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Glucosylation of benzyl alcohols by the cultured suspension cells of *Nicotiana tabacum* and *Catharanthus roseus*

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Abstract

The cultured cells of *Nicotiana tabacum* (white cells) converted regioselectively exogenous 2-, 3-, and 4-hydroxybenzyl alcohols into corresponding hydroxybenzyl-β-D-glucopyranoside. (*RS*)-1-Phenylethanol having chiral center in its substituent was also glucosylated to give 1-phenylethyl-β-D-glucopyranoside by the cultured cells of *N. tabacum* (white and green cells) and *Catharanthus roseus*. The glucosylation with the green cells of *N. tabacum* occurred enantioselectively to give the glucoside of (*S*)-alcohol preferentially, while the glucosylation with the white cells of *N. tabacum* and the *C. roseus* cells gave preferentially the glucoside of (*R*)-alcohol.

Keywords: Glucosylation; Chiral discrimination; Hydroxybenzyl alcohol; 1-Phenylethanol; *Nicotiana tabacum*; *Catharanthus roseus*; Cultured cells

1. Introduction

Biological transformation has enabled the accomplishment of many stereochemical reactions hitherto possible only under numerous and difficult chemical steps. As a result, many stereoselective reactions are now carried out with the use of biocatalysts to achieve structural modification of important pharmacologically active compounds. Studies on glucosylation of phenols [1–6], steroids [7,8], terpenoids [9–17], organic acids [18–22] have been carried out using cultured cells. Although biological transformation is usually regio- and enantioselective, until recently little attention has been given to regio- and enantioselectivity of glucosylation reaction [21]. We have investigated the regio- and enantioselectivities of glucosylation occurring in the cultured cells of green and white cells of *Nicotiana tabacum* and green cells of *Catharanthus roseus*, using benzyl alcohols such as 2-, 3-, and 4-hydroxybenzyl alcohols (**1–3**) having both of a primary and a phenolic hydroxyl group in its molecule and racemic 1-phenylethanol (**4**) and its acetate (**5**) [23].

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2. Experimental

2.1. Analysis

NMR spectra were measured in $CDCl_3$ on a JEOL GSX500 [500 MHz (^1H) and 125.8 MHz (^{13}C)] or a GSX270 [270 MHz (^1H) and 67.9 MHz (^{13}C)] NMR spectrometer. FAB-MS were taken on a JEOL SX102A mass spectrometer. GLC was carried out with CP-cyclodextrin β-236-M-19 (Chrompack) column, and HPLC with Puresil C18 (Waters) column using MeOH:H_2O = 1:3 (v/v) as eluent.

2.2. Plant materials

White suspension cells of *N. tabacum* [24] were cultured in 500 ml conical flasks containing 200 ml Murashige and Skoog's (MS) medium [25] supplemented with 3% sucrose and 10 mM 2,4-dichlorophenoxyacetic acid (2,4-D) in the dark. Green suspension cells of *N. tabacum* were obtained by cultivating the white cells under illumination (4000 lux) in MS medium with 1% sucrose and 5 mM 2,4-D. The suspension cells of *C. roseus* [26] were cultured in 500 ml conical flasks containing 200 ml of SH medium [27] supplemented with 3% sucrose and 10 mM 2,4-D under illumination (4000 lux). Each suspension cells were cultivated on a rotary shaker (75 rpm) at 25°C for 3 weeks prior to use for biotransformation experiments.

2.3. Biotransformation of hydroxybenzyl alcohols (*1*–*3*) by the cultured cells of N. tabacum

Five milligrams of the substrates such as 2-, 3- and 4-hydroxybenzyl alcohols (**1–3**) dissolved in 50 μl EtOH was administered to the flask containing the white cells (150 g) of *N. tabacum* in 500 ml of MS medium at 1-day intervals. A total of 0.2 mmol of the substrate (30 mg) was administered. The cultures were incubated for additional 5 days after final administration at 25°C on a rotary shaker (75 rpm). After incubation, the cells and medium were separated by filtration. The cells were extracted with MeOH and the extract was concentrated by evaporation in vacuo. The methanolic fraction was partitioned between H_2O and *n*-BuOH. On the other hand, the filtered medium was extracted with *n*-BuOH. The combined butanolic fractions were subjected to preparative TLC with MeOH: $CHCl_3$ (1:4) to give glucosides (1.5 mg of 2-hydroxybenzyl-β-D-glucopyranoside (**6**), 13 mg of 3-hydroxybenzyl-β-D-glucopyranoside (**7**), 7 mg of 4-hydroxybenzyl-β-D-glucopyranoside (**8**)). The structures of the products were identified on the basis of ^1H and ^{13}C NMR and FAB mass spectra [28].

2-Hydroxybenzyl-β-D-glucopyranoside (**6**): FAB-MS *m/z* 309 $[M + Na]^+$; ^1H NMR (D_2O) δ 3.31–3.49 (m, 4H, H-2′, 3′, 4′, 5′), 3.75 (dd, 1H, J = 12.2, 5.4 Hz, H-6a′), 3.94 (d, 1H, J = 12.2 Hz, H-6b′), 4.56 (d, 1H, $J_{1'\text{-}2'}$ = 8.3 Hz, H-1′), 4.80 (d, 1H, J = 11.2 Hz, H-7a), 4.96 (d, 1H, J = 11.2 Hz, H-7b), 6.92 (dd, 1H, J = 7.6, 1.2 Hz, H-3), 6.95 (td, 1H, J = 7.6, 1.2 Hz, H-5), 7.34 (td, 1H, J = 7.7, 1.2 Hz, H-4), 7.41 (dd, 1H, J = 7.6, 1.2 Hz, H-6); ^{13}C NMR (D_2O) δ 60.4 (C-6′), 66.7 (C-7), 69.2 (C-4′), 72.7 (C-2′), 75.4 (C-3′), 75.5 (C-5′), 100.9 (C-1′), 115.4 (C-3), 120.2 (C-5), 122.7 (C-1), 130.0 (C-4), 130.9 (C-6), 154.0 (C-2).

3-Hydroxybenzyl-β-D-glucopyranoside (**7**): FAB-MS *m/z* 309 $[M + Na]^+$; ^1H NMR (D_2O) δ 3.32–3.48 (m, 4H, H-2′, 3′, 4′, 5′), 3.73 (dd, 1H, J = 12.2, 4.9 Hz, H-6a′), 3.94 (d, 1H, J = 12.2 Hz, H-6b′), 4.52 (d, 1H, $J_{1'\text{-}2'}$ = 7.8 Hz, H-1′), 4.70 (d, 1H, J = 11.7 Hz, H-7a), 4.89 (d, 1H, J = 11.7 Hz, H-7b), 6.88 (dd, 1H, J = 7.9, 1.5 Hz, H-4), 6.94 (s, 1H, H-2), 6.99 (d, 1H, J = 7.6 Hz, H-6), 7.32 (t, 1H, J = 7.6 Hz, H-5); ^{13}C NMR (D_2O) δ 60.4 (C-6′), 69.3 (C-4′), 70.8 (C-7), 72.7 (C-2′), 75.4 (C-3′), 75.6 (C-5′), 100.8 (C-1′), 115.2 (C-4), 115.3 (C-2), 119.8 (C-6), 129.7 (C-5), 138.1 (C-1), 156.2 (C-3).

4-Hydroxybenzyl-β-D-glucopyranoside (**8**): FAB-MS *m/z* 309 $[M + Na]^+$; ^1H NMR (D_2O)

δ 3.28–3.47 (m, 4H, H-2′, 3′, 4′, 5′), 3.73 (dd, 1H, $J = 12.2$, 4.6 Hz, H-6a′), 3.92 (d, 1H, $J = 12.2$ Hz, H-6b′), 4.50 (d, 1H, $J_{1'\text{-}2'} = 7.9$ Hz, H-1′), 4.66 (d, 1H, $J = 11.6$ Hz, H-7a), 4.85 (d, 1H, $J = 11.6$ Hz, H-7b), 6.89 (d, 2H, $J = 8.2$ Hz, H-2, 6), 7.34 (d, 2H, $J = 8.2$ Hz, H-3, 5); ^{13}C NMR (D_2O) δ 60.4 (C-6′), 69.3 (C-4′), 70.9 (C-7), 72.8 (C-2′), 75.5 (C-3′), 75.6 (C-5′), 100.5 (C-1′), 115.4 (C-3, 5), 127.5 (C-1), 130.5 (C-2, 6), 156.3 (C-4).

2.4. Biotransformation of (RS)-1-phenylethanol (4) by the cultured cells of N. tabacum and C. roseus

To the suspension cultures (150 g) (white cells of *N. tabacum*, green cells of *N. tabacum*, or *C. roseus* cells), 10 ml of glucose solution (250 mg/ml H_2O) and 0.32 ml of **4** (67 mg/ml EtOH) were administered. Three additional administrations were performed at 2-day intervals. A total of 2.1 mmol of the substrate (256 mg) was administered to three conical flasks containing the cultured cells. These cultures were then incubated for 7 days at 25°C on a rotary shaker (75 rpm). The incubation mixture was extracted in the same procedure as the biotransformation of hydroxybenzyl alcohols. The butanolic fraction was purified by chromatography on a silica gel column eluted with MeOH:EtOAc (1:19 to 1:4) to give glucosides, **9** (white *N. tabacum* cells, 65 mg; green *N. tabacum* cells, 112 mg; *C. roseus* cells, 59 mg) and **10** (green *N. tabacum* cells, 21 mg). Part of the glucoside **9** (and **10**) was further purified by HPLC on Puresil C_{18}-column [flow rate, 0.7 ml/min; solvent system, MeOH–water (1:3)] several times to give (*R*)-1-phenylethyl-β-D-glucopyranoside (**9a**) (R_t 22.4 min) and (*S*)-1-phenylethyl-β-D-glucopyranoside (**9b**) (R_t 22.8 min) [or (*R*)- and (*S*)-1-phenylethyl-6-*O*-(2-hexenoyl)-β-D-glucopyranosides, **10a** (R_t 16.9 min) and **10b** (R_t 17.6 min)]. The structures of the glucosides, **9a**, **9b**, **10a** and **10b** were determined by means of physicochemical measurements and comparison with the reported data [28–30].

(*R*)-1-Phenylethyl-β-D-glucopyranoside (**9a**): FAB-MS: *m/z* 285 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 4.77 (q, 1H, $J = 6.4$ Hz, H-1), 1.46 (d, 3H, $J = 6.4$ Hz, H-2), 7.21–7.34 (5H, m, H-4, 5, 6, 7, 8), 4.40 (d, 1H, $J_{1'\text{-}2'} = 7.3$ Hz, H-1′), 3.40–3.44 (m, 3H, H-2′, 3′, 4′), 3.10 (t, 1H, $J = 7.5$ Hz, H-5′), 3.59 (bs, 2H, H-6′); ^{13}C NMR($CDCl_3$) δ 78.1 (C-1), 24.3 (C-2), 143.3 (C-3), 128.2 (C-4, 8), 126.7 (C-5, 7), 127.6 (C-6), 101.5 (C-1′), 73.4 (C-2′), 76.4 (C-3′), 69.5 (C-4′), 75.5 (C-5′), 61.5 (C-6′).

(*S*)-1-Phenylethyl-β-D-glucopyranoside (**9b**): FAB-MS: *m/z* 285 $[M + H]^+$; ^{1}H NMR ($CDCl_3$) δ 4.91 (q, 1H, $J = 6.4$ Hz, H-1), 1.47 (d, 3H, $J = 6.4$ Hz, H-2), 7.21–7.34 (m, 5H, H-4, 5, 6, 7, 8), 4.10 (d, 1H, $J_{1'\text{-}2'} = 8.2$ Hz, H-1′), 3.42 (t, 1H, $J = 8.2$ Hz, H-2′), 3.29 (t, 1H, $J = 9.2$ Hz, H-3′), 3.55 (t, 1H, $J = 9.2$ Hz, H-4′), 3.02 (d, 1H, $J = 9.2$ Hz, H-5′), 3.76 (dd, 2H, $J = 19.2$, 8.2 Hz, H-6′); ^{13}C NMR ($CDCl_3$) δ 75.6 (C-1), 22.2 (C-2), 142.0 (C-3), 128.6 (C-4, 8), 126.5 (C-5, 7), 127.8 (C-6), 99.7 (C-1′), 73.3 (C-2′), 76.2 (C-3′), 69.3 (C-4′), 75.3 (C-5′), 61.2 (C-6′).

(*R*)-1-Phenylethyl-6-*O*-(2-hexenoyl)-β-D-glucopyranoside (**10a**): FAB-MS *m/z* 403 $[M + Na]^+$, 381 $[M + 1]^+$; ^{1}H NMR ($CDCl_3$) δ 4.89 (q, 1H, $J = 6.3$ Hz, H-1), 1.53 (d, 3H, $J = 6.4$ Hz, H-2), 7.28–7.39 (m, 5H, H-4, 5, 6, 7, 8), 4.44 (d, 1H, $J_{1'\text{-}2'} = 7.8$ Hz, H-1′), 3.30–3.60 (m, 4H, H-2′, 3′, 4′, 5′), 4.50 (dd, 1H, $J = 12.2$, 3.9 Hz, H-6a′), 4.16 (d, 1H, $J = 12.7$ Hz, H-6b′), 5.84 (d, 1H, $J = 15.6$ Hz, H-2″), 7.00 (dt, 1H, $J = 15.6$, 7.3 Hz, H-3″), 2.19 (q, 2H, $J = 7.3$ Hz, H-4″), 1.52 (m, 2H, H-5″), 0.94 (t, 3H, $J = 7.3$ Hz, H-6″); ^{13}C NMR ($CDCl_3$) δ 75.9 (C-1), 21.6 (C-2), 142.7 (C-3), 128.3 (C-4, 8), 126.5 (C-5, 7), 127.6 (C-6), 100.9 (C-1′), 73.6 (C-2′), 75.9 (C-3′), 69.9 (C-4′), 74.3 (C-5′), 63.0 (C-6′), 167.3 (C-1″), 120.6 (C-2″), 150.9 (C-3″), 34.3 (C-4″), 21.2 (C-5″), 13.7 (C-6″).

(*S*)-1-Phenylethyl-6-*O*-(2-hexenoyl)-β-D-glucopyranoside (**10b**): FAB-MS *m/z* 403 $[M + Na]^+$, 381 $[M + 1]^+$; ^{1}H NMR ($CDCl_3$) δ 4.93 (q, 1H, $J = 6.4$ Hz, H-1), 1.50 (d, 3H, $J = 6.4$

Hz, H-2), 7.20–7.38 (m, 5H, H-4, 5, 6, 7, 8), 4.08 (d, 1H, $J_{1'\text{-}2'} = 6.4$ Hz, H-1′), 3.20–3.50 (m, 4H, H-2′, 3′, 4′, 5′), 4.48 (dd, 1H, $J = 11.7$, 4.8 Hz, H-6a′), 4.34 (d, 1H, $J = 11.2$ Hz, H-6b′), 5.90 (d, 1H, $J = 15.6$ Hz, H-2″), 7.06 (dt, 1H, $J = 15.6$, 6.8 Hz, H-3″), 2.22 (q, 2H, $J = 6.8$ Hz, H-4″), 1.52 (m, 2H, H-5″), 0.96 (t, 3H, $J = 7.3$ Hz, H-6″); ^{13}C NMR ($CDCl_3$) δ 75.7 (C-1), 23.9 (C-2), 141.9 (C-3), 128.6 (C-4, 8), 126.7 (C-5, 7), 128.0 (C-6), 99.4 (C-1′), 73.6 (C-2′), 76.0 (C-3′), 70.0 (C-4′), 74.0 (C-5′), 63.2 (C-6′), 167.3 (C-1″), 120.7 (C-2″), 150.9 (C-3″), 34.3 (C-4″), 21.2 (C-5″), 13.7 (C-6″).

*2.5. Biotransformation of (RS)-1-phenylethyl acetate (**5**) by the cultured cells of N. tabacum and C. roseus*

In the same method descrived above, 10 ml of glucose solution (250 mg/ml H_2O) and 0.32 ml of **5** (90 mg/ml EtOH) were administered to the flask containing the suspended cells (150 g) (white cells of *N. tabacum*, green cells of *N. tabacum*, or *C. roseus* cells). After three additional administrations, a total of 2.1 mmol of the substrate (344 mg) was administered to three conical flasks containing the cultured cells. Products were extracted and purified in the same way as the biotransformation of **4**. Column chromatography on a silica gel of the reaction mixture gave glucosides, **9** (white *N. tabacum* cells, 85 mg; green *N. tabacum* cells, 131 mg; *C. roseus* cells, 60 mg) and **10** (green *N. tabacum* cells, a trace amount).

*2.6. Reductive hydrolysis of the glucoside **10***

A solution of **10** (10 mg) in tetrahydrofuran was added to the suspension of lithium aluminium hydride (10 mg) in tetrahydrofuran and the mixture was refluxed for 30 min. Usual work-up of the reaction mixture gave a glucoside **9** (6 mg): FAB-MS m/z 285 $[M + H]^+$.

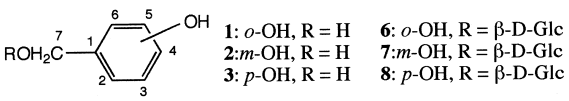
*2.7. Enzymatic hydrolysis of the glucoside **9***

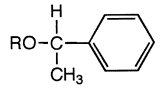
Glucoside **9** (5 mg) was incubated for 22 h at 37°C with β-glucosidase (93 U) from sweet almond and 0.8 ml of phosphate buffer (0.1 M, pH 6.0). The mixture was extracted with diethyl ether to give a crude product, which was purified by TLC to yield 1-phenylethanol (2 mg). The enantiomeric composition of the alcohol was determined by GLC on CP-cyclodextrin-β-236-M-19 column (30 m × 0.25 mm i.d. × 0.25 mm) operated at column temp. 100–150°C (programming 2°C/min), injector temp. 180°C, detector temp. 180°C, split ratio of 50:1, flow rate 50 ml/min with He (28 psi) as carrier gas. The R_t of (*R*)- and (*S*)-alcohols were 17.6 and 18.0 min, respectively.

*2.8. Time course experiments in the glucosylation of (RS)-1-phenylethanol (**4**)*

The white and green cells of *N. tabacum* were cultured for 2–3 weeks. Each 50 g of the cells was portioned to seven flasks containing 100 ml of the MS medium. (*RS*)-1-Phenylethanol (**4**) (21 mg) and glucose (1.3 g) was administered to the flasks and the mixtures were incubated with rotary shaker (75 rpm) at 25°C. At a regular time interval, the cultures were extracted with MeOH and then the methanolic fraction was partitioned between H_2O and EtOAc. The aqueous layer was further extracted with *n*-BuOH. The filtered medium was extracted with EtOAc and then with *n*-BuOH. The amounts of the glucosides in the butanolic fractions were calculated on the basis of the peak area of their HPLC analyses. The butanolic fraction was further subjected to preparative TLC on a silica gel plate with MeOH:EtOAc (1:10) to give a product **9**. The diastereomeric excess of the product **9** was determined by the ratio of peak intensities of the anomeric proton signals for **9a** and **9b** in the ^{1}H NMR spectrum of the product **9**.

3. Results and discussion





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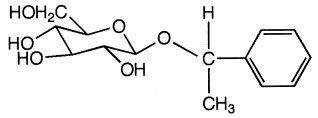
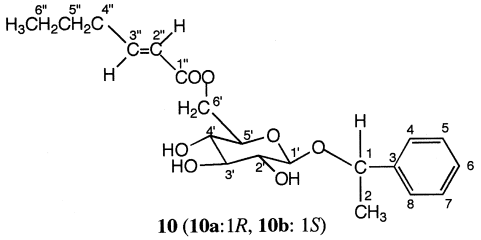
5: R = Ac



9 (**9a**:1*R*, **9b**: 1*S*)



10 (**10a**:1*R*, **10b**: 1*S*)

3.1. Regioselectivity in the glucosylation of hydroxybenzyl alcohols

In the biotransformation of 2-, 3-, and 4-hydroxybenzyl alcohol (**1–3**) by the white cells of *N. tabacum*, the primary hydroxyl group was regioselectively glucosylated to give their corresponding hydroxybenzyl glucosides, and no glucosylation of phenolic hydroxyl group was found. The structure of the glucosides obtained was confirmed by the measurement of ^{1}H and ^{13}C NMR spectra. The $J_{1'\text{-}2'}$ values in the ^{1}H NMR signal for anomeric proton of these glucosides showed that the sugar moiety of these glucosides is β-orientation. 3-Hydroxybenzyl alcohol (**2**) was effectively glucosylated to give 3-hydroxybenzyl-β-D-glucopyranoside (**7**) in 19% yield. However, glucosylation of 2-hydroxybenzyl alcohol (**1**) hardly occurred; only 2% of **1** was glucosylated. When 4-hydroxybenzyl alcohol (**3**) was used as substrate, the yield of glucoside (**8**) was half in comparison to the case of 3-hydroxybenzyl alcohol. These observations indicate that the glucosylation of the hydroxybenzyl alcohol having phenolic hydroxyl group in meta position occurred preferentially, because of the highest electron density of the primary hydroxyl group among these substrates. It may be postulated that the steric hindrance by a phenolic hydroxyl group in ortho position decreased the glucosylation.

3.2. Enantioselectivity in the glucosylation of (RS)-1-phenylethanol

Biotransformation of **4** (or its acetate **5**) having a chiral center in its molecule by green *N. tabacum* cultures gave glucosides, **9** and **10**, as shown in Table 1. On the other hand, transformation of **4** (or **5**) with white *N. tabacum* cultures gave **9**. The structure of **9** [m/z 285 (M + 1) from FAB mass spectrum] was determined as the glucoside of **4** by means of ^{1}H and ^{13}C NMR measurements. The ^{13}C NMR of **9** showed 24 peaks all coming in pairs, indicating a mixture of two diastereomeric glucosides (**9a** and **9b**) differing only in the absolute configuration of the aglycone part. The anomeric carbon of both diastereomers assumed the β-orientation, as indicated by the $J_{1'\text{-}2'}$ values of 7.3 and 8.2 Hz. The enantiomeric excess of the aglycone part of the glucoside was determined from the diastereomeric excess of the glucoside, which was determined on the basis of the intensities of corresponding anomeric proton signals in the ^{1}H NMR. The glucosides were effectively hydrolyzed by β-glucosidase, thus enabling us to confirm the stereochemistry of the sugar moiety. The enantiomeric compositions of the aglycones, which were obtained by hydrolysis with β-glucosidase, were confirmed with the GLC analysis on a chiral capillary column.

Compound **10** [m/z 381 (M + 1) from FAB mass spectrum] was also a mixture of two diastereomers (**10a** and **10b**). The ^{13}C NMR of **10** showed an ester group conjugated with a double bond. Reductive hydrolysis of **10** with lithium aluminium hydride gave glucoside **9**. From the ^{1}H–^{1}H COSY and the ^{13}C–^{1}H COSY spectra,

10 was determined as the 6-*O*-(2-hexenoyl)-β-D-glucopyranoside of **4**.

Table 1 shows the preferred absolute configurations and enantiomeric compositions of the aglycone parts of **9** and **10** as well as those of the residual alcohol in the cultured medium. The aglycones of glucoside **9** and 6-*O*-(2-hexenoyl)glucoside **10** obtained from the biotransformation of **4** and **5** with the green *N. tabacum* cells showed preferentially the *S* configuration, while the preferred configuration of residual alcohol in the cultured medium was *R*. On the other hand, the conversion of **4** and **5** with the white *N. tabacum* cells selectively gave glucoside **9** of the opposite *R* configuration. However, the preferred configuration of the alcohol obtained as a result of the hydrolysis of the acetate **5** with the white *N. tabacum* cells was *R*. This indicates that green and white cells transform stereoselectively the (*S*)- and (*R*)-alcohols, respectively, into their corresponding glucosides, while the acetate may be subjected to a two-step reaction involving first the enantioselective hydrolysis of the (*R*)-acetate to a corresponding alcohol in the cells and then the glucosylation of the latter. On the other hand, it was found that the *C. roseus* cells glucosylated preferentially (*R*)-substrate, as shown in Table 1.

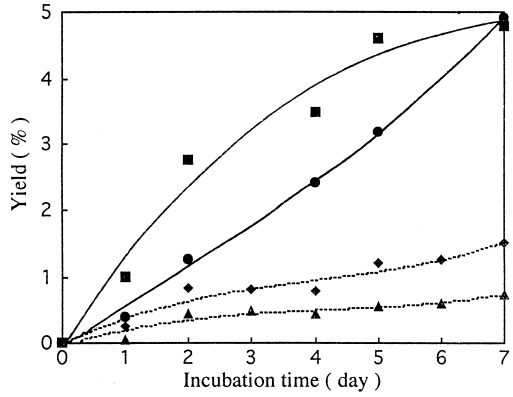


Fig. 1. Time courses of the glucosylation of **4** with the green and white cells of *N. tabacum*: —●—, glucoside of (*R*)-alcohol and —■—, glucoside of (*S*)-alcohol with the green cells; –◆–, glucoside of (*R*)-alcohol and –▲–, glucoside of (*S*)-alcohol with the white cells.

Next, the time course of the yield and enantioselectivity in the glucosylation of (*RS*)-1-phenylethanol (**4**) with the green and white cells of *N. tabacum* were investigated. At a regular

Table 1
Yields and enantiomeric compositions of the transformation products obtained from racemic **4** and **5** by the cultured cells of *N. tabacum* and *C. roseus*

Cultured cells	Substrate	Product	Yield (%)[a]	Aglycone moiety of product		Residual alcohol		
				Ee (%)[b]	Config.[c]	Yield (%)[d]	Ee (%)[e]	Config.[c]
N. tabacum (Green)	**4**	**9**	18.9	14	*S*	18	8	*R*
		10	0.8	15	*S*			
N. tabacum (Green)	**5**	**9**	21.9	18	*S*	19	11	*R*
		10	trace	n.d.[f]	n.d.			
N. tabacum (White)	**4**	**9**	10.9	12	*R*	19	6	*S*
N. tabacum (White)	**5**	**9**	14.2	20	*R*	15	7	*R*
C. roseus	**4**	**9**	9.9	28	*R*	22	18	*S*
C. roseus	**5**	**9**	10.1	24	*R*	n.d.	n.d.	n.d.

[a] Of isolated product.
[b] Enantiomeric excess calculated from the diastereomeric excess of the glucoside, which was determined on the basis of the intensities of anomeric proton signals in the ^{1}H NMR of the glucoside.
[c] Preferred configuration of the alcohol.
[d] Determined from the peak area GLC of the residual alcohol in the cultured medium.
[e] Enantiomeric excess calculated from chiral GLC of the residual alcohol in the cultured medium.
[f] Not determined.

time interval, the diastereomeric ratio of the glucosides obtained was examined by the integration ratio of the anomeric protons of their ^{1}H NMR spectra. As shown in Fig. 1, the green *N. tabacum* cells preferentially produced the glucoside of (*S*)-alcohol, but the diastereomeric excess approached to $R/S = 1/1$ with lapse of incubation time. On the contrary, the white *N. tabacum* cells preferentially produced the glucoside of (*R*)-alcohol, but the diastereomeric excess of the glucosides unchanged with lapse of incubation time. The decrease of diastereomeric excess found in the case of the green *N. tabacum* cells may be explained as increase of a enzyme having opposite stereoselectivity during long incubation term.

It is very interesting that the enantioselectivity differs by the kind of plant cells and the culture condition. The difference in enantioselectivity between white and green *N. tabacum* cells suggests that different enzyme systems arise from subtle variations in cell culturing conditions. The investigation of the enzyme system of this glucosylation is now in progress.

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