Advances and Challenges in Biomanufacturing of Glycosylation of Natural Products

Shunyang Hu 1,†, Bangxu Wang 1,†, Liang Pei 2, Jisheng Wang 3, Ya Gan 1, Liangzhen Jiang 1, Bingliang Liu 1,*, Jie Cheng 1,*, and Wei Li 2,*

1 Meat Processing Key Laboratory of Sichuan Province, College of Food and Biological Engineering, Chengdu University, Chengdu 610106, China; hushunyang@stu.cdu.edu.cn (S.H.); wangbangxu@stu.cdu.edu.cn (B.W.); ganya@cdu.edu.cn (Y.G.); jiangliangzhen@cdu.edu.cn (L.J.); liubingliang@cdu.edu.cn (B.L.)
2 Sichuan Ingia Biosynthetic Co., Ltd., Chengdu 610200, China; peiliang@scingia.com
3 The 9th Geological Brigade of Sichuan, Deyang 618300, China; 18582368898@163.com
* Correspondence: chengjie@cdu.edu.cn (J.C.); liwei@scingia.com (W.L.)
† These authors contributed equally to this work.

Abstract: Glycosylation is one of the most common and important modifications in natural products (NPs), which can alter the biological activities and properties of NPs, effectively increase structural diversity, and improve pharmacological activities. The biosynthesis of glycosylation in natural products involves multiple complex biological processes, which are coordinated by many enzymes. UDP-glycosyltransferases (UGTs) play a crucial role in glycosylation modification, and have attracted long-term and widespread research attention. UGTs can catalyze the O-, C-, S-, and N-glycosylation of different substrates, producing a variety of glycosides with broad biological activity, while improving the solubility, stability, bioavailability, pharmacological activity, and other functions of NPs. In recent years, the rapid development of synthetic biology and advanced manufacturing technologies, especially the widespread application of artificial intelligence in the field of synthetic biology, has led to a series of new discoveries in the biosynthesis of NP glycosides by UGT. This work summarizes the latest progress and challenges in the field of NP glycosylation, covering the research results and potential applications of glycosylated derivatives of terpenes, flavonoids, polyphenols, aromatic compounds, and other compounds in terms of biogenesis. Looking to the future, research may leverage artificial intelligence-driven synthetic biology techniques to decipher genes related to the synthetic pathway, which is expected to further promote the large-scale synthesis and application of glycosylated NPs, and increase the diversity of NPs in the pharmaceutical, functional food, and cosmetic industries.

Keywords: natural product; glycosylation; UDP-glycosyltransferases; structural diversity; biomanufacturing

1. Introduction

Natural products (NPs) are a family of compounds that are widely distributed in nature and are produced by organisms using biological catalysts. These include primary and secondary metabolites [1]. This family includes alkaloids, terpenes, phenolic compounds, flavonoids, and lignans. NPs are numerous, structurally diverse, and many NPs possess a variety of biological activities such as anti-inflammatory, antibacterial, antiviral, antioxidant, anticancer, antiradiation, and immunomodulatory effects, and are widely applied in food, health supplements, pharmaceuticals, feed, and other fields [2] (as shown in Table 1). However, many NPs have poor solubility, insufficient biological activity, and low bioavailability. NPs can undergo various structural modifications through biochemical reactions, such as acetylation [3], esterification [4], glycosylation [5], hydroxylation [6], and methylation [7], which endow NPs with greater structural and functional diversity.
Different types and quantities of modifying groups, as well as modifications with different regional and stereochemical selectivities, can produce NPs with different structures [8].

Glycosylation can significantly improve the physical and chemical properties and biological activities of many NPs, such as enhancing selectivity, solubility, stability, reducing the toxicity of compounds, enhancing pharmacological activity, and prolonging the duration of drug action [9]. The alteration of the sugar side chain structure in NPs has a significant impact on the activity, selectivity, and pharmacokinetic properties of the parent compound [10]. The addition of hydrophilic groups can make NPs more easily absorbed, thereby enhancing their pharmacological properties. Sun et al. [11] reported that glycosylation is an important method for modifying various flavonoid compounds, with glycosides having higher solubility, stability, and bioavailability compared to the corresponding aglycone. The introduction of sugar moieties into non-glycoside compounds not only confers different molecular and spatial-electronic properties to NPs, but also the glycosylated modified NPs usually have a more stable structure. In recent years, the glycosylation of NPs has demonstrated attractive characteristics and a broad application prospect in new drug development. Glycosylation-modified NPs, such as Urdamycin [12], Adriamycin [13], Aclarubicin [14], Erythromycin [15], megalomycin C [9], calicheamicin [10], Staruosporine [16], Vancomycin [17], Digitoxin [18,19], Amphotericin B [19], and rebeccamycin [20], are important sources of drugs or drug precursors and are widely used in the pharmaceutical field. Glycosides have various applications in the treatment of respiratory diseases, lowering cholesterol, analgesic and sedative effects, preventing scurvy, and treating cancer [17]. Currently, glycosylated NPs are used as antibiotics (e.g., pikromycin, kanamycin, vancomycin), anti-tumors (e.g., kaempferol, apigenin), anti-inflammatory (e.g., quercetin), and antioxidants (e.g., naringenin). In addition to the pharmaceutical industry, glycosylated NPs are also widely used in the food, feed, cosmetics, materials, and other industries [21].

UDP-glycosyltransferases (UGTs) are key enzymes in the glycosylation process, responsible for attaching sugar moieties to the O-, C-, S-, and N-positions of acceptor molecules [22]. UGTs serve as biocatalysts that can catalyze glycosylation reactions in aqueous solutions under mild and environmentally friendly conditions [23]. UGTs have been identified in plants, bacteria, fungi, yeast, and edible fungi. UGTs identified from plants have the ability to glycosylate almost all major types of secondary metabolites, such as phenylpropionoids [24], alkaloids [25], terpenes [26], and polyketide compounds [27]. In the glycosylation reaction, apart from glucose, the sugar donors can also include xylose, rhamnose, galactose, N-acetyl-D-glucosamine, arabinose, etc. Huang et al. [28] used UDP-glucose as a sugar donor to produce β-glucosides, and co-expression of VvGT15c and GmSUS improved the glycosylation of geraniol and enhanced the cell resistance to toxic terpenes. Chen et al. [29] used UDP-rhamnose to regenerate the system to generate isorhamnetin-3-O-rhamnopyranoside, which exhibited strong inhibitory effects on the cytotoxicity of HepG2, MCF-7, and A549 cells. Gao et al. [23] used UDP-galactose transferase GmSGT2 to introduce sugar portions into glycurrhetinic acid (GA) at C-3OH to produce GA glycosides. Wen et al. [30] reported that glycosyltransferase HtUGT72AS can directly accept N-acetylglucosamine to catalyze the glycosylation of flavonols at 3-OH. Li et al. [31] introduced SIUGT91R1 and UDP-arabinose into Escherichia coli (E. coli) to achieve the production of rosavin, which exhibited high levels of activity. The regulation of post-translational mechanisms in microalgae was explored, which are essential for ensuring the proper production and glycosylation of antibodies [32]. Glycosylation is a universal and sustainable biotechnological method that plays a key role in the production of bioactive compounds. Andreeu et al. [33] reported on enzymatic glycosylation strategies for the production of bioactive compounds and noted the advantages of enzymatic glycosylation including its high regioselectivity, stereoselectivity, and sustainability, as well as the possibility of incorporating immobilization techniques or chemical or genetic modifications to improve the glycosylation process.
This review provides an extensive overview of the latest developments and challenges in the glycosylation of NPs in biomanufacturing. The glycosylation of various natural products, including terpenoids, flavonoids, polyketides, and aromatic compounds, is described and discussed. Furthermore, the diverse applications of biomanufacturing of glycosylation in the fields of food, pharmaceuticals, and feed are also summarized (Figure 1).

Figure 1. Glycosylation modification of natural products: sugar donors, compound classification, properties, and applications.
Table 1. Progress in microbial synthesis of glycosylated natural products.

<table>
<thead>
<tr>
<th>Types of Natural Products</th>
<th>Product</th>
<th>Microbial Sources</th>
<th>GTs</th>
<th>Titer (g/L)</th>
<th>Engineered Strategy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terpenoids and its</td>
<td>Reb M</td>
<td>E. coli</td>
<td>UGT76G1-T284S/M88L/L200A</td>
<td>0.023</td>
<td>The UGT76G1-T284S/M88L/L200A variant was obtained by structure-guided evolution</td>
<td>[34]</td>
</tr>
<tr>
<td>derivatives</td>
<td>Rh2, PPD</td>
<td>S. cerevisiae</td>
<td>UGTPg45</td>
<td>2.25, 11.02</td>
<td>Modular engineering of the mevalonate pathway was constructed and the expression</td>
<td>[35]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>level of P450 was optimized</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ro</td>
<td>S. cerevisiae</td>
<td>GT73F3, UGT73P40</td>
<td>0.52</td>
<td>Seven enzymes from five species with high catalytic efficiency and substrate</td>
<td>[36]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>specificity were screened through in vitro and in vivo characterization</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ginsenoside Rh2</td>
<td>S. cerevisiae</td>
<td>UGT51</td>
<td>0.3</td>
<td>The semi rational design strategy of UGT51 was conducted, preventing Rh2</td>
<td>[37]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>degradation and increasing UDP-glucose precursor supply</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CK</td>
<td>S. cerevisiae</td>
<td>UGTPg1</td>
<td>5.74</td>
<td>optimizing the expression of UGTPg1, enhancing the biosynthesis of UDP-glucose,</td>
<td>[38]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>and reducing the consumption of UDP-glucose</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rg1, R1, R2</td>
<td>S. cerevisiae</td>
<td>PgUGT71A53, PgUGT94Q13,</td>
<td>1.95, 1.62,</td>
<td>A set of UGT94 family UGTs were identified from ginseng and Panax notoginseng</td>
<td>[39]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>PgUGT71A54,</td>
<td>1.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rh2</td>
<td>Bacillus subtilis</td>
<td>UGT51</td>
<td>3.7</td>
<td>The regioselectivity of Yjic for Rh2 synthesis was successfully improved using a</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>semi rational design</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rh1, PPT</td>
<td>E. coli</td>
<td>UGTBL1</td>
<td>20.48, 18.04</td>
<td>A structure-directed mutagenesis strategy was proposed to modify the enzyme UGT1</td>
<td>[41]</td>
</tr>
<tr>
<td></td>
<td>Asiaticoside</td>
<td>S. cerevisiae</td>
<td>CaUGT73C7, CaUGT73C8</td>
<td>0.00077</td>
<td>knocking out EGH1, a glycoside hydrolase that degrades asiaticoside, and introducing key pathway enzymes</td>
<td>[42]</td>
</tr>
<tr>
<td></td>
<td>Baicalein-7-O-glucoside,</td>
<td>E. coli</td>
<td>EbUGT75L25, AtUGT98C1</td>
<td>0.57, 0.88</td>
<td>A whole-cell biocatalytic system that lacks competing genes and incorporates an</td>
<td>[43]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>exogenous UDP-glucose supply pathway, as well as a glucose transferase, rhamnose</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>transferase, and UDP-rhamnose synthesis pathway has been developed</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7-O-glucoside</td>
<td>S. cerevisiae</td>
<td>SbGT34</td>
<td>1.20</td>
<td>Using the endogeneous glucosidase of brewing yeast as a whole-cell biocatalyst</td>
<td>[44]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fisetin 3-O-glucoside,</td>
<td>E. coli</td>
<td>UGT78K1</td>
<td>1.18, 1.03</td>
<td>Utilizing a multi-nucleotide synthesis vector to assemble multiple genes of</td>
<td>[45]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>nucleoside diphosphate (NDP)-sugar biosynthetic pathways, a robust genetic circuit</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>for producing valuable flavonoid glycosides in E. coli was constructed</td>
<td></td>
</tr>
</tbody>
</table>
Table 1. Cont.

<table>
<thead>
<tr>
<th>Types of Natural Products</th>
<th>Product</th>
<th>Microbial Sources</th>
<th>GTs</th>
<th>Titer (g/L)</th>
<th>Engineered Strategy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoids and its derivatives</td>
<td>Astragalosides</td>
<td><em>E. coli</em></td>
<td>AtUGT78D2</td>
<td>3.6</td>
<td>An efficient UDP-glucose synthesis pathway was reconstructed in recombinant strains by introducing sucrose permease, sucrose phosphorylase, and uridylic acid transferase</td>
<td>[46]</td>
</tr>
<tr>
<td></td>
<td>Populoside, Orientin, Flavopiridoside, Quercetin, Chrysin, Trichoside</td>
<td><em>E. coli</em></td>
<td>TcCGT1, GtfC, PhUGT</td>
<td>17.2, 36.5, 5.2, 14.1, 6.4, 11.4</td>
<td>A glycosylation platform strain was established in <em>E. coli</em> through multiple metabolic engineering with UDPG supply</td>
<td>[47]</td>
</tr>
<tr>
<td></td>
<td>Hyperoside</td>
<td><em>E. coli</em></td>
<td>HmGAT</td>
<td>0.025</td>
<td>Four key enzymes were identified by gene screening and functional validation</td>
<td>[48]</td>
</tr>
<tr>
<td></td>
<td>Salidroside</td>
<td><em>E. coli</em></td>
<td>UGT72B14</td>
<td>0.006</td>
<td>The UGT72B14 from Rhodiola was optimized by codons and expressed in <em>E. coli</em></td>
<td>[49]</td>
</tr>
<tr>
<td>Polyketide and its derivatives</td>
<td>Polydatin</td>
<td><em>S. cerevisiae</em></td>
<td>PcR3GAT</td>
<td>0.55</td>
<td>Key glycosyltransferases for resveratrol production were identified by transcriptome analysis. By combining the resveratrol biosynthetic module, UDP-glucose supply module, and glycosyltransferase expression module, the biosynthesis of glycosylated resveratrol was achieved</td>
<td>[50]</td>
</tr>
<tr>
<td></td>
<td>MEB</td>
<td><em>E. coli</em></td>
<td>rfbA</td>
<td>0.048</td>
<td>Blocking the competing pathway of precursor glucose-1-phosphate</td>
<td>[51]</td>
</tr>
<tr>
<td></td>
<td>Quercitrin</td>
<td><em>E. coli</em></td>
<td>AtUGT78D1</td>
<td>7.7</td>
<td>Coupling the UDP-rhamnose generating system to Arabidopsis thaliana rhamnosyltransferase (AtUGT78D1)</td>
<td>[52]</td>
</tr>
<tr>
<td>Aromatic and its derivatives</td>
<td>Gastrodin</td>
<td><em>Yarrowia lipolytica</em></td>
<td>SyUGT, RsUGT, ArUGT</td>
<td>13.4</td>
<td>More than fifty genetic modifications were introduced into the yeast genome</td>
<td>[53]</td>
</tr>
<tr>
<td></td>
<td>Rosavin</td>
<td><em>E. coli</em></td>
<td>SfUGT91R1</td>
<td>7.54</td>
<td>Incorporation of SfUGT91R1 and the UDP-arabinose pathway into resin-generating stains</td>
<td>[31]</td>
</tr>
<tr>
<td>Steroidal saponins</td>
<td><em>E. coli</em></td>
<td>PpUGT6</td>
<td>-</td>
<td>Molecular docking of the PpUGT6 protein with ligands was performed, and key residues interacting with ligands were predicted</td>
<td>[54]</td>
<td></td>
</tr>
<tr>
<td>Steroid and its derivatives</td>
<td>Dioscin</td>
<td><em>E. coli</em></td>
<td>DzGT1</td>
<td>-</td>
<td>The cDNA encoding the trillin rhamnosyltransferase from <em>D. zingiberensis</em> was isolated</td>
<td>[55]</td>
</tr>
<tr>
<td></td>
<td>CTS 3-O-glycosides</td>
<td><em>E. coli</em></td>
<td>UGT74AN2</td>
<td>-</td>
<td>Key residues for sugar donor recognition and preference were identified</td>
<td>[56]</td>
</tr>
</tbody>
</table>
2. Glycosylation of Terpenoids and Its Derivatives

Terpenoids are the most diverse class of NPs and possess important physiological activities, such as anti-tumor, antiproliferative, multidrug resistance reversal, cytotoxicity inhibition, antibacterial, vasoactive, immunomodulatory, anti-inflammatory, antioxidant, neuroprotective, and pro-inflammatory effects [57]. The physiological activities of terpenoids, such as polarity, volatility, solubility, and reactivity, can be modulated through glycosylation [24]. Steviol glycosides (SGs), derived from the leaves of the Stevia plant, are a series of highly sweet, low-calorie compounds. The sweetness of steviol glycosides is approximately 200–300 times that of sucrose, but the calorie content is only 1/300 of sucrose [25]. Saponins are a highly diverse class of triterpenoid glycosides with broad biological activities and play crucial roles in commercial applications in the fields of medicine, food, and cosmetics [58]. Ginsenoside Ro is the only oleanolic-type saponin in ginseng and possesses functions such as anti-tumor activity [59,60] and anticoagulant effects [61]. Asiaticoside is the major functional component of *Centella asiatica* and is a pentacyclic triterpene glycoside. It has wound healing and neuroprotective properties, as well as anti-inflammatory, antiviral, antidiabetic, and cardiomyocyte-protective activities [62–64].

SGs, such as stevioside and rebaudioside A, are widely used as sweeteners in consumer foods and beverages [65]. SG like Reb D and Reb M have a pleasant taste and are considered good sugar substitutes, but their natural production is limited [66]. However, biosynthesis offers a more promising application prospect for large-scale production of Reb D/M. Zhang et al. [67] characterized the biochemical and structural features of the rice glycosyltransferase OsUGT91C1 and found that the double mutant F379A/F208M had a catalytic efficiency 3–6 times higher than that of the wild-type OsUGT91C1. Liu et al. [68] reported the crystal structure of SrUGT76G1 in complex with various ligands and utilized SrUGT76G1 to efficiently synthesize the rare high-intensity sweetener Reb M from Reb D, while significantly reducing the formation of the byproduct Reb I. Guo et al. [64] obtained the variant UGT76G1-T284S/M88L/L200A through structure-guided evolution, which exhibited 2.38 times higher catalytic activity towards Reb D compared to UGT76G1-T284S and synthesized 23.37 mg/mL of Reb M.

Ginsenosides are considered the primary active ingredients responsible for various pharmacological activities [69]. Wei et al. [70] reported that UGTPg100 can glycosylate the C6-OH of ginsenoside PPT to produce bioactive ginsenoside Rh1, while UGTPg101 catalyzes the production of F1 from PPT, which is further converted to ginsenoside Rg1. Ren et al. [36] constructed an artificial dual glycosylation pathway for de novo biosynthesis of ginsenosides in Saccharomyces cerevisiae. In a 5 L fed-batch fermentation, the titer of Ro reached 528.0 mg/L, which is over 370,000 times higher than previously reported. Wang et al. [35] integrated UGTp45 and UGTp29 from ginseng into yeast, resulting in a titer of ginsenoside Rh2 reaching 179.3 mg/L. Zhuang et al. [37] synthesized 0.3 g/L of ginsenoside Rh2 in yeast utilizing engineered chimeric glycosyltransferase UGT51, which exhibited a catalytic efficiency 1800 times higher than that of the wild-type enzyme. Wang et al. [38] redesigned the complete biosynthetic pathway of CK to optimize UGTPg1 expression and achieved a production titer of 5.74 g/L of ginsenoside CK by enhancing the biosynthesis of UDP-glucose. Li et al. [39] successfully produced 1.95 g/L of ginsenoside Rg1, 1.62 g/L of notoginseng saponin R1, and 1.25 g/L of notoginseng saponin R2 in yeast by introducing UGTs such as PgUGT71A53, PgUGT94Q13, and PgUGT71A54. Ma et al. [40] improved the regio selectivity of Yjic for Rh2 synthesis through semi-rational design and found that the mutant M315F could effectively synthesize 3.7 g/L and block further glycosylation at C12-OH. Chu et al. [41] proposed a mutagenesis strategy to modify the glycosyltransferase UGTBL1 through loop engineering, PSPG motif evolution, and access tunnel engineering, which, combined with recharge batch cascade reaction for UDPG cycles, yielded 20.48 g/L of ginsenoside Rh1 and 18.04 g/L of 3-β-O-Glc-PPT.

Asiaticoside is a triterpenoid compound in *Centella asiatica* with wound healing and anti-inflammatory activities [71]. Zhao et al. [42] analyzed and identified 51 sugar transferases, deleted the glycosidase EGH1, and introduced key pathway enzymes CaUGT73C7.
and CaUGT73C8, ultimately synthesizing 772.3 µg/L of asiaticoside de novo in a 5 L fermentation tank. Kim et al. [72] discovered that UGT73AH1 catalyzes the glycosylation reaction of asiatic acid monosaccharides and found that it is very likely to be glycosylated at the C-28 position. Han et al. [73] screened out 75 UGTs and identified the sugar transferase CaUGT1, which can specifically transfer glucose to the C-28 carboxyl groups of asiatic acid and hydroxyasiatic acid. Costa et al. [74] identified UGT73AD1 as a UDP-glucose 28-O-glucosyltransferase, which has a relatively narrow specificity and can glycosylate monosaccharides in the C28 carboxyl group. This enzyme may be involved in the biosynthesis of saponins in the genus Centella. Cyanohydrin-type diterpenes are a unique class of naturally occurring compounds derived from mushrooms, possessing distinctive chemical scaffolds and diverse biological activities [75]. Ma et al. [76] synthesized cyanohydrin-type diterpenes de novo in Saccharomyces cerevisiae and obtained engineered yeast strains that produced seven "non-natural" cyanohydrin xylosides through combinatorial biosynthetic strategies. Li et al. [77] discovered that UGTPn87 exhibited mixed sugar donor specificity toward UDP-Glc and UDP-Xyl and elongated the second sugar chain at the C3 or/and C20 positions of proto-ginseng diol-type saponins. GA is a pentacyclic triterpene glycoside ligand and a major functional component of licorice, primarily existing in the form of functional glycosides in licorice. Gao et al. [23] identified GmSGT2, which specifically transfers a glucuronic acid to GA-3-O-mono-glucuronide (GAMG) to produce Gal-GAMG. Sun et al. [78] found that UGT99D1 can transfer arabinose to the C-3 position of pentacyclic triterpenes. They further constructed a sequential four-enzyme cascade system comprising sucrose synthase, UDP-Glc dehydrogenase, UDP-glucuronic acid decarboxylase, and UDP-Glc 4-epimerase to convert sucrose to UDP-Ara.

3. Glycosylation of Flavonoid and Its Derivatives

The majority of flavonoids exist in the form of glycosides, and due to their unique structures, they exhibit a wide range of physiological and pharmacological activities [79]. Glycosylation can alter the biological activities of flavonoids, increase their water solubility, reduce toxicity, and enhance specificity [80]. The main flavonoid glycosides include baicalin, naringin, astragalus sinensis, vincetoxin, and orientin, among others. Baicalin is an effective component of the root of the traditional Chinese medicine Scutellaria baicalensis, which has actions including antibacterial, diuretic, anti-inflammatory, spasm-relieving, and anticancer effects [81]. Naringin is an important medicinal flavonoid glycoside produced by various plants, which has pharmacological activities such as preventing cardiovascular diseases, antioxidant, antidiabetic, and anticancer effects [82–84]. Astragaloside, known as an effective compound in the herb Astragalus, has significant biological activity in protecting the liver, antiviral, cardiovascular protection, and immune regulation [85]. Flavonols, which have a ketone group and are a class of flavonoids, have antioxidant potential and reduce the risk of vascular diseases [86]. Compared to flavones, flavonols have a hydroxyl group at the third position of the C ring, which can also be glycosylated [87].

3.1. Glycosylation of Flavonoid Glycosides

Flavonoid glycosides are an important class of natural functional and flavor components. Zhang et al. [43] introduced the exogenous uridine diphosphate (UDP) glucose supply pathway, glucose transferase, rhamnose transferase, and UDP-rhamnose synthesis pathway, resulting in a 568.8 mg/L and 877.0 mg/L increase in the production of baicalein-7-O-glucoside and baicalein-7-O-rhamnoside, respectively. Chen et al. [29] used a three-enzyme cascade involving rhamnosyltransferase, Glycine max sucrose synthase, and UDP-rhamnose synthase to efficiently synthesize 231 mg/L of isorhamnetin-3-O-rhamnose. Wang et al. [44] overexpressed the flavonoid glycoside SbGT34 from Scutellaria baicalensis and produced 1.20 g/L 7-O-glucoside (S7G) by optimizing fermentation conditions, which demonstrated the feasibility of in vivo glycosylation scale-up in Staphylococcus aureus. Yuan et al. [88] identified six new flavonoid 7-O-glucosyltransferase genes (CgUGT89D30, CgUGT90A31, CgUGT89AK1, and CgUGT73AC12, PtUGT89AK1,
PtUGT90A31) and found that CgUGT89AK1 had the highest catalytic efficiency. Over-expression of CgUGT90A31 and CgUGT89AK1 led to increased synthesis of flavonoid glycosides (FG) in grapefruit leaves. Xu et al. [89] found that DcUGT88C3 can utilize lactose and anthocyanins to synthesize anthocyanin-3-O-lactoside, exhibiting optimal activity towards anthocyanins at 30 °C and pH 8.6. He et al. [90] reported that rCsUGT78A15 can catalyze the synthesis of anthocyanin 3-O-lactoside and delphinidin 3-O-lactoside using UDP-galactose as the sugar donor, with higher catalytic efficiency towards delphinidin. In a recent report, Zhao et al. [91] identified anthocyanin biosynthetic genes ABGs in different rice varieties, revealing that OsUGT88C3 can produce delphinidin 3-O-lactoside using UDP-galactose and cyanidin as substrates. Parajuli et al. [45] overexpressed glycosyltransferase UGT78K1, the ArGt-3 gene from Arabidopsis thaliana, and biosynthetic genes for UDP-glucose, TDP-rhamnose (glf, glk, and pgm2) in E. coli BL21, yielding approximately 1.18 g/L of fisetin 3-O-glucoside and 1.03 g/L of fisetin 3-O-rhamnoside in a 3 L bioreactor. Chen et al. [92] overexpressed the 3-O-glucosyltransferase AmGT5, the 3-hydroxy group transferase AmGT1G146V/I, the 25-O-glucosyltransferase AmGT9, the 20-O-glucosyltransferase AmGT8, and the 6-O-glucosyltransferase AmGT8A394F in Astragalus root, producing 13 astragalosides with a conversion rate increasing from 22.6% to 98.7%. Pei et al. [46] introduced the Arabidopsis AtUGT78D2 and UDP-glucose synthesis pathway into E. coli, achieving a yield of 3600 mg/L of astragaloside through fed-batch fermentation. Liu et al. [47] overexpressed the glycosyltransferase TcCGT1, rhamnosyltransferase GtfC, and galactosyltransferase PhUGT, resulting in production yields of salicin, verbascoside, aucubin, quercitr, hyperoside, and trifolioside reaching 17.2, 36.5, 5.2, 14.1, 6.4, and 11.4 g/L, respectively. Bao et al. [93] discovered a highly efficient flavonoid 6-C-glucosyltransferase GcCGT from Gentiana crassicaulis, and through homology modeling and site-directed mutagenesis obtained the mutant F387K, which catalyzes the 6-C glucosylation of flavonoid 8-C-glucoside, producing 6,8-di-C-glucoside. Xue et al. [94] designed the mutant 49A/50A-Cm1, 2RhaT based on semi-rational design and error-prone PCR mixed strategy, increasing the catalytic efficiency towards naringenin 7-O-glucoside by 136-fold, with a new naringenin yield reaching 7.63 g/L. He et al. [95] reported that glycosyltransferase TcCGT1 efficiently and selectively catalyzes the regional and efficient 8-C glucosylation of 36 flavonoids and other flavonoid compounds, as well as the O-glycosylation of various phenolic compounds. UGT708C1 from Fagopyrum esculentum (buckwheat) uses UDP-glucose as the sugar donor and catalyzes the C-glucosylation of 2-hydroxyflavones [96]. Xiao et al. [97] constructed a UDP-rhamnose regeneration system, which synthesizes 131.3 mg/L sanchinoside, 179.9 mg/L rutin, 276.6 mg/L hesperidin, 249.0 mg/L neohesperidin, 30.4 mg/L Diosmin, and 100.7 mg/L neosanchinoside. Zong et al. [98] determined the crystal structure of the rhamnose transferase UGT89C1 in Arabidopsis plants. UGT89C1 exhibited activity against various flavones and their derivatives, such as quercetin, kaempferol, and benzenediols. Thoma et al. [99] characterized and identified the N-acetylglucosamine transferase GnT-I, which catalyzes the transfer of N-acetylglucosamine from UDP-N-acetylglucosamine to the α-1,3 Man antenna of Man5GlcNAc2.

### 3.2. Glycosylation of Flavonols

Ginseng contains various flavonol glycosides with multiple biological activities such as antioxidant, anticancer, anti-inflammatory, and anti-hyperglycemic activities [100]. Yin et al. [101] used proteomics data analysis to reveal that UGT73A18, UGT74T4, and UGT79W1 are involved in the second galactosylation of flavonoid glycosides, which clarified the mechanism of flavonol glycosylation. Chen et al. [102] explored the promiscuity of the novel benzoin C-glycosyltransferase MiCGT and found that MiCGT can generate C-xyloside and C-glucoside from UDP-xylose. Wen et al. [103] used quercetin as a model substrate to evolve regionally selective mutants of MiCGT and produced two glycosylation products, which are quercetin 7-O-glycoside and quercetin 3-O-glycoside. Wang et al. [48] identified four key enzymes, HmF3H1-2, HmFLS, HmF3H, and HmGAT, among which HmF3H and HmFLS catalyze the conversion of flavanones to dihydroflavonols, and can
also catalyze the conversion of dihydroflavonols to flavonols. Ultimately, the synthesis of hyperoside with a titer of 25 mg/L was successfully achieved using naringin as the substrate in *E. coli* BL21. Li et al. [104] conducted transcriptomic and phylogenetic analysis of UGTs and found that KT324624 was a crucial enzyme involved in rutin synthesis, converting 3-O-glucoside of syringin/quercetin to 3-O-rutinoside of syringin and rutin. Wen et al. [30] reported that the glycosyltransferase HuUGT72AS can directly accept six sugar donors (UDP-glucose/-arabinose/-galactose/-xylose/-N-acetylglucosamine/-rhamnose) to catalyze the 3-OH glycosylation of flavonols. Through structural modeling and mutagenesis analysis, the Tyr377 mutation to Ara377 enhanced the catalytic efficiency of HtUGT72AS1 towards UDP-N-acetylglucosamine, and the V146S mutant improved the region selectivity for the 7-OH of flavonoid compounds.

### 4. Glycosylation of Polyketide and Its Derivatives

Pyrone-type compounds have important biological activities such as antiviral, antibacterial, antineoplastic, metabolic regulation, immune modulation, and anti-insecticidal activities, and are widely used in medicine, food, and other fields [105]. These compounds are mainly synthesized by pyrone polymerase to form a pyrone core, which is then modified by complex rare sugar-mediated glycosylation, hydroxylation, and methylation to ultimately form active molecules. Therefore, glycosylation modification contributes to the structural diversity, complexity, and specific biological activity of pyrone-type compounds [106]. As one of the main pyrone glycosides in Rhodiola, salidroside exhibits antioxidant, adaptogenic, anti-stress, antibacterial, immune-modulating, vasoregulatory, and anti-tumor effects [107,108]. Polydatin is a kind of polyketide glycoside drug with anticancer, anti-aging and anti-inflammatory effects [109–111]. Erythromycin is a typical member of the macrolide antibiotic family, and the modification of rare sugars determines its antimicrobial activity, while the oxidation of the cyclic peptide confers its antibiotic activity. Pactamycin is an anticancer drug produced by Streptomyces pactum, which has potent antibacterial, anti-tumor, antiviral, and antiprotozoal activities [112,113].

Xue et al. [49] reported the production of 6.7 mg/L of astaxanthin in *E. coli* through the overexpression of UGT72B14, which is 3.2-fold higher than that of the wild-type GT. Liu et al. [50] identified the key enzyme PcR3GAT in the synthesis of polydatin, which increased the yield of polydatin from 70 mg/L to 545 mg/L. Liu et al. [51] constructed a biosynthetic pathway for 3-O-α-mycarosylerythronolide B (MEB) in *E. coli*, using multi-strategy metabolic engineering to increase its titer to 48.3 mg/L, which is 11.5-fold higher. Eida et al. [114] reported that glycosylation can occur on the polyketide intermediate bound to acyl carrier protein (ACP) in pactamycin biosynthesis. The oxo-propionyl-ACP intermediate is glycosylated by the N-glycosyltransferase PtmJ, providing the sugar precursor for the formation of the aminocyclopentitol core structure of pactamycin. Kudo et al. [115] glycosylated 3ABA-PctK with uridine diphosphate-N-acetyl-D-glucosamine (UDP-GlcNAc) using the glycosyltransferase PctL to produce GlcNAc-3ABA-PctK. Gu et al. [52] introduced the disaccharide phosphate degradation pathway and the Arabidopsis UDP-rhamnose synthase (AtRHM) into the recombinant strain, achieving a maximum production of UDP-rhamnose reaching 82.2 mg/L. Through the optimization of biotransformation conditions, they were able to reach a peak production of quercetin at 7.6 g/L.

### 5. Glycosylation of Aromatic and Its Derivatives

Aromatic compounds and their derivatives are widely used in areas such as medicine, flavoring, and feed. Through glycosylation, the pharmacokinetics and pharmacological properties of aromatic compounds can be improved, leading to the design of more effective drugs. Gastrodin, a phenolic glycoside, is the main component of *Gastrodia elata* and is well known for its sedative, hypnotic, anticonvulsant, and neuroprotective activities [116,117]. Rosavin is a natural active product from the root of *Rhodiola rosea* and has various biological activities such as anti-tumor, antibacterial, anti-depression, and antioxidant [117,118]. Zhang et al. [119] identified and explained the catalytic mechanism of a CGT enzyme in
licorice named GgCGT, which can catalyze two-step C-glycosylation reactions of substrates containing trihydroxyphenylacetone groups. Li et al. [120] used a sugar bromide as a sugar precursor to synthesize various C-aryl glycosides with an aryl group at the meta position. Gu et al. [53] introduced 50 gene modifications into the yeast genome and successfully obtained 13.4 g/L of Gastrodin in a 5 L bioreactor. Li et al. [31] identified four UGTs capable of catalyzing the formation of rosavin from agarwood, belonging to the UGT91R subfamily, with SlUGT91R1 showing the highest activity level. Subsequently, by integrating SlUGT91R1 and the UDP-arabinose pathway into the production of rosavin, the yield of rosavin reached 7.54 g/L.

6. Glycosylation of Steroid and Its Derivatives

Steroidal saponins are one of the most abundant and biologically active groups of drugs found in plants, and they are also one of the most widely distributed secondary metabolites in plants. Glycosylation plays an important role in the structural complexity and diversity, chemical stability, water solubility, and biological activity of saponins [70]. Chen et al. [121] characterized six UGTs (UGT80A40, UGT80A41, UGT80A33, UGT80A34, UGT73CE1, UGT91AH 1-3), and found that 2′-O-rhamnoglycosyltransferase UGT73CE1 and 6′-O-glycosyltransferase UGT91AH 1–3 catalyze the sequential glycosylation to synthesize steroid disaccharides and trisaccharide glycoside, resulting in the production of 24 terpenoid glycosides. He et al. [54] cloned and identified the sterol glycosyltransferase PpUGT6, which exhibits promiscuous glycosylation at the C-3 of pennogenin sapogenin of polyphyllin, the C-17 position of testosterone and methyltestosterone, and the C-3 position of GA. Song et al. [122] identified three types of UGTs involved in the glycosylation of saponins in Paris polyphylla plants. They found that UGT73CR1, UGT80A33, and UGT80A34 can catalyze the attachment of glucose to the C-3 position of the diosgenin to form oleanolic acid. Among them, UGT73CR1 has good catalytic activity for steroidal saponins and steroidal alkaloids. Li et al. [55] isolated a three-leaf rhamnogalacturonan glycosyltransferase DzGT1 from D. zingiberensis and expressed it heterologously in E. coli. The results showed that DzGT1 could glycosylate three-leaf saponins to form proapogenin A of diooscin (PSA).

Huang et al. [56] identified the catalytic mechanism of the glycosyltransferase UGT74AN2 in bitter melon, and used it to catalyze the C-3 glycosylation of different structural cardiac glycosides. UGT74AN2 can also catalyze the glycosylation of other types of NPs such as flavonoids. Huang et al. [123] identified a glycosyltransferase UGT74AN3 from chrysanthemum that has strong cardiac glycoside glycosylation ability, and found that UGT74AN3 has a strong substrate versatility. UGT74AN3 can utilize UDP-glucose to glycosylate 78 different types of small molecules (flavonoids, steroids, terpenes, and alkaloids). Additionally, UGT74AN3 also has promiscuity towards sugar donors, being able to utilize six different UDP sugar donors for glycosylation reactions, with UGT74AN3 showing a clear preference for UDP-Glc. Wen et al. [124] identified a steroidal glycosyltransferase UGT74AN1 from Asclepias curassavica, which was found to have catalytic efficiency and regioselectivity for the glycosylation of cardenolide and bufadienolide glycosides, producing 3-O-β-D-glucosides. They also found that UGT74AN1 can catalyze the glycosylation of C21 steroid precursors and exhibit extensive promiscuity in generating on various drug-like scaffolds.

7. Conclusions

Glycosylation, as one of the most important biochemical reactions in nature, plays a crucial role in many fundamental processes. Due to its importance and specificity, it has long been a widely studied topic. UGTs form glycosidic bonds in a variety of compounds such as polysaccharides, proteins, lipids, antibiotics, flavonoids, and steroids, thereby improving their chemical and biological properties. The role of UGTs in disease development and their potential as therapeutic targets is also gaining attention. This review provides a comprehensive overview of the progress in research on the glycosylation of natural products, covering the involvement of glycosylation in the biosynthesis of steviol
glycosides, ginsenosides, asiaticosides, baicalin, naringin, astragaloside IV, rhodioloside, and polygonum cuspidatum glycosides.

However, despite some progress, research on natural product glycosylation still faces many challenges. Producing specific NPs glycosides depends on the biosynthetic pathway of NPs precursors, which are often incomplete. In this case, candidate pathway design, enzyme selection, and pathway testing all present different challenges. When introducing UGIs into heterologous hosts, they may not function optimally or at all due to reasons such as low expression, improper folding, and incorrect localization. Given the current urgent need for affordable, effective, and synthetically inefficient drugs in nature, conducting in-depth research on UGT functions necessary for modifying glycosylated NPs synthesis, and adjusting the biosynthetic system to increase the production of these NPs, has become an increasingly important research focus.

In the future, utilizing artificial intelligence-driven synthetic biology to decipher synthesis pathway-related genes, rate-limiting steps, co-factor supply, and metabolic flux balance will further promote large-scale synthesis and the application of glycosylated NPs, increasing the diversity of NP glycosides used in pharmaceuticals, functional foods, and cosmetics in industrial production.

Author Contributions: Writing—original draft preparation, S.H., B.W., L.P. and J.W.; writing—review and editing, Y.G., L.J., B.L., W.L. and J.C.; visualization, J.C.; supervision, J.C.; project administration, J.C.; funding acquisition, W.L. and J.C. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Chengdu Science and Technology Project (2024-YF08-00022-GX), the Open Fund Projects for Key Laboratory of Coarse Processing, Ministry of Agriculture and Rural Affairs (2020CC019), the Open Fund Projects for Key Laboratory of Medicinal and Edible Plants Resources Development (10Y202107), 2024 Sichuan Provincial College Student Innovation Training Program Project, and the Open Funding Project of Meat Processing Key Laboratory of Sichuan Province (23-R-07).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study.

Conflicts of Interest: Authors Liang Pei and We Li were employed by Sichuan Ingia Biosynthetic Co., Ltd. Authors Jisheng Wang was employed by the 9th Geological Brigade of Sichuan. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References
4. Majhi, S. Applications of yamaguchi method to esterification and macrolactonization in total synthesis of bioactive natural products. ChemistrySelect 2021, 6, 4178–4206. [CrossRef]
Fermentation 2024, 10, 349


29. Chen, A.; Gu, N.; Pei, J.; Su, E.; Duan, X.; Cao, F.; Zhao, L. Synthesis of isorhamnetin-3-0-rhamnoside by a three-enzyme (rhamnosyltransferase, glycine max sucrose synthase, udp-rhamnose synthase) cascade using a udp-rhamnose regeneration system. *Molecules* 2019, 24, 3042. [CrossRef]


55. Li, J.; Mosongo, I.; Li, H.; Wu, Y.; Li, C.; Yang, S.; Zhang, Y. Identification and characterization of a trillin rhamnosyltransferase from *Dioscora zingiberensis*. *Front. Plant Sci.* 2021, 12, 713036. [CrossRef]


63. Wong, J.H.; Barron, A.M.; Abdullah, J.M. Mitoprotective effects of *Centella asiatica* (L.) Urb.: Anti-inflammatory and neuroprotective opportunities in neurodegenerative disease. *Front. Pharmacol.* 2021, 12, 687935. [CrossRef]


68. Liu, Z.; Li, J.; Sun, Y.; Zhang, P.; Wang, Y. Structural insights into the catalytic mechanism of a plant diterpene glycosyltransferase srugt76g1. Plant Commun. 2020, 1, 100004. [CrossRef]


70. Wei, W.; Wang, P.; Wei, Y.; Liu, Q.; Yang, C.; Zhao, G.; Yue, J.; Yan, X.; Zhou, Z. Characterization of panax ginsengudp-glycosyltransferases catalyzing protopanaxatriol and biosynthesis of bioactive ginsenosides f1 and f2 in metabolically engineered yeasts. Mol. Plant 2015, 8, 1412–1424. [CrossRef]

71. Rachpirom, M.; Pichayakorn, W.; Puttarak, P. Preparation, development, and scale-up of standardized pentacyclic triterpenoid-rich extract from Centella asiatica (L.) urb. and study of its wound healing activity. Helixagon 2023, 9, e17807. [CrossRef] [PubMed]


73. Han, X.; Zhao, J.; Chang, X.; Li, Q.; Deng, Z.; Yu, Y. Revisiting the transcriptome data of osugt78a15 catalyzes the anthocyanidin 3-o-galactoside biosynthesis in tea plants. Plant Physiol. Biochem. PPB 2022, 166, 738–749. [CrossRef]


80. Jiang, N.; Doseff, A.I.; Grotewold, E. Flavonoids: From biosynthesis to health benefits. Plants 2016, 5, 27. [CrossRef]


84. Jerger, M.; Boje, K.; Huwel, S.; Lohmann, C.; Gall, H.J.; Nahrstedt, A. In vitro studies indicate that miquelianin (quercetin 3-o-beta-d-glucuronopyranoside) is able to reach the cns from the small intestine. Planta Medica 2003, 69, 1013–1017. [CrossRef]


Yin, Q.; Han, X.; Chen, J.; Han, Z.; Shen, L.; Sun, W.; Chen, S. Identification of specific glycosyltransferases involved in flavonoid c-glucosyltransferases from Fagopyrum esculentum M. (buckwheat) cotyledon. Plant J. 2014, 80, 437–448. [CrossRef] [PubMed]


Zong, G.; Fei, S.; Liu, X.; Li; Gao, Y.; Yang, X.; Wang, X.; Shen, Y. Crystal structures of hmannosyltransferase ugt89c1 from Arabidopsis thaliana reveal the molecular basis of sugar donor specificity for udp-β-l-rhamnose and hmannosylation mechanism. Plant J. 2019, 99, 257–269. [CrossRef] [PubMed]


Recio, M.C.; Giner, R.M.; Mañez, S. Immunomodulatory and antiproliferative properties of Rhodiola species. Planta Medica 2016, 82, 952–960. [CrossRef]


Wang, H.L.; Gao, J.P.; Han, Y.L.; Xu, X.; Wu, R.; Gao, Y.; Cui, X.H. Comparative studies of polydatin and resveratrol on mutual transformation and antioxidative effect in vivo. Phytotherapy Research. 2015, 22, 553–559. [CrossRef]


120. Li, J.; Korvorapun, K.; De Sarkar, S.; Rogge, T.; Burns, D.J.; Warratz, S.; Ackermann, L. Ruthenium(II)-catalysed remote C-H alkylation as a versatile platform to meta-decorated arenes. *Nat. Commun.* 2017, 8, 15430. [CrossRef]


**Disclaimer/Publisher’s Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.