Enantioselective Glucosylation of (*R,S*)-1-Phenylethanol by the Cultured Suspension Cells of Higher Plants

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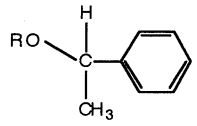
(*R,S*)-1-Phenylethanol and its acetate were transformed into their β-D-glucopyranoside by the cultured suspension cells of *Nicotiana tabacum* and *Catharanthus roseus*. The glucosylation with the green *N. tabacum* cells occurred enantioselectively to give the glucoside of the (*S*)-alcohol, while the biotransformation with the white *N. tabacum* cells and *C. roseus* cells gave preferentially the glucoside of the (*R*)-alcohol.

The biotransformation of exogenous substrates by plant cell cultures is a useful method for the structural modification of compounds having pharmacological activities. Glucosylation of substrates such as phenols,[1,2] steroids,[3,4] terpenoids[4,5] and others[6,7] by cultured plant cells *en route* to the preparation of therapeutically active products has been reported. The biological transformation is usually enantioselective, thus making it possible to obtain products difficult to synthesize chemically. However, despite its potential usefulness for the synthesis of chiral compounds, little attention has been given to the enantioselectivity of the reaction.[8,9] We have investigated the enantioselectivity of the glucosylation reaction occurring in plant cell cultures, using as substrates a secondary alcohol having a chiral center, (*R,S*)-1-phenylethanol (**1**), and its acetate (**2**), both known as perfumery ingredients.

White and green cells of *Nicotiana tabacum*[10] and *Catharanthus roseus* cells[13] were used for the biotransformation of **1** and **2**.[16] The yields of products are shown in Table 1. The structure of **3** [m/z 285 (M+1) from FAB mass spectrum] was determined as the monoglucoside of **1** by means of ^{1}H and ^{13}C NMR measurements and comparison with the reported data for the glucoside.[8,9,17] The ^{13}C NMR of **3** showed 24 peaks all coming in pairs, indicating a mixture of two diastereomeric glucosides diferring only in the absolute configuration of the aglycone moiety. Part of the compd **3** was subjected to preparative HPLC to give (*R*)-1-phenylethyl β-D-glucopyranoside (**3a**)[18] and (*S*)-1-phenylethyl β-D-glucopyranoside (**3b**).[19] The glucosides, **3a** and **3b**, were effectively hydrolyzed by β-glucosidase,[20] thus confirming the stereochemistry of the sugar moiety.



1: R = H
2: R = Ac

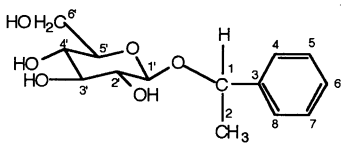


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Table 1 shows the diastereomeric compositions of **3** and the preferred absolute configurations of the aglycone moieties[20,21] in the biotransformation with the white and green tobacco cells and the *C. roseus* cells. The aglycones of glucoside **3** obtained from the biotransformation of **1** and **2** with the green cells showed preferentially the *S* configuration (14 and 18% de, respectively), while the conversion of **1** and **2** with the white cells gave selectively **3** of the opposite (*R*) configuration (12 and 20% de, respectively). This indicates that the green and white cells transform preferentially the (*S*)- and (*R*)-alcohols, respectively, into their corresponding glucosides. On the other hand, the *C. roseus* cells transformed preferentially the (*R*)-alcohol into **3**.

In addition, the conversion of the acetate **2** into **3** may involve a two-step reaction with hydrolysis[10,22] of the acetate to the alcohol and then the glucosylation of the latter. On the other hand, the difference in enantioselectivity between white and green tobacco cells suggests that enzyme systems in the cells may be affected by subtle variations in the cell culturing conditions. Research on this topic is now in progress.

Table 1. Biotransformation of racemic **1** and **2** into glucoside **3** by the cultured suspension cells of *N. tabacum* and *C. roseus*

Cultured cells	Substrate	Product	Yield / % [a]	Diastereomeric excess / % de [b]	Prefered config. of aglycone
N. tabacum (Green)	**1**	**3**	18.9	14	*S*
	2	**3**	21.9	18	*S*
N. tabacum (White)	**1**	**3**	10.9	12	*R*
	2	**3**	14.2	20	*R*
C. roseus	**1**	**3**	9.9	28	*R*

[a] Of isolated product.
[b] % De were determined on the basis of the intensities of anomeric proton signals in the ^{1}H NMR of glucoside **3**.

References and Notes

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10 White cells of *Nicotiana tabacum*[11] were cultured in 500 ml conical flasks containing 200 ml Murashige and Skoog's (MS) medium[12] supplemented with 3% sucrose and 10 mM 2,4-dichlorophenoxy acetic acid (2,4-D). Green cells were obtained by culturing the white cells under illumination (4000 lux) in MS medium with 1% sucrose and 5 mM 2,4-D. The flasks were shaken on a rotary shaker (75 rpm) at 25 °C.

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13 Suspension cells of *Catharanthus roseus*[14] were cultured in 500 ml conical flasks containing 200 ml of SH medium[15] supplemented with 3% of sucrose and 10 mM 2,4-D under illumination (4000 lux). The flasks were shaken on a rotary shaker (75 rpm) at 25 °C.

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16 Ten ml of glucose solution (25 g in 100 ml H_2O) and 0.32 ml of **1** or **2** (1g in 15 ml EtOH) were administered to the cell suspension cultures. Three additional administrations of the substrate were performed at two-day intervals. The cultures were then incubated for seven days at 25 °C on a rotary shaker (75 rpm). After the incubation, the cells and media were separated by filtration with suction. The cells were extracted with MeOH and the extract was concentrated by evaporation *in vacuo*. The methanolic fraction was partitioned between H_2O and EtOAc. The aqueous layer was further extracted with BuOH. The filtered medium was extracted with EtOAc and then with BuOH. The BuOH extracts from both cells and culture medium gave compound **3** by column chromatography on silica gel.

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18 Glucoside **3a**: FAB-MS m/z 285 $[M+1]^+$. ^1H NMR ($CDCl_3$): δ 4.77 (1H, q, J=6.4Hz, H-1), 1.46 (3H, d, J=6.4Hz, H-2), 7.21~7.34 (5H, m, H-4,5,6,7,8), 4.40 (1H, d, J=7.3Hz, H-1'), 3.40~3.44 (3H, m, H-2', 3', 4'), 3.10 (1H, t, J=7.5Hz, H-5'), 3.59 (2H, bs, 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').

19 Glucoside **3b**: FAB-MS m/z 285 $[M+1]^+$. ^1H NMR ($CDCl_3$): δ 4.91 (1H, q, J=6.4Hz, H-1), 1.47 (3H, d, J=6.4Hz, H-2), 7.21~7.34 (5H, m, H-4,5,6,7,8), 4.10 (1H, d, J=8.2Hz, H-1'), 3.42 (1H, t, J=8.2Hz, H-2'), 3.29 (1H, t, J=9.2Hz, H-3'), 3.55 (1H, t, J=9.2Hz, H-4'), 3.02 (1H, d, J=9.2Hz, H-5'), 3.76 (2H, dd, J=19.2, 8.2Hz, 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').

20 Glucoside **3** was incubated for 22 h at 37 °C with 93U β-glucosidase from sweet almond and 0.8 ml phosphate buffer (0.1 M, pH 6.0). The mixture was extracted with ether and the crude product was purified by TLC to give 1-phenylethanol.

21 The intensities of the pair of anomeric proton signals at δ 4.40 (d, J=7.3 Hz, for **3a**) and 4.10 (d, J=8.2, for **3b**) in the ^1H NMR of **3** were used for the determination of the diastereomeric excess. The enantiomeric compositions of the aglycone moieties were confirmed by analysis of the hydrolyzed alcohol with GLC on CP-cyclodextrin-β-236-M19 column.

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