Arabidopsis thaliana*	Quercetin/UDP-xylose	Quercetin-3-*O*-xyloside					In vivo	[114]
Maltosyltransferase	2,084	*Caldicellulosiruptor bescii* DSM 6725	Piceid/maltose	D-maltosyl-(α-1,4)-piceid	80	6.0	70	17,200 mg	In vitro	[119]

Table 1 continued

Enzyme	Gene size (bp)	Strains	Acceptor/donor	Products	MW (kDa)	pH	Tem (°C)	Yield of product (mg/L) or conversion rate (%)	Synthetic methods	References
Glucosyltransferase (DicGT4)	1,561 for mRNA	*Dianthus caryophyllus*	Naringenin/UDP-glucose	Naringenin-7-*O*-glucoside (1); naringenin-4′-*O*-glucoside (2)			30	87 mg for (1) and 82 mg for (2)	In vivo	[174]
UGT73A16	1,413 of mRNA	*Withania somnifera*	Apigenin, genistein, biochanin A, daidzein, naringenin, 3-hydroxy flavone, baicalein and kaempferol/UDP-glucose, UDP-glucuronic acid	Naringenin 7-, naringenin 4′-, kaempferol 3-, daidzein 7-, biochanin A 7-, genistein 7-, biacalein 7-, flavone 3-*O*-glucoside; baicalin	52	8.0	37	>95 %	In vitro and In vivo	[145]
Amylosucrase		*Deinococcusgeothermalis* DSM 11300	(+)-catechi/sucrose	(+)-catechin-3′-*O*-α-D-glucopyranoside, (+)-catechin-3′-*O*-α-D-maltoside		7.0	30			[21]

compounds exist such as ginsenoside Rg3, Rc, and Rb1 and are usually used as substrates for the enzymatic synthesis of rare derivatives such as Rd, compound K, Rh2, or F2. This bioconversion has gained much interest, as the uncommon ginsenosides show stronger activities (cardioprotective effects, antioxidative or anti-inflammatory properties) more than that of their common precursors [24, 170]. Table 2 summarizes updated information on use of the enzymatic approach for producing ginsenoside derivatives. The success of the biotransformation method using *E. coli* as the host may open up large-scale production of valuable ginsenosides in the near future.

Cyanogenic glycosides

Cyanogenic glycosides are amino acid-derived secondary metabolites that play an essential role protecting plants from insects and other herbivores. These compounds are significantly distributed in sorghum (*Sorghum bicolor*), cassava (*Manihot esculenta*), and barley (*Hordium vulgare*) [64]. Biosynthesis of cyanogenic glycosides usually includes three phases in which phases I and II are characterized by cytochrome P450 to convert L-amino acids into aldoximes and aldoximes into hydroxynitrile, respectively; a UDP-glucosyltransferase in phase III attaches the glucosidic moiety to the hydroxynitrile acceptor to produce a cyanogenic glycoside [34]. Many investigations have demonstrated this concept [61, 84, 141, 142]. The biosynthesis of linamarin and lotaustralin has been studied extensively in cassava by Jorgensen et al. [62], and the proposed synthesis is shown in Fig. 7.

Vitamin glycosides

Vitamin C glucosides and derivatives

Vitamin C or L-ascorbic acid is an important nutrient for humans and animals. This compound is a cofactor for collagen synthesis reactions; therefore, it causes severe scurvy symptoms, if lacking [166]. The L-ascorbic acid is also an antioxidant against oxidative stress [114]. However, vitamin C is an unstable compound in aqueous solution as it is easily degraded under oxidative conditions, such as light, metal ions, or heat. Glycosylation by chemical or biochemical approaches can be used to enhance stability and activity of vitamin C. As chemical synthesis of vitamin C glucosides is complicated, enzymatic synthesis is the main method. There are several types of vitamin C glucosides enzymatically produced such as ascorbic acid-2-, 3-, 5- or 6-*O*-α-D-glucoside (Fig. 8). Some types of enzymes such as α-glucosidases [106], amylase [92], or α-CGTase [102] are used to produce ascorbic acid glucosides. Immobilized α-CGTase is

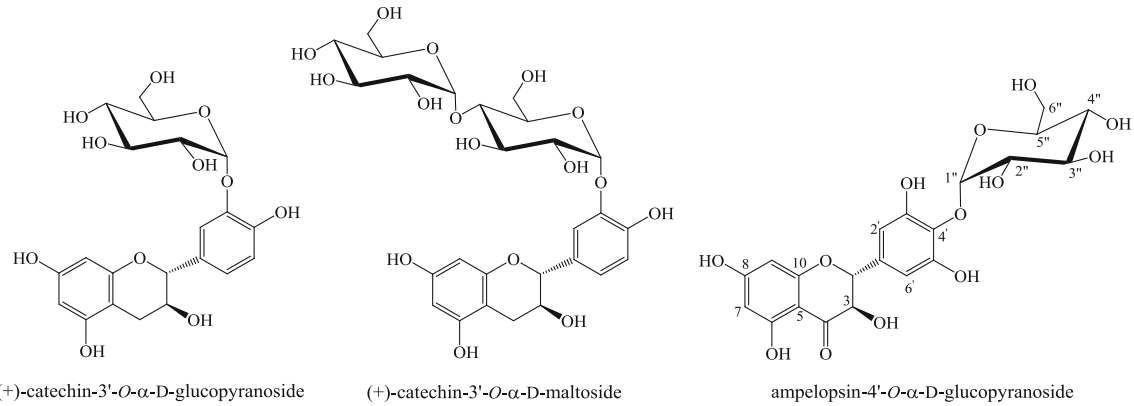


Fig. 3 Catechin and ampelopsin glycosides were synthesized by *Deinococcus*-origined amylosucrase [21] and *Leuconostoc*-derived dextransucrase [179]

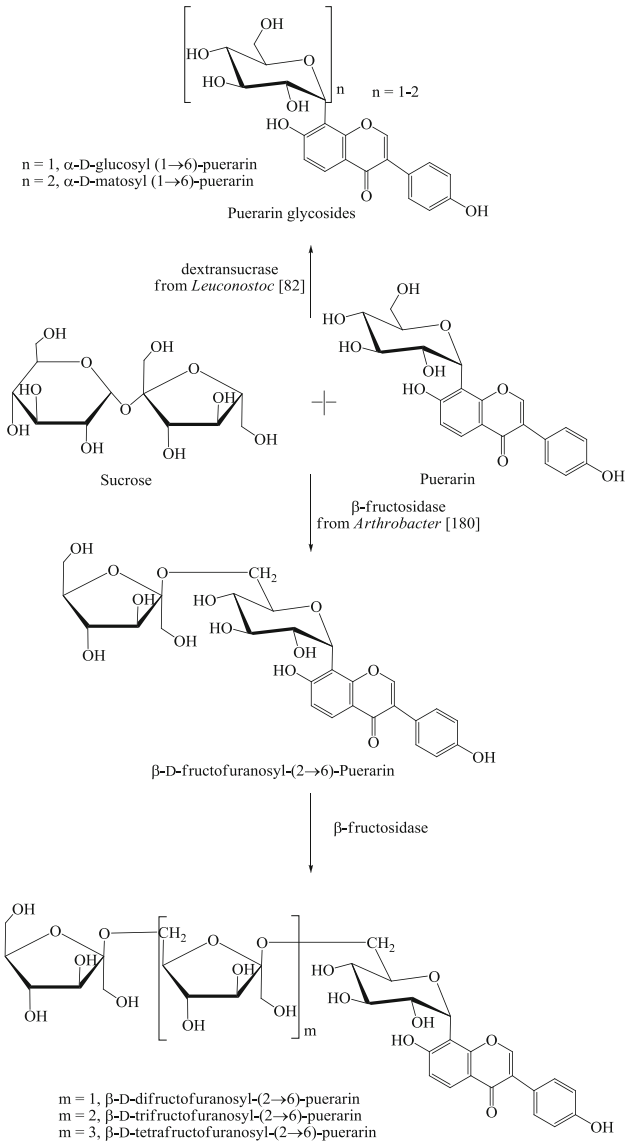


Fig. 4 Production of puerarin glucosides from *Lecoconostoc* dextransucrase [81] and *Arthrobacter* β-glucosidase [180]

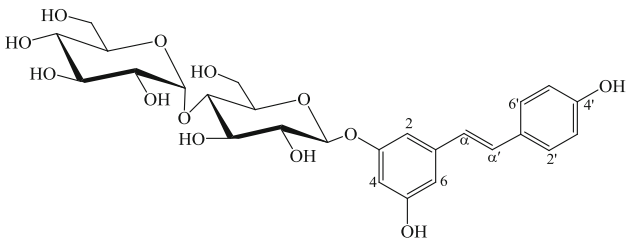


Fig. 5 Structure of piceid glycoside [118]

utilized for continuous production of ascorbic acid 2-*O*-α-D-glucoside and the highest yield of target compound is 21 g/L. Such a record is two-fold higher than of free α-CGTase [186]. Acylation at the C-5 or C-6 position of the aglycon structure or C-6′ at the sugar part is preformed to improve bioactivity of vitamin C glucosides [156, 157]. A recent update on vitamin glucosides is listed in Table 3.

Pyridoxine glycosides

Pyridoxine is one of the component of vitamin B6, along with pyridoxal and pyridoxamine. This compound supports the balance of hormonal changes in women and aids in the immune system [67, 175]. Pyridoxine is a light- and heat-sensitive compound and the glycosylated pyridoxine is less sensitive than its aglycon [40, 68]. Enzymatic synthesis of pyridoxine glycosides using β-glucosidase from sweet almond has been performed and has generated a series of products (Fig. 9) [14].

α-Tocopherol glucoside

The α-Tocopherol and the tocotrienol are the main components of vitamin E; but they are poorly soluble in water, less stable, and show poor absorbtivity. The major function of α-tocopherol is as a potent antioxidant, free-radical

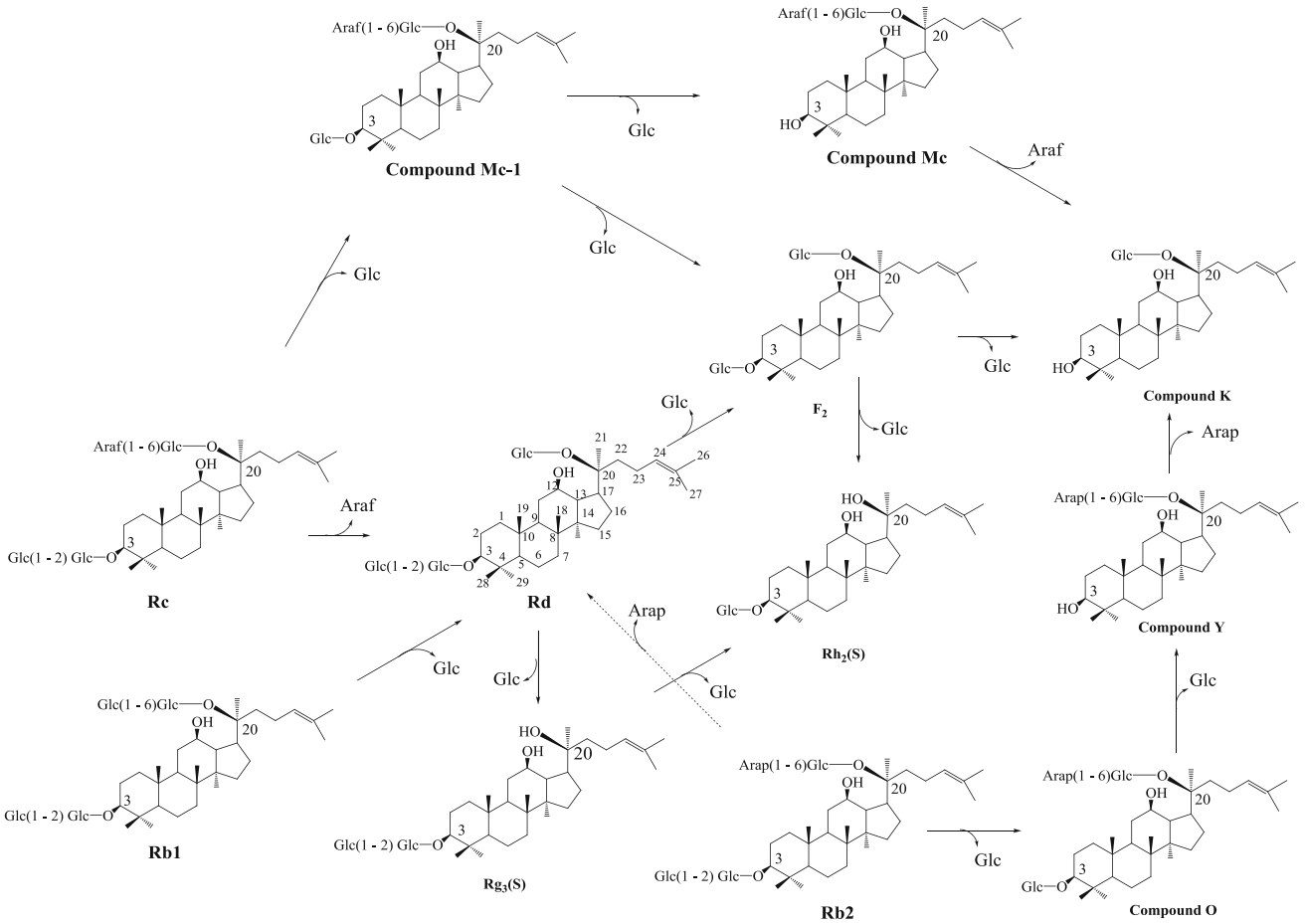


Fig. 6 Biotransformation pathway of ginsenoside via trimming hydrolysis by glycosidases. *Glc* β-D-glucopyranosyl, *Arap* α-L-arabinopyranosyl, *Araf* α-L-arabinofuranosyl

scavenger [177] and to reduce oxidative stress [91]. Glycosylation of this compound may enhance its solubility leading to changes in bioactivity. Divakar et al. [101] successfully synthesized glycosylated tocopherols using the transglycosylation reaction and amyloglucosidase from *Rhizopus* mold or β-glucosidase from sweet almond (Fig. 10) [124].

Glycosylated hydroquinones as skin lightening agents

Due to the inhibitory effect against tyrosinase, an enzyme that catalyzes the formation and deposition of melanin pigment, hydroquinone and arbutin (4-hydroxyphenyl-β-D-glucopyranoside) are used in cosmetics as skin lightening agents. However, hydroquinones have some side effects such as skin irritation [58], dermatitis, and atrophy [9]. Arbutin is more water soluble and less harmful compared to its aglycon and inhibits tyrosinase activity without affecting its mRNA expression [120]. Enzymatic synthesis of arbutin glucosides as well as hydroxyphenyl glycosides has received much interest using microbial enzymes such as *Leuconostoc*-derived glucansucrase [105] or oligoxylosyl transfer enzyme (OxtA from *Bacillus* sp. strain KT12) [20]. Arbutin has been synthesized using an enzymatic method with hydroquinone and sugars such as maltopentaose, starch, or sucrose [105, 152]. Numerous glycosidases have been characterized that glycosylate hydroquinone such as amylosucrase [139], β-galactosidase [76], and levansucrase [65]. The main characteristics of these enzymes are listed in Table 4 (Fig. 11).

Stevioside and rubusoside

Stevioside and rubusoside are natural sweeteners isolated from the leaves of *Stevia rebaudiana*. These compounds can be used clinically as inhibitors of atherosclerosis by improving insulin signaling and antioxidant defense in obese insulin-resistant mice [37], as superior scavengers of both hydroxyl and superoxide radicals against oxidative stress [149], or a stimulants for the immune system by enhancing apoptosis [158]. However, the commercial products are relatively expensive; therefore, production of

Table 2 Trimming hydrolysis of ginsenoside using glycosidases

Enzymes	Gene size (bp)	Strains	Substrates	Products	MW (kDa)	pH	Tem (°C)	K_m (mM)	V (μM/min.mg protein)	References
β-Glucosidase		*Esteyavermicola* CNU120806	Ginsenoside Rg3	Ginsenoside Rh2		5.0	50			[50]
β-Glucosidase		*Esteya vermicola* CNU 120806	Ginsenoside Rb1	Gypenoside LXXV		5.0	50			[51]
β-Glucosidase	1,947	*Terrabacter ginsenosidimutans* sp. nov.	*p*-nitrophenyl-β-D-glucopyranoside/ginsenoside Rb1	Gypenosides XVII and LXXV		7.0	37	4.2 ± 0.8 and 0.14 ± 0.05 mM for p-nitrophenyl-β-D-glucopyranoside and Rb1, respectively	100.6 ± 17.1 and 329 ± 31 μmol/min.mg protein*p*-nitrophenyl-β-D-glucopyranoside and Rb1, respectively	[2]
β-Glucosidase	1,344	*Sphingomonas* sp. strain 2F2	*p*-nitrophenyl-β-D-β-glucopyranoside/ginsenosides Rb(1), Rb(2), Rc and Rd	Ginsenosidesgypenoside XVII, ginsenoside C-O, ginsenoside C-Mc(1) and ginsenoside F(2), ginsenosides Gyp XVII, C-O, C-Mc(1) and F(2)	49,399	5.0	37	2.9 ± 0.3 mM for the donor substrate	515.4 ± 38.3 μmol/min.mg protein	[171]
Glycosidase	1,857	Sanguibacterkeddieii	Major ginsenosides Rb_1, Rb_2, Rc, Rd, Re and Rg_1	Active rare ginsenosides such as C-Y, C-Mc, C-K, Rg_2(S), and F_1				0.456 ± 0.009 and 0.167 ± 0.003 mM for *p*-nitrophenyl-β-D-glucopyranoside and Rb(1), respectively	30.2 ± 0.7 and 4.1 ± 0.1 μmol/min.mg protein for *p*-nitrophenyl-β-D-glucopyranoside and Rb(1), respectively	[77]
β-Glucosidase	2,271	*Microbacteriumesteraromaticum*	Ginsenoside Rb1	Compound K	80	7.0	40			[126]
β-Glucosidase	2,271	*Microbacteriumesteraromaticum*	Ginsenoside Rb2	Compound Y and K		7.0	40			[125]
α-L-Arabinofuranosidase		*Rhodanobacterginsenosidimutans*strain Gsoil 3054(T)	Ginsenoside Rc	Ginsenoside Rd				0.53 ± 0.07 and 0.30 ± 0.07 mM*p*-nitrophenyl-α-L-arabinofuranoside and ginsenosideRc, respectively	27.1 ± 1.7 and 49.6 ± 4.1 μmol/min. mg of protein for *p*-nitrophenyl-α-L-arabinofuranoside and ginsenoside Rc, respectively	[3]
α-L-Arabino furanosidase (Abf22-3)	1,527	*Leuconostoc* sp. strain 22-3	*p*-nitrophenyl-α-L-arabinofuranoside/ginsenoside Rc	Ginsenoside Rd	58.486	6.0	30	0.95 ± 0.02 μM for the donor	1.2 ± 0.1 μmol/min.mg protein	[97]
β-Glucosidase	1,830	*Actinosynnemamirum*KACC 20028(T)	GinsenosideRb(1), protopanaxatriol-type ginsenoside Re and Rg(1)	Gypenoside XVII and Rd to F(2), Rg(2)(*S*) and Rh(1)(*S*)	65.277	7.0	37	0.69 ± 0.06 mM for Rb1 and 0.45 ± 0.02 mM for Rd	16.13 ± 0.29 μmol/min.mg protein for Rb1 and 51.56 ± 1.35 μmol/min.mg protein for Rd	[12]

MW molecular weight, *Tem* temperature, K_m *and* V_{max} Michaelis–Menten constant and maximum velocity

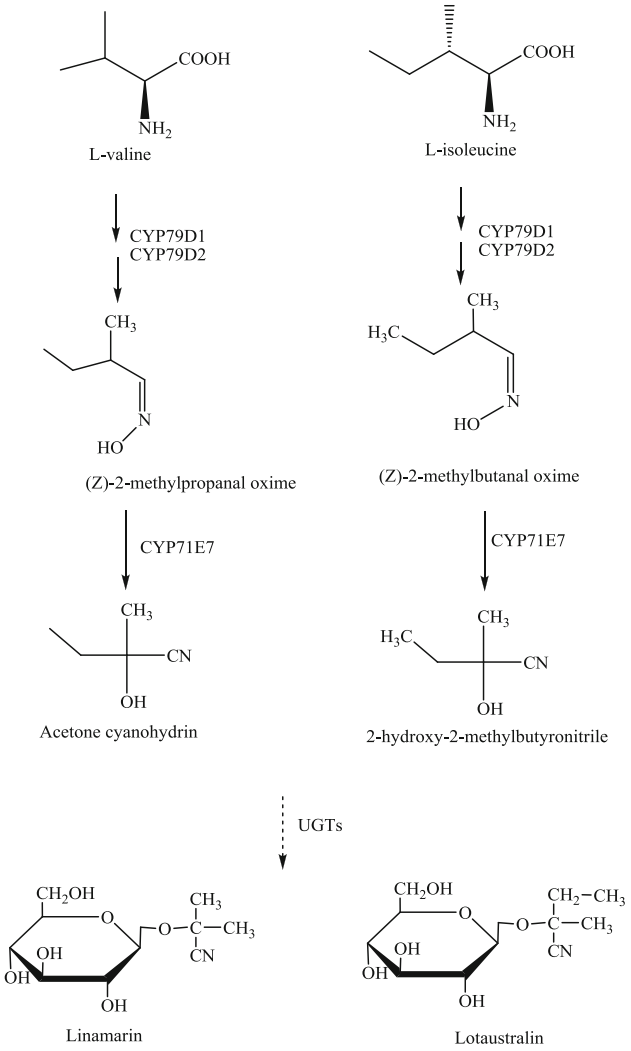


Fig. 7 Proposed biosynthesis of cyanogenic glycosides

these sweeteners has gained much attention through an enzymatic approach. In addition, as stevioside and rubusoside have a slightly bitter aftertaste, many efforts have concentrated on modifying the structure of stevioside. For example, a new *Aspergillus aculeatus*-derived β-glucosidase has been applied for mass production of rubusoside under optimal conditions of 280 mM stevioside and 16.6 μL of enzyme at pH 5.1 and 63 °C [82]. Similarly, Xia et al. [169] showed the highest bioconversion of stevioside to rubusoside of 98.3 %, accompanied by a rubusoside yield of 91.4 %. Using β-cyclodextrin glucanotransferase isolated from an alkalophilic strain of *Bacillus firmus*, two derivatives of stevioside such as 4′-*O*-α-D-glycosyl stevioside and 4″-*O*-α-D-maltosyl stevioside have been produced via three different methods including traditional, ultrasound-, and microwave-assisted reactions [59]. In addition to the synthesis of stevioside derivatives by the enzymatic method, a new series of steviosides have been identified in leaves of *Stevia rebaudiana* [17, 18]. These studies may identify corresponding enzymes for post-modification in the near future (Fig. 12).

Vanillyl glucosides

Vanillyl alcohol is the main component of *Gastrodia elata* Blume extract, an herb that has been used for centuries in Oriental countries. This compound has been demonstrated as a neuroprotective agent by suppressing oxidative stress and as an anti-apoptotic agent in toxin-induced dopaminergic MN9D cells [74]. Vanillyl also shows anti-angiogenic, anti-inflammatory, and anti-nociceptive activities in mice [63] and suppresses cancer cell migration and metastasis in animals [94]. Additionally, vanillin possesses anti-microbial potential by inhibiting growth of *Saccharomyces cerevisiae, Zygosaccharomyces bailii*, and *Zygosaccharomyces rouxii*, which are associated with food spoilage [32]. Glycosylation of vanillin and its derivatives has been a focus due to decreased toxicity and increased stability, bioactivity, and solubility. For example, vanillin becomes toxic when it accumulates in the cellular cytoplasm; therefore, bioconversion of vanillin into vanillin glucoside (less toxic) enhances the yield of this compound in *Schizosaccharmyces pombe* [4, 10, 46].

Vanillin and 8-nordihydrocapsaicin are glycoslated by cultured *Eucalyptus perriniana* cells to generate vanillin-4-*O*-β-D-glucopyranoside, 4-*O*-β-D-glucopyranosylvanillyl alcohol, 8-nordihydrocapsaicin-4-*O*-β-D-glucopyranoside, and 8-nordihydrocapsaicin-4-*O*-β-D-gentiobioside [136].

Using maltase from *S. cerevisiae* as a catalyst, a new glucoside of vanillyl alcohol called 4-hydroxy-3-methoxybenzyl-α-D-glucopyranoside was synthesized from vanillyl alcohol and maltose (donor) via a trans-glycosylation reaction. Under optimal conditions, the yield of glucoside was 90 mM with no by-product formed. The compound also showed potent antioxidant activity against ABTS [2, 20-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid)] [165] (Fig. 13). In addition, a vanillyl alcohol isomaltoside has been synthesized in a second reaction reported by the same group [29]. The current biological synthesis of vanillin glucosides is updated in Table 5.

Coumarin and their glycoside derivatives

Coumarins (1,2-benzopyrone), a group of natural products, are distributed in vanilla grass (*Anthoxanthum odoratum*), sweet grass (*Hierochloe odorata*), and deer tongue (*Panicum clandestinum*). These compounds show anti-neoplastic effects in a number of systemic malignancies [66], as well as antifungal and antioxidant activity [135]. Furthermore, 4-methylesculetin, a coumarin derivative, possesses

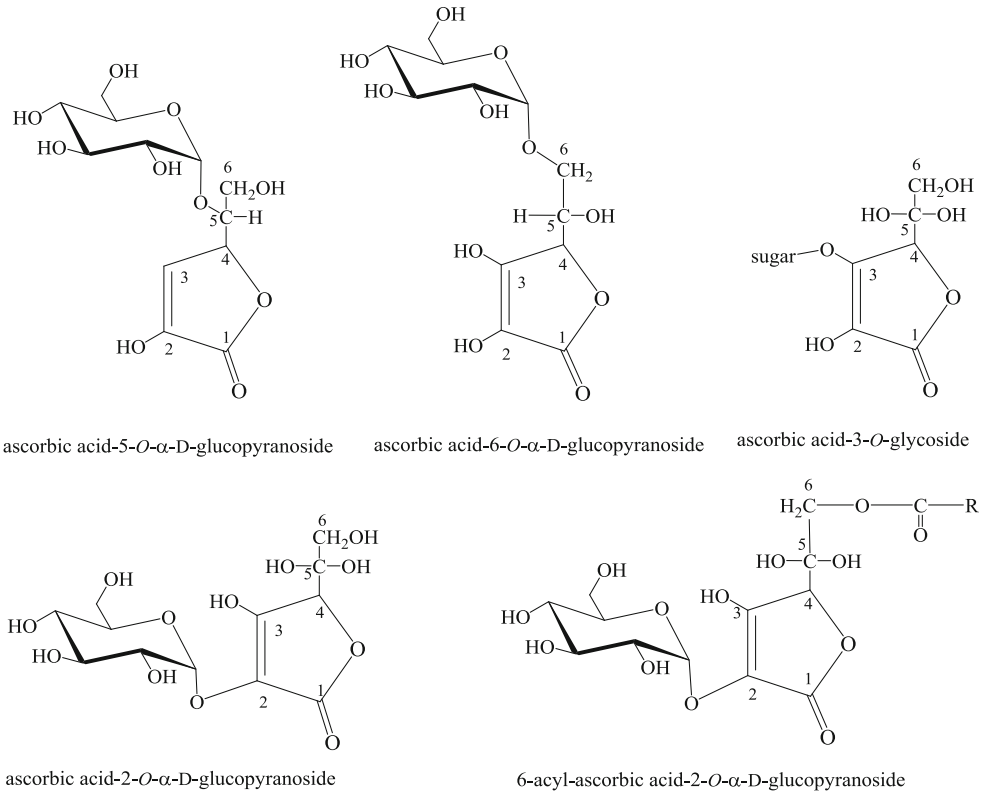


Fig. 8 Structure of L-ascorbic acid glucosides (C) ascorbic acid-3-*O*-α-D-glycoside in which the sugar can be maltose, chitobiose, cellubiose, etc.

Table 3 Glucosylation of vitamins

Enzymes	Strains	Acceptors/donors	Products	MW (kDa)	pH	Tem (°C)	References
Glucosidase and amyloglucosidase	*Rhizopus,*sweet almond	Pyridoxine, (vitamin B6), ergocalciferol (D2), thiamin (B1), riboflavin (B2)/D-glucose	Variety of pyridoxine glucosides		4–8	68	[14]
Amyloglucosidase	*Rhizopus* mold	Alpha-tocopheryl/D-glucose	6-*O*-(D-galacto, gluco, manno) pyranosyl α-tocopherol		7	25	[124]
Cyclodextrin andglycosyltransferase	*Paenibacillus macerans* CCTCC M203062	Ascorbic acid/β-cyclodextrin	2-*O*-α-D-glucopyranosyl-L-ascorbic acid		5.5	50	[186]
β-Glucosidase	Sweet almond	Retinol/D-glucose, D-galactose, D-mannose, D-fructose, and D-sorbitol	Retinol glycosides (18-*O*-(D-glucopyranosyl)retinol, 18-*O*-(D-fructofuranosyl)retinol, etc.)	64.6		50	[15]
Cyclodextrin and glucanotransferases	*Saccharomyces cerevisiae, Bacillus stearothermophilus, B. circulans, B. halophilus*	Ascorbic acid/starch, maltodextrin, γ-cyclodextrin, and maltose	2-*O*-α-D-glucopyranosyl-L-ascorbic acid		5.5–6.0	50–60	[102]
Sucrose phosphorylase	*Bifidobacterium longum*	L-ascorbic acid/sucrose	2-*O*-α-D-glucopyranosyl-L-ascorbic acid		7.5	37	[89]

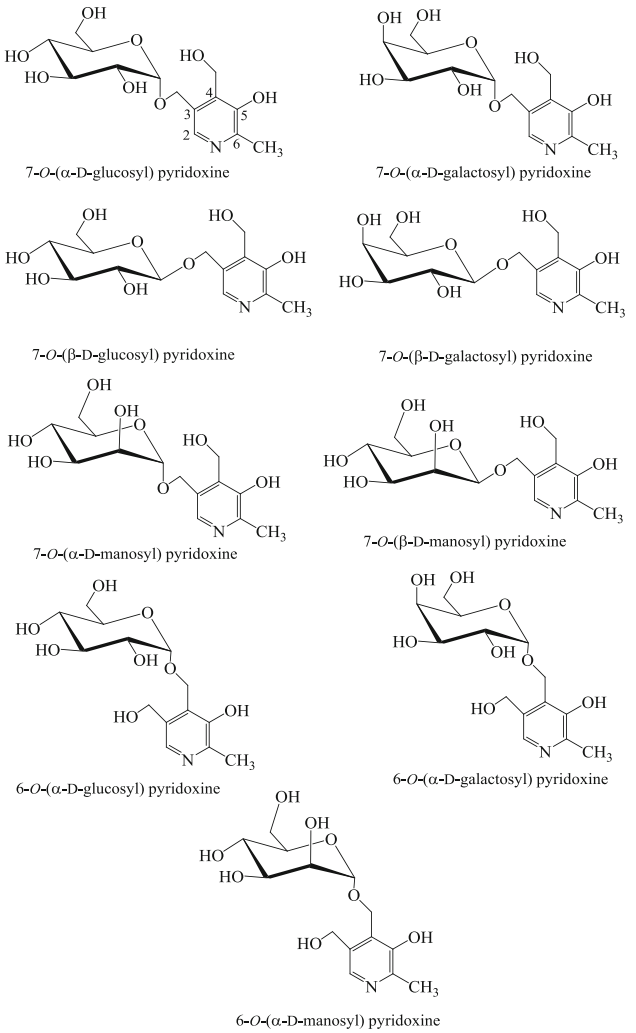


Fig. 9 Different pyridoxine glycosides were synthesized by β-glucosidase [14]

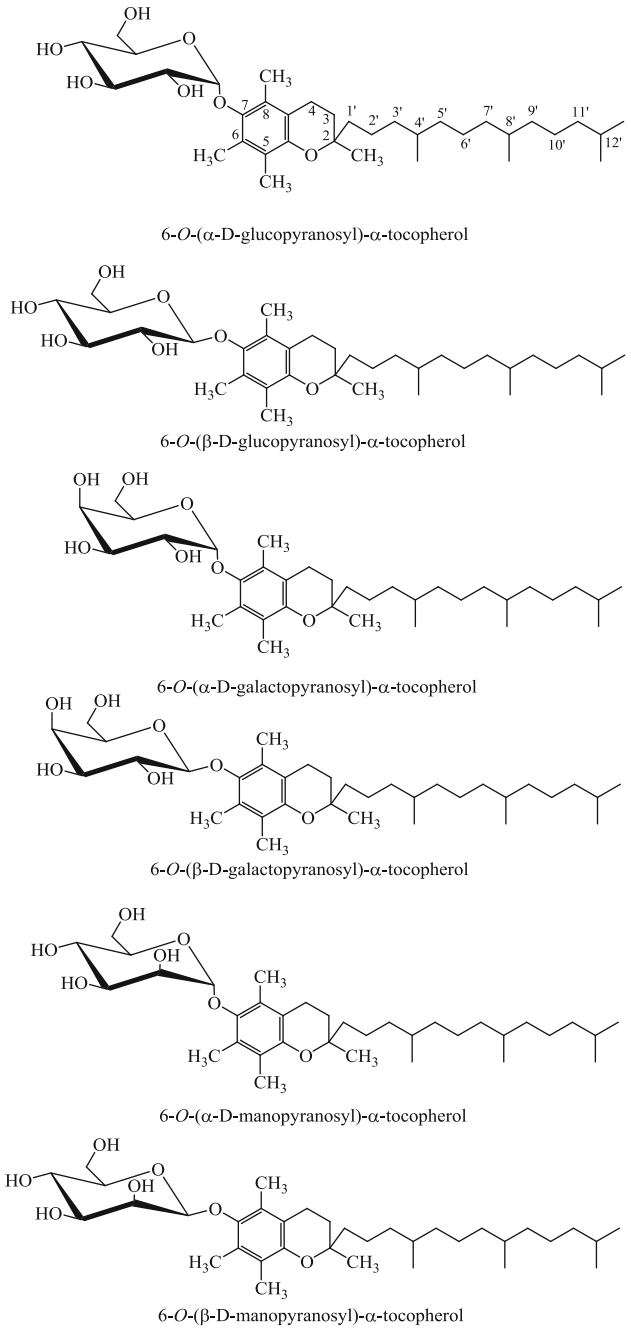


Fig. 10 Glycosylated tocopherol derivatives were enzymatically synthesized by β-glucosidase [124]

anti-arthritic and anti-inflammatory properties [47]. Coumarin glycosides show better solubility and stability than their aglycon derivatives and play important roles in pharmaceutical studies. Isoarnottinin 4′-glucoside, a glycosylated derivative of coumarin, has been isolated from *Prangos uloptera* (Apiaceae) leaves. This compound exhibits significant phytotoxic activity against lettuce and modest cytotoxic activity against the HeLa cell line [131]. In other studies, aviprin and aviprin-3″-*O*-glucoside, two linear furano coumarin from Apiaceae, show antibacterial activity, cytotoxic effects, and phytotoxic activity [130, 185]. Therefore, the biosynthesis of coumarin glycosides has gained much interest in pharmaceutical engineering. The most popular method to biotransform coumarin is by cultured plant cells. For example, three novel glycoside derivatives and four known coumarin compounds can be produced from *Catharanthus roseus* cultured suspension cells [182]. Yu et al. [187] reported glycol diversification of coumarin glycosyltransferase in transgenic hairy roots of *Polygonum multiflorum* using esculetin and various coumarin-derived synthetic substrates (Fig. 14). Two new potential antifungal coumarin glycosides such as 6-chlorocoumarin 7-*O*-β-D-glucopyranoside and 7-hydroxy-4-trifluoromethyl-coumarin 5-*O*-β-D-glucopyranoside were chemoenzymatically synthesized by the same group. In this case, the authors found that hairy root cells of *P. multiflorum* worked as an independent whole-cell biocatalyst

Table 4 Biological synthesis of several skin lightening agents

Enzymes	Gene size (bp)	Strains	Acceptor/ donor	Products	MW (kDa)	pH	Tem (°C)	Ki (Tyr)	IC50 (mM) (DPPH)	References
Glucansucrase		*Leuconostoc mesenteroides* B-1299CB	Arbutin/ sucrose	4-hydroxyphenyl β-isomaltoside (**1**); 4-hydroxyphenyl β-isomaltotrioside		5.2	28	3.7 for (**1**)		[105]
β-Xylanase		*Bacillus* sp. KT12	Hydroquinone/ xylan	4-hydroxyphenyl-β-D-xylopyranoside (1); 4-hydroxyphenyl-β-D-[xylopyranosyl-(1–4) β-D-xylopyranoside (2); 4-hydroxyphenyl-β-D-[xylopyranosyl-(1–4)]$_2$ β-D-xylopyranoside (3); 4-hydroxyphenyl-β-D-[xylopyranosyl-(1–4)]$_3$ β-D-xylopyranoside (4)		4–11	40	0.20, 0.29, 0.057 for (**2**), (**3**), and (**4**), respectively		[20]
Lactase		*Kluyveromyces lactis*	Hydroquinoe/ lactose	Hydroquinone galactoside		7.0	28	0.75	3.31	[76]
Levansucrase	1,275	*Leuconostocmesenteroides*	Hydroquinoe/ sucrose	Hydroquinone fructoside	47.1	5.2	28	1.53	5.83	[65]
Amylosucrase (ASase)		*Deinococcus geothermalis*	Hydroquinone/ sucrose	Hydroquinone-*O*-α-D-glucopyranoside	73	7.0	35			[139]

MW molecular weight, *Tem* temperature, *IC50* half maximal inhibitory concentration of diphenylpicrylhydrazyl (DPPH) scavenging activity, *Ki (Tyr)* inhibition constant against tyrosinase

instead of isolating the enzyme and characterizing it using a traditional method [187].

Glycosylated polyketides as antibiotics

These famous glycosides have been used as antibiotics including enediyne, polyene, amino glycosides, macrolides, and anthacyclines, and about 60 % of the antibiotics have been found in *Streptomyces* spp. Many biosynthetic gene clusters for antibiotics have been discovered and provide a huge genetic source as well as a genetic base for functional characterization of particular biosynthetic pathways. The development of DNA and protein engineering in combination with computer-based modeling programs allows for an increase in the production of target compounds via metabolic engineering and rational design, as well as to create novel derivatives using combinatorial biosynthesis and directed evolution. The GTs involved in the biosynthesis of plant-derived or *Streptomyces*-originating secondary metabolites are at the center of biotechnological evolution to produce bioactive compounds. Here, we list an update on glycosides produced as antibiotics using typical examples.

Polyketides are widely popular secondary metabolites in plants, bacteria, fungi, and animals. They possess clinically important properties such as antibacterial, anticancer, and anti-tumor effects [43, 44]. The core structure of polyketides is usually synthesized via the decarboxylative condensation of malonyl-CoA, methylmalonyl-CoA, and ethylmalonyl-CoA originated extender units in a similar process to that of fatty acid synthesis. The multi-enzyme complex responsible for this is called modular polyketide synthase (PKS) and contains different modules of enzyme domains and synthesizes each component of the structure in a stepwise chain assembly [140]. The diversity of polyketides depends on the structure of the aglycone and the type of attached sugars.

Streptomyces venezuelae YJ028 is a mutant containing a deletion of the entire biosynthetic gene cluster encoding pikromycin PKS and desosamine biosynthetic enzymes. This strain harbors plasmids that bear various sugar biosynthetic pathways and flexible enzymes such as DesVII/DesVIII and has been used as a host for exogenously adding tylactone to produce different glycosylated tylosin derivatives [44]. Similarly, a narbomycin-produced *S. venezuelae* YJ003 mutant, containing a deletion of thymidine-5′-diphospho-D-desosamine, has been used as a host after inserting various deoxy-sugar gene cassettes and glycosyltransferase DesVII. Several narbomycin glycosylated derivatives have been produced using intracellular narbornolide and activated sugars (TDP-D-boivinose, TDP-L-olivose, TDP-L-rhamnose, TDP-D-quinovose, TDP-D-desosamine, TDP-L-digitoxose, and TDP-3-*O*-demethyl-

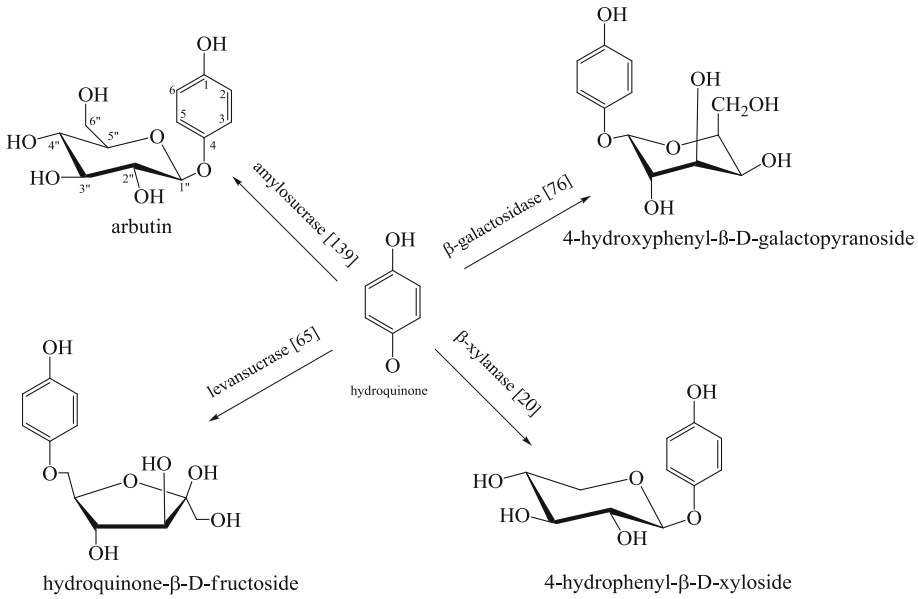


Fig. 11 Biosynthesis of variety of hydroquinone glycosides [76]

D-chalcose) as acceptors and donors, respectively [45]. Using a similar combinatorial biosynthetic approach, those authors successfully produced various types of glycosylated rhodomycinones using *S. venezuelae* YJ028, a mutant lacking the pikromycin PKS genes and genes for biosynthesis of TDP-D-desosamine, as a host containing a flexible glycosyltransferase AknS and a TDP-4-ketohexose reductase, AvrE, which efficiently supports TDP-4-*epi*-L-daunosamine biosynthesis. In addition, a variety of sugar gene cassettes were used independently to provide glyco-diversified aglycons [43] (Fig. 15).

Mitoxantrone, a polyketide type II anthraquinone, is famous as an anti-cancer drug for metastatic breast cancer, acute myeloid leukemia, and non-Hodgkin's lymphoma [75, 137]. However, the drug also exhibits some side effects such as nausea, hair loss, and immune suppression [138]. Glycosylation of mitoxantrone extends the therapeutic window by decreasing cardiotoxicity and enhancing activity. Thorson et al., succeeded in asymmetric enzymatic glycosylation of mitoxantrone to produce mitoxantrone 4′-β-D-glucoside using a promiscuous engineered glycosyltransferase derived from the macrolide-inactivating OleD. This product exhibits high cytotoxicity to different cancer cell lines [188].

Mithramycin is an aureolic acid antibiotic with strong antitumor activity. It is composed of a tricyclic aglycone and five deoxysugars that form a disaccharide and a trisaccharide chain. Several mithramycin derivatives, which differ in glycosylation patterns, have been produced from *Streptomyces argillaceus* by combinatorial biosynthesis. They show high antitumor activity and less toxicity in hollow fiber assays and in subcutaneous colon and melanoma cancers xenograft models [108, 122] (Fig. 16). In a similar study, the chromomycin CmnA is an acetyltransferase that attaches two acetyl groups during chromomycin A3 biosynthesis. This compound and mithramycin are anthrocyclines but differ in their glycosylation profiles and functional sugar group substitution. The CmnA has been inserted in the mithramycin-produced *S. griseus* mutant and generated a series of new acetyl-containing mithramycin derivatives with antitumor activity [36].

Diosgenin

Diosgenin, a derivate of isospirostane, is a compound that exhibits potent anti-cancer activity [56] and is anti-neoplastic and apoptotic in squamous cell carcinoma [26]. In addition, diosgenyl analogues have been chemically synthesized such as amino acid diosgenyl esters and diosgenyl salicylate conjugates, which also show anti-cancer and anti-inflammatory activities [52]. Diosgenin is produced from *Dioscorea zingiberensis* C. H. Wright (DZW) tubers by acid hydrolysis. But, the disadvantage of this method is the abundant by-products, which cause environmental pollution. Therefore, much effort has concentrated on developing a clean and applicable method to produce diosgenin on a large-scale. Microbial biotransformation and enzymatic processing are the most useful methods to convert precursors such as spirostanosides of DZW into diosgenin by β-glucosidase from *Aspergillus fumigates* [93] or the DZW tuber is directly processed through

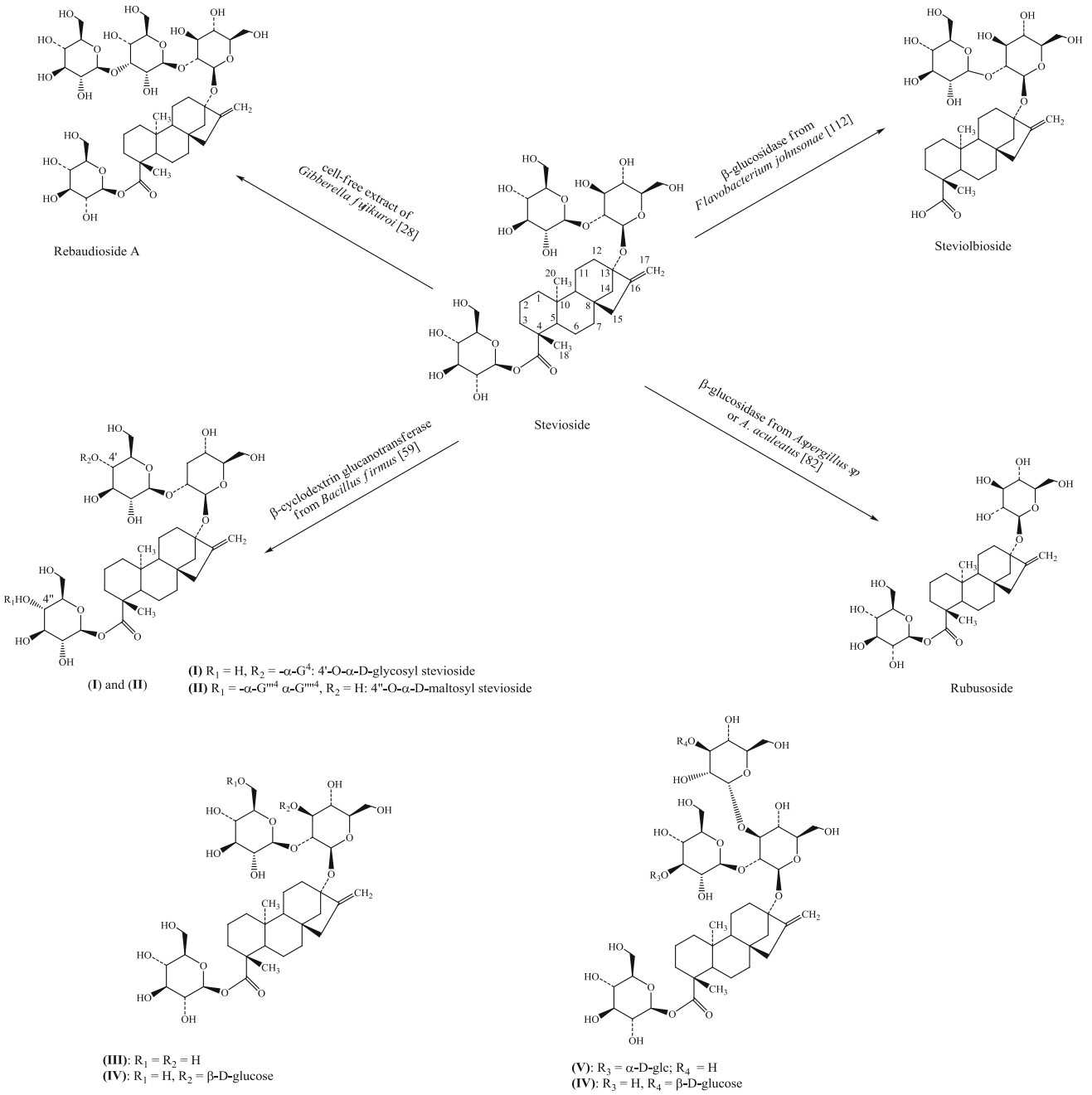


Fig. 12 Synthesis of steviosides and rubususides using different types of glucosidases [28, 112]. *G* glucose

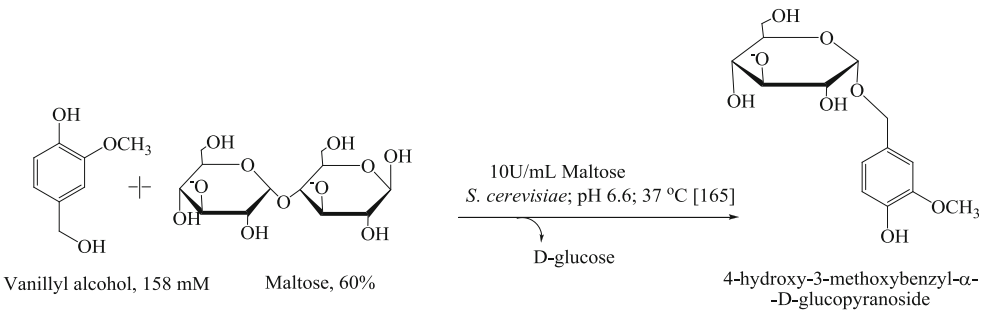
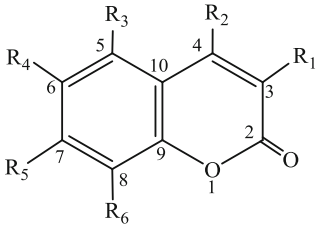


Fig. 13 Enzymatic synthesis of 4-hydroxy-3-methoxybenzyl-α-D-glucopyranoside [165]

Table 5 Glucosylation of vanillin

Enzymes	Resources or host	Acceptor/donor	Products	pH	Tem (°C)	References
Cell suspension culture	*Eucalyptus perriniana*	Biotransformation of vanillin and 8-nordihydrocapsaicin as acceptor	Vanillin 4-*O*-β-D-glucopyranoside; 4-*O*-β-D-glucopyranosylvanillyl alcohol; 8-nordihydrocapsaicin 4-*O*-β-D-glucopyranoside; 8-nordihydro-capsaicin 4-*O*-β-D-gentiobioside			[136]
Amyloglucosidase, β-glucosidase	*Rhizopus*, sweet almond	N-vanillyl-nonanamide/ D-glucose, D-galactose, D-ribose, maltose and lactose	N-vanillyl-nonanamide-D-glucoside; N-vanillyl-nonanamide-D-galactoside; N-vanillyl-nonanamide β-D-mannoside; N-vanillyl-nonanamide-D-riboside; N-vanillyl-nonanamidemaltoside; N-vanillyl-nonanamide β-lactoside	4–8		[146]
Glycosyltransferase	*Arabidopsis thaliana, S. cerevisiae*	Vanillin/glucose	Vanillin glucoside			[46]
α-1,4-Glucosidase	*S. cerevisiae*	Vanillyl alcohol/maltose	4-hydroxy-3-methoxybenzyl-α-D-glucopyranoside	6.6	37	[165]



1: $R_1 = R_2 = R_3 = R_6 = H$; $R_4 = OGlc$; $R_5 = OH$
7-hydroxycoumarin - 6-*O*-β-D-glucopyranoside

2: $R_1 = R_3 = R_4 = R_6 = H$; $R_5 = OGlc$; $R_2 = OGlc$
4-methylcoumarin-7-*O*-β-D-glucopyranoside

3: $R_3 = R_4 = R_6 = H$; $R_1 = R_2 = CH_3$; $R_5 = OGlc$
3,4-dimethylcoumarin-7-*O*-β-D-glucopyranoside

4: $R_1 = R_4 = R_6 = R = H$; $R_2 = CH_3$; $R_3 = OGlc$; $R_5 = OH$
2-methylcoumarin-*O*-β-D-glucopyranoside

5: $R_4 = R_6 = H$; $R_1 = R_2 = CH_3$; $R_3 = OGlc$; $R_5 = OH$
3,4-dimethylcoumarin-*O*-β-D-glucopyranoside

6: $R_1 = R_3 = R_6 = H$; $R_2 = CH_3$; $R_4 = Cl$; $R_5 = OGlc$
6-chloro-4-methylcoumarin-7-*O*-β-D-glucopyranoside

7: $R_1 = R_3 = R_6 = H$; $R_2 = C_6H_5$; $R_4 = Cl$; $R_5 = OGlc$
6-chloro-4-phenylcoumarin-7-*O*-β-D-glucopyranoside

8: $R_1 = R_3 = R_4 = H$; $R_2 = CH_3$; $R_5 = OGlc$; $R_6 = OH$
8-hydroxy-4-methylcoumarin-7-*O*-β-D-glucopyranoside

9: $R_1 = R_3 = R_4 = H$; $R = CH_3$; $R_5 = OGlc$; $R_6 = Allyl$
8-allyl-4-methylcoumarin-7-*O*-β-D-glucopyranoside

Fig. 14 Variety of coumarin glycoside derivatives that were synthesized by transgenic hairy roots of *Polygonum multiflorum* [187]

enzymatic saccharification and microbial transformation in *Trichoderma reesei* under optimal conditions for 156 h [190]. Process optimization for the production of diosgenin with *T. reesei* has been further carried out by response surface methodology (and the highest diosgenin yield of 90.57 % was achieved under suitable conditions [190]. Additionally, *T. harzianum* was applied to convert steroidal saponins into diosgenin and achieved 30.05 ± 0.59 mg/g diosgenin, which was slightly higher than that obtained from an acid hydrolysis approach [96]. Dioscin-hydrolyzed glucosidase has been used as a biocatalyst for diosgenin synthesis. This is exemplified by the enzyme dioscin-glycosidase, which was isolated from *Absidia* sp.d38 under optimal temperature and pH to produce a new dioscin-glycosidase at 40 °C and pH 5.0, respectively [32] (Fig. 17).

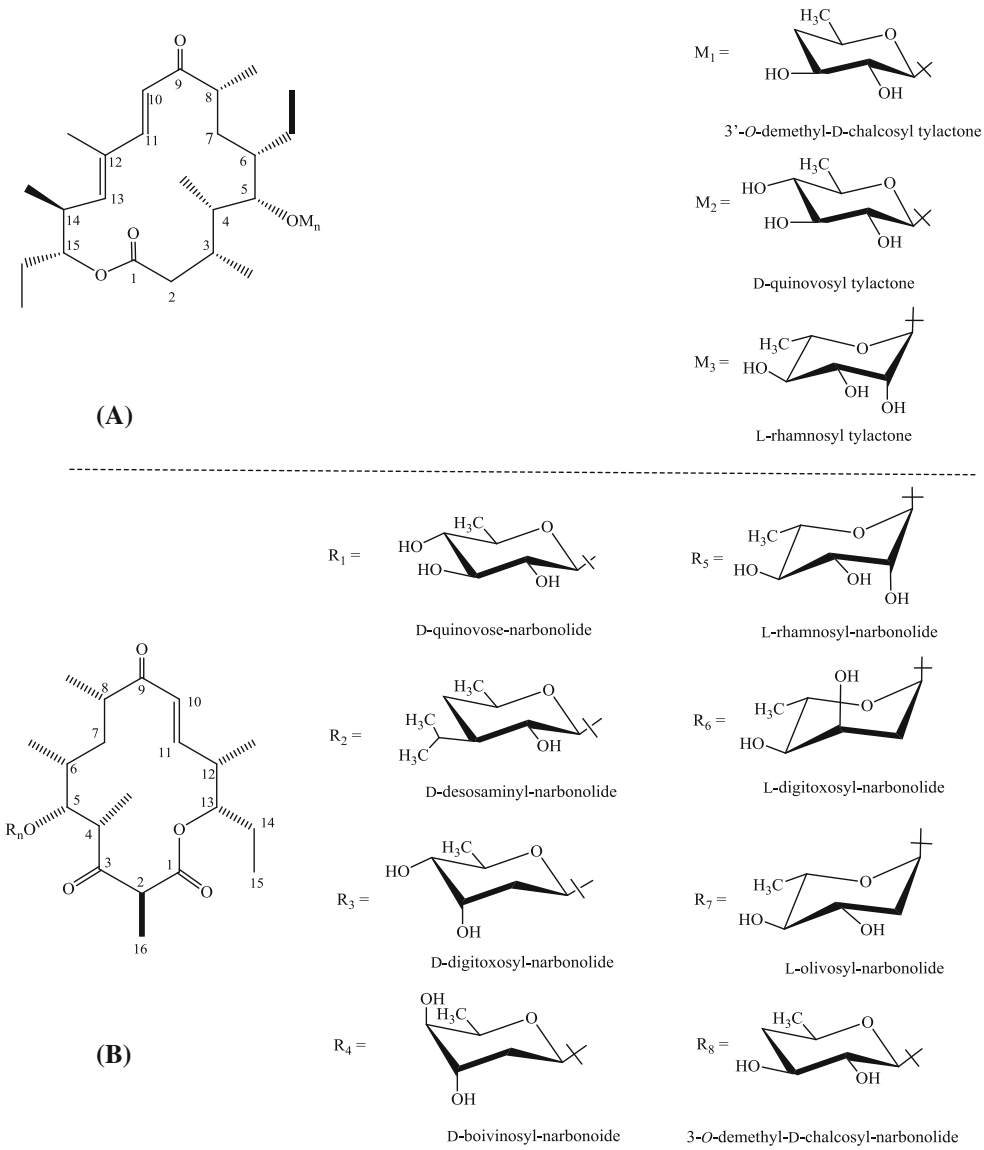


Fig. 15 Structure of tylactone glycosides (**a**) and narbonolide glycosides (**b**)

Alkyl glucosides and alkyl polyglucoside as surfactants

Alkyl glucosides and their derivatives are eco-friendly and industrially important non-ionic surfactants with high surface activity and good biodegradability as well as foaming control, wetting, detergent, and emulsifying properties [128]. Alkyl-glucosides are composed of aliphatic alcohols and glucose obtained from a renewable resource. These compounds are used in cosmetic and hair conditioning agents or as emulsion stabilizers. A series of synthetic alkyl glucoside vesicles has been used as a drug carrier [81] or as effective solubilizing agents for bovine rhodopsin [150]. Chemical synthesis of these compounds requires several steps and often includes a mixture of both anomers of two possible cyclic forms of the glycosides; therefore, it is difficult to obtain a pure compound [6]. The enzymatic approach is cheap and effective due to its stereo- and regio-selectivity [98]. Alkyl-β-D-glucosides can be biosynthesized by whole-cell conversion. For example, the thermo-tolerant *Pichia etchellsii* containing β-glucosidase has been used as a host to produce octyl-β-D-glucopyranoside via trans-glucosylation between p-nitrophenyl β-D-glucopyranoside and octanol as an acceptor [128]. In another study, the production of three glycosides using middle chain aliphatic alcohols (hexanol and heptanol) was achieved by reverse hydrolysis catalyzed by defatted meal from almond, apricot, and peach kernels [6]. A new *Dictyoglomus*-derived β-glucosidase exhibits extremely high thermostability, was glucose tolerant in aqueous solution,

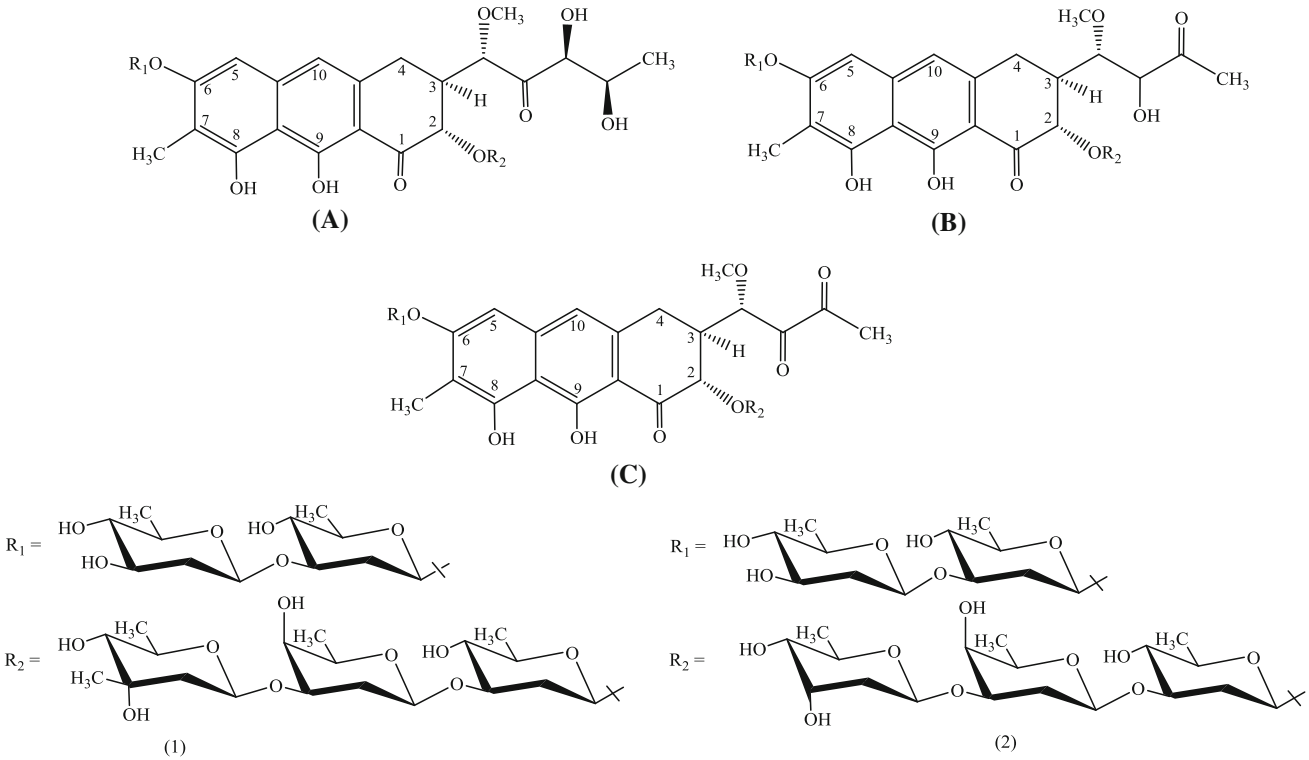


Fig. 16 Mithramycin (**1**) and demycarosyl-3-D-β-digitoxosylmithramycin (**2**)

and was glucosylated with n-octanol at 70 °C instead of 50 °C. Thus, this enzyme is promising for synthesizing various glucosides [191]. Other studies on the synthesis of hexyl α-glucoside and α-polyglucoside have been performed with α-glucosidase from *Microbacterium paraoxydans* [79, 111] (Table 6) (Fig. 18).

Production of cardiac glycosides

Cardiac glycosides (CGs) are steroids produced by plant cells and mammalian adrenocortical cells and are widely used to treat certain forms of cardiac insufficiency. They are composed of either a C_{23} (cardenolides) or a C_{24} (bufadiennolides) genin and sugar moieties. The most important natural source of these compounds are leaves from the *Digitalis* genus (Scrophulariaceae), which contain hundreds of different cardenolide-type glycosides built up from five types of genins and ten different sugars [4, 42, 161]. Digoxin and digitoxin have been extensively used for treating arrhythmias, contractility disorders, and congestive heart failure [104]. Oubain and digoxin are strong inducers of P-glycoprotein, a transmembrane transporter that extrudes several drugs such as doxorubicin, and, hence, affects the absorption of drugs in colon epithelia [133]. Commercial production of CGs by chemical extraction from natural plants is insufficient. Therefore, biotechnological approaches have been applied to improve the product yield. The CG production has been carried out using plant tissue culture and extraction. This is exemplified by digoxin and digitoxin from in vitro shoot cultures of *Digitalis lanata* using a temporary immersion system [123]. Another case is the production of two cardenolides (lanatoside C and digoxin) from *D. davisiana* Heywood [41]. Furthermore, calcium chloride and polysaccharides have been used to enhance production of total CGs in *D. lanata* [38] (Fig. 19).

Future outlook

Studies on small glycosides have been accumulating due to their broad applications in pharmaceutical, cosmetic, agriculture, and medicine. A number of novel glycoside activities have been updated, indicating their importance. The discovery of new glycosides as natural products based on chemical extraction, purification, and identification from natural sources; enzymatic processing using advances in protein engineering and DNA recombinant technology; as well as computer-based modeling

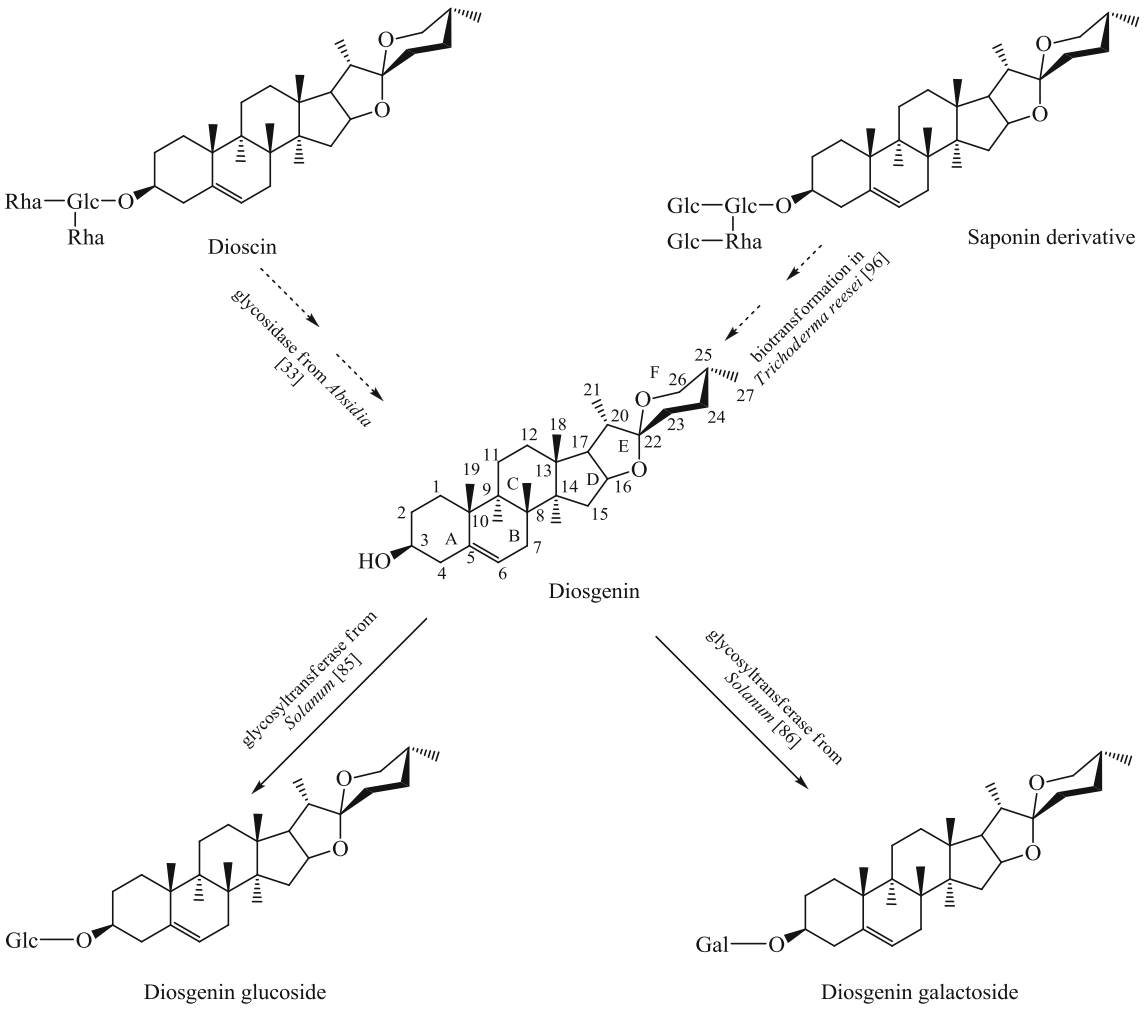


Fig. 17 Biotransformation of diosgenin and its derivatives [33, 85, 86]

programs has become a main force. We have outlined some trends and perspectives that are being used presently as well as in the future. First, efforts have concentrated on modifying the aglycon structure and activated sugars using enzymatic approaches and accumulating knowledge on the biosynthesis of acceptors such as polyketides, terpenes, flavonoids, vitamins, steroids, and new derivatives of amino sugars, deoxy sugars, and pseudo sugars as donors. Second, exploitation of promiscuous enzymes has focused on glycosyltransferases and glycosidases in addition to engineering techniques using site-directed mutagenesis and domain exchange in combination with high-throughput screening or other biochemical-based detection systems to recruit suitable enzymes as biocatalysts. Modeling programs are critical for studying the interaction between substrates and enzymes before setting up reactions. Third, diversification of the glycosylation method such as the development of highly tolerant organic solvent microorganism strains to produce puerarin glucosides [180] has significantly improved the solubility of puerarin and controlled the main product by increasing solvent concentration. This is a promising method to scale-up the production of target compounds by optimizing culture conditions. Fourth, detection methods for in vitro enzyme assays such as chemosensors for released NDP (NDP-sugar) [178] or pH-dependent color assays for high-throughput screening of both in vitro and in vivo reactions [121] are sensitive, easy, inexpensive and applicable techniques.

Table 6 Synthesis of alkyl glucosides

Enzymes	Gene size (bp)	Strains	Acceptor/donor	Products	MW (kDa)	pH	Tem (°C)	K_m (mM)	V_{max} (mM/min.mg protein)	References
β-Glucosidase		*Pichia etchellsii*	n-Octanol, n-dodecanol, n-decanol, methanol/ p-nitrophenyl β-D-glucopyranoside, Methyl-β-D-glucopyranoside	Octylglucoside, methyl-β-D-glucoside, hexyl-β-D-glucoside, decyl-β-D-glucoside, dodecyl-β-D-glucoside			40			[129]
β-D-Glucosidases		*Thermotoga neapolitana* DSM4359	Hexanol/glucose		81.1	5.8	60			[39]
Glucosidase	2,166	*Thermotoga neapolitana* DSM4359	Hexanol/p-nitrophenyl-beta-glucopyranoside	Hexyl glucoside	81.1	5.3–5.8	60			[160]
Glucosidase		*Amygdalus communis* var. 'Dulcis'	Cholecalciferol	D-Glucose, D-galactose, D-mannose						[101]
Glucosidase	1,400	*Microbacterium paraoxydans*	Methanol to decanol/ maltose, sucrose, etc	Alkyl glucoside	30 ± 5	7.0	50	4.71 ± 0.24 mM	0.5	[111]
Dextransucrase		*Leuconostoc mesenteroides*	Ethanol, methanol/ sucrose	Methyl-α-D-glucopyranoside and ethyl-α-D-glucopyranoside		5.2	28			[79]
β-Galactosidases		*Aspergillusoryzae,* apricot meal	Hexanol, heptanol/D-lactose	Hexyl and heptyl β-D-galactopyranosides		5				[6]
β-Glucosidase		*Pichiaetchellsii*	Ooctanol/*p*-nitrophenyl β-dglucopyranoside	Octylglucoside			40			[128]
Cyclodextringlycosyltransferase		*Bacillus macerans*	Alkyl polyglycoside/α-cyclodextrin	n-Tetra-decyl-glucopyranoside (α and β); n-dodecyl-glucopyranoside (α and β); ect		5.2	60			[154]
Cyclodextringlycosyltransferase		*Bacillus macerans; Thermoanaerobactersp*	n-Dodecyl-(1,4)-β-maltopyranoside/α-cyclodextrin	n-Dodecyl-(1,4)-β-maltooctaoside		5.15	60			[155]

MW molecular weight, *Tem* temperature, K_m *and* V_{max} Michaelis–Menten constant and maximum velocity

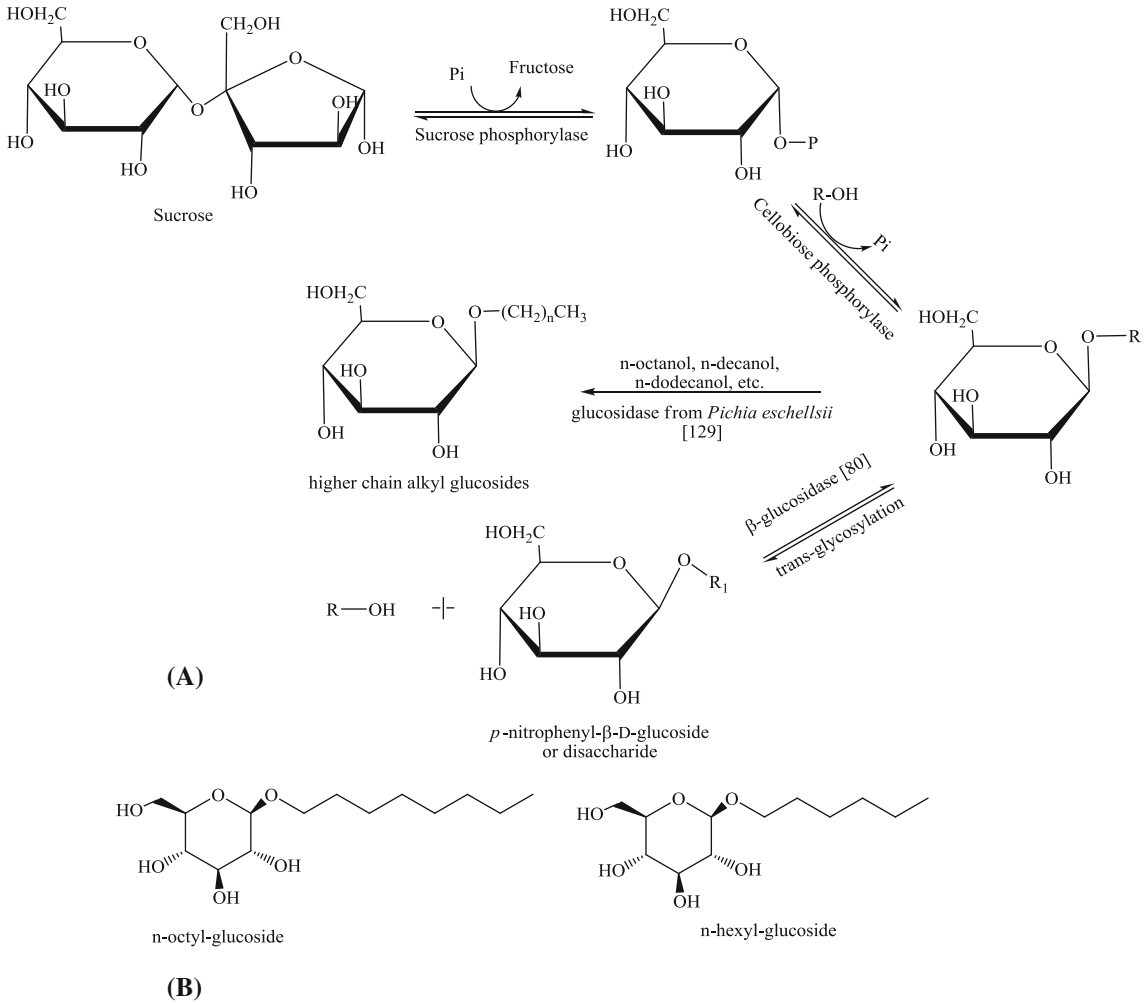
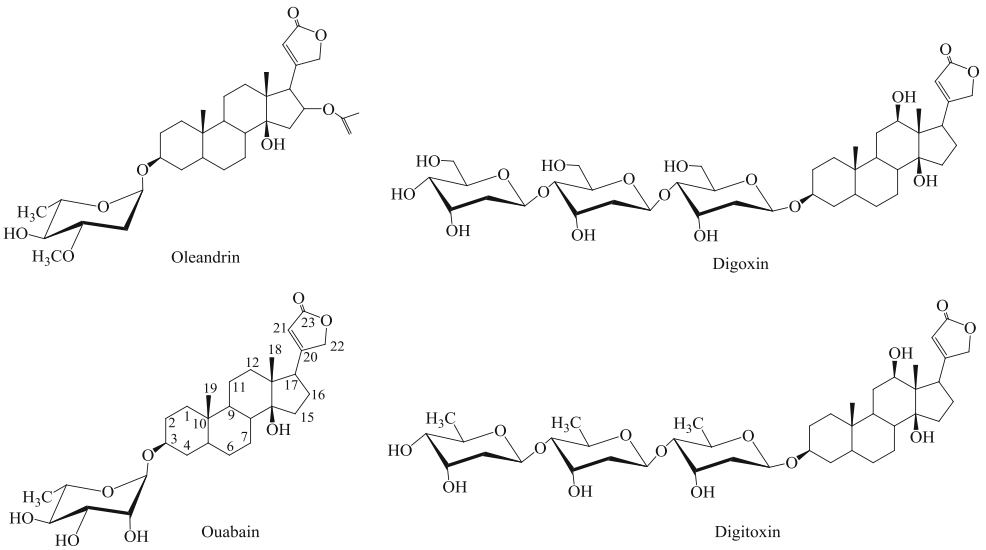


Fig. 18 Synthesis of alkyl glucoside. R-OH, alcohol; R_1, p-nitro-phenyl or monosaccharide (**a**) and structures of n-hexyl- and n-octyl glucoside (**b**) [80]

Fig. 19 Structures of some typical cardiac glycosides



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