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Enantioselective glucosylation of (±)-secondary alcohols with plant glucosyltransferases

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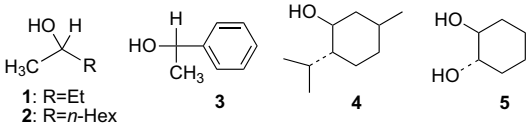
Abstract—Two glucosyltransferases were isolated from plant cell cultures of *Catharanthus roseus* and *Nicotiana tabacum*. The enzyme from *C. roseus* enantioselectively glucosylated (±)-secondary alcohols to give the glucosides of (*R*)-alcohols, while the glucosylation with that from *N. tabacum* gave preferentially the glucosides of (*S*)-alcohols.

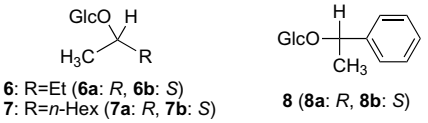
1. Introduction

Glycosylation using biocatalysts is very attractive in the practical preparation of alkyl glycosides, because one-step enzymatic glycosylation is more advantageous than chemical glycosylation, which requires tedious steps such as protection and deprotection of sugar hydroxyl groups. The enantioselectivities of enzymatic glycosylation have been studied in the transglycosylation and reverse hydrolysis with glycosidases.[1,2] However, little attention has been paid to the enantioselective glycosylation by glycosyltransferases. Recently, enantioselective glucosylation of a (±)-secondary alcohol with plant cell cultures has been reported; the glucosylation with *Catharanthus roseus* occurred enantioselectively to give the glucoside of the (*R*)-alcohol, while the glucosylation with *Nicotiana tabacum* preferentially gave the glucoside of the (*S*)-alcohol.[3] Over the course of developing a new enzymatic asymmetric synthesis method, we investigated the enantioselective glucosylation with plant glucosyltransferases from *C. roseus* and *N. tabacum*.

2. Results and discussion

Two glucosyltransferases named GTF-I and II were isolated from the corresponding plant cell cultures of *C. roseus* and *N. tabacum*, respectively, by three steps of column chromatographies.[4–7] First, (±)-secondary alcohols **1**–**5** (10 mg each) were administered to 10 mL of 50 mM HEPES buffer (pH 7.0) containing ca. 50 μg of isolated GTF-I from *C. roseus* and 40 mg of UDP-glucose and incubated at 35 °C for 24 or 36 h. The yields of the product glucosides were determined by HPLC analyses.[8] Extraction from the reaction mixture with 1-butanol followed by purification using column chromatography on silica gel with $CHCl_3$–MeOH (95:5, v/v) gave the products. The *J* values in the ^1H NMR signal for the anomeric protons of the resulting glucosides showed that the sugar moiety of the products was of β-orientation. The absolute configurations of the aglycone moieties of the products were confirmed by direct comparison of the ^1H NMR spectra with the authentic glucosides synthesized from enantiomerically pure





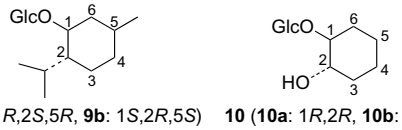


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Table 1. Enantioselective glucosylation of (±)-secondary alcohols with GTF-I from *C. roseus*

Substrates	Products	Reaction time (h)	Conversion (%)	De (%)[a]	E[b]	Configuration[c]
1	**6a**	24	25	78	11	*R*
2	**7a**	24	22	84	15	*R*
3	**8a**	36	28	87	20	*R*
4	**9a**	36	17	98	>100	*R*
5	**10a**	24	39	>99	>100	*R*

[a] % De(s) were determined on the basis of the intensities of the 1-methyl proton signals in the ^{1}H NMR of glucosides **6** and **7** and the anomeric proton signals in the ^{1}H NMR of glucosides **8**–**10**.
[b] Calculated from $ee_{substrate}$ and $ee_{product}$ using standard equation.[15]
[c] Preferred configuration at the aglycone moieties of the products.

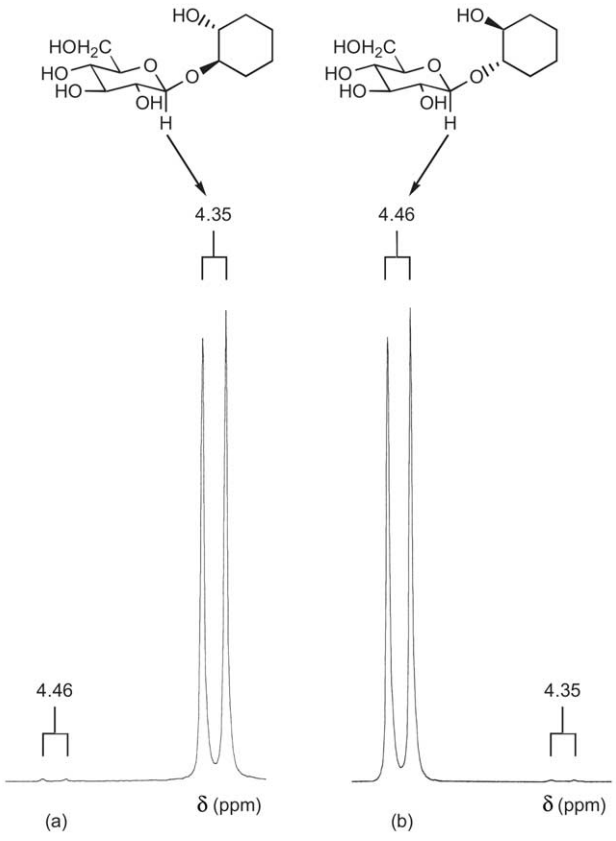

Figure 1. Pairs of anomeric proton signals in ^{1}H NMR of the products **10** obtained by the glucosylation of **5** with (a) GTF-I from *C. roseus* and (b) GTF-II from *N. tabacum*.

(*R*)- and (*S*)-alcohols by chemical glucosylation.[9,10] The diastereomeric compositions of the products were determined based on the intensities of the 1-methyl or anomeric proton signals in the ^{1}H NMR spectra.[11–13] The enantioselectivity was greatly improved upon when using the isolated GTF-I, whereas glucosylation with the microsomal crude enzyme resulted in lower de(s) of 57–65%, suggesting that the enantioselectivity of the glucosylation with the microsomal enzyme might be affected by impurities such as glucosidases or other glucosyltransferases. It was found that **1**–**4** could be glucosylated to give the corresponding glucosides of (*R*)-alcohols by GTF-I (Table 1). Although the glucosylation of **1** showed relatively low enantioselectivity (78% de), it was improved to 84% de when **2**, which has a long alkyl chain, was used as the substrate. In the case of **4**, the glucosylation resulted in a high diastereomeric excess of 98%. A considerably challenging diol-substrate for enantioselective glucosylation, **5**, was also glucosylated to the mono-glucoside with an (*R*)-configuration in its aglycone part by GTF-I, allowing us to achieve the highest de of >99% (Fig. 1a).[14] These results demonstrate that glucosylation with the glucosyltransferase from *C. roseus* occurred enantioselectively to give the glucosides of (*R*)-alcohols and that the substrates with hydroxyl group(s) attached to the cyclohexane ring could be glucosylated with excellent enantioselectivity.

Substrates **1**–**5** were next subjected to glucosylation with GTF-II from *N. tabacum* and then glucosylated to the corresponding glucosides having an (*S*)-configuration at their aglycone moieties (Table 2). It is noteworthy that GTF-II glucosylated **3**–**5** to (*S*)-alkyl glucosides **8b**–**10b** with very high enantioselectivity [de(s) of >99% and 100%] (Fig. 1b), suggesting that enantioselective glucosylation with GTF-II is useful, as a new enzymatic enantiomer discriminating reaction, for the practical preparation of alkyl glucosides in diastereomerically pure form. The results obtained herein reveal that the glucosylation with the glucosyltransferase from

Table 2. Enantioselective glucosylation of (±)-secondary alcohols with GTF-II from *N. tabacum*

Substrates	Products	Reaction time (h)	Conversion (%)	De (%)[a]	E[b]	Configuration[c]
1	**6b**	24	24	90	25	*S*
2	**7b**	24	20	93	35	*S*
3	**8b**	36	26	>99	>100	*S*
4	**9b**	36	21	100	>100	*S*
5	**10b**	24	33	>99	>100	*S*

[a] % De(s) were determined on the basis of the intensities of the 1-methyl proton signals in the ^{1}H NMR of glucosides **6** and **7** and the anomeric proton signals in the ^{1}H NMR of glucosides **8**–**10**.
[b] Calculated from $ee_{substrate}$ and $ee_{product}$ using standard equation.[15]
[c] Preferred configuration at the aglycone moieties of the products.

N. tabacum affords the glucosides of the (*S*)-alcohols with excellent enantioselectivity.

3. Conclusion

The enantioselective glucosylation with two glucosyltransferases from *C. roseus* and *N. tabacum* has been accomplished with high enantioselectivity. It should be emphasized that the enantioselectivities in the glucosylation of the (±)-secondary alcohols were opposite between these enzymes and that each diastereomer of alkyl glucosides can be synthesized by selective use of these glucosyltransferases.

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4. Homogenates of cultured cells of *C. roseus*[5] in 100 mM HEPES buffer (pH 7.0) were centrifuged at 10,000*g* for 30 min to give a cell free extract, which itself was centrifuged at 100,000*g* for 2 h to give a microsomal enzyme fraction as the precipitation. After solubilization of the enzymes from the microsomal fraction with 0.1% Triton X-100, purification of the solubilized enzymes by chromatographies on a REACTIVE GREEN 19 agarose gel column, a Sephadex G-200 column and then a diethylaminoethyl-Toyopearl column gave homogeneous glucosyltransferase as judged by SDS-PAGE: GTF-I, monomeric form with molecular mass of ca. 72 kDa. The glucosyltransferase was isolated from cultured cells of *N. tabacum*[5] by the same procedure: GTF-II, monomeric form with molecular mass of ca. 51 kDa.
5. The suspension cells of *C. roseus* were cultured in 500 mL conical flasks containing 300 mL of SH medium supplemented with 3% sucrose and 10 mM 2,4-dichlorophenoxyacetic acid (2,4-D) under illumination (4000 lux).[6] The suspension cells of *N. tabacum* were cultured in 500 mL conical flasks containing 300 mL of Murashige and Skoog's (MS) medium supplemented with 1% sucrose and 5 mM 2,4-D under illumination (4000 lux).[7] Each suspension cells were incubated on a rotary shaker (75 rpm) at 25 °C for 3 weeks prior to use for enzyme preparation.
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8. Conditions for HPLC analysis: column, Puresil C18 column (Waters); detector, differential refractometer (Waters); solvent, MeOH–H_2O (1:3, v/v); flow rate, 1 mL min^{-1}.
9. Authentic glucosides **6**–**10** were prepared according to the previously reported procedure.[10] Enantiomerically pure alcohols (12 mg each) were added to a mixture of 2,3,4,6-tetra-*O*-acetyl α-D-glucopyranosyl fluoride (53 mg), 1,1,3,3-tetramethylguanidine (35 mg) and BF_3–OEt_2 (0.05 mL) in acetonitrile (2 mL) and stirred for 2 h at room temperature. Extraction of organic materials with ethyl acetate followed by purification using column chromatography on silica gel with hexane–ethyl acetate (9:1, v/v) afforded alkyl 2,3,4,6-tetra-*O*-acetyl D-glucopyranosides as a ca. 1:9 α/β-mixture, which were hydrolyzed with saturated K_2CO_3 to give 5–10 mg of authentic glucosides as a mixture of α- and β-anomers (ca. 1:9).
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11. The intensities of the pair of 1-methyl proton signals in the ^{1}H NMR (CD_3OD) spectra were used for the determination of the diastereomeric excesses of **6** and **7**. 1-Methyl proton signals of the products were as follows: δ 1.15 (d, J = 6.4 Hz, for **6a**) and 1.23 (d, J = 6.1 Hz, for **6b**); δ 1.15 (d, J = 6.4 Hz, for **7a**) and 1.21 (d, J = 6.4 Hz, for **7b**). The intensities of the pair of anomeric proton signals in the ^{1}H NMR (CD_3OD) spectra were used for the determination of the diastereomeric excesses of **8**–**10**. Anomeric proton signals of the products were as follows: δ 4.40 (d, J = 7.3 Hz, for **8a**) and 4.10 (d, J = 8.0 Hz, for **8b**); δ 4.36 (d, J = 7.6 Hz, for **9a**) and 4.32 (d, J = 7.6 Hz, for **9b**); δ 4.35 (d, J = 7.6 Hz, for **10a**) and 4.46 (d, J = 7.6 Hz, for **10b**). The enantiomeric compositions of the aglycone moieties of products **6**–**10** were confirmed by chiral GLC analysis of the hydrolyzed alcohols, which had been prepared by hydrolysis of the products with almond-β-glucosidase.[12,13]
12. Product glucosides were incubated with almond-β-glucosidase (90 U) and 1 mL of phosphate buffer (0.1 M, pH 6.0) at 37 °C for 24 h. After 24 h incubation, the glucosides were completely hydrolyzed to the alcohols as judged by HPLC and TLC analyses of the reaction mixture. The reaction mixture was extracted with diethyl ether to give a crude alcohol fraction, which was purified by column chromatography on silica gel with pentane-ethyl acetate (9:1, v/v) to yield the hydrolyzed alcohols.
13. Conditions for capillary GLC analysis: column, Rt-βDEX (Restek, 0.25 mm × 30 m); injector, 180 °C; detector, 180 °C; oven, 100 °C; carrier gas, N_2 (50 mL min^{-1}). Retention times for alcohols **1**–**5** in the GLC were as follows: (*S*)- and (*R*)-**1**, 9.7 and 10.2 min; (*S*)- and (*R*)-**2**, 12.6 and 13.2 min; (*R*)- and (*S*)-**3**, 15.1 and 16.5 min; (1*S*,2*R*,5*S*)- and (1*R*,2*S*,5*R*)-**4**, 20.0 and 20.3 min; (1*S*,2*S*)- and (1*R*,2*R*)-**5**, 27.7 and 28.9 min.
14. Product **10a** converted from **5** by GTF-I: $[\alpha]_D^{25} = -15$ (*c* 0.20, MeOH) {lit.[2] $[\alpha]_D^{20} = -17$}; **10b** glucosylated by GTF-II: $[\alpha]_D^{25} = -23$ (*c* 0.18, MeOH) {lit.[2] $[\alpha]_D^{20} = -22$}. The optical rotation data of the products **6**–**9** could not be obtained due to the low transformation rate and lack of products.
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