Diastereoselective Formation of Disaccharides from (*RS*)-1-Phenylethanol by Cultured Cells of *Catharanthus roseus*

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The biotransformation of (*RS*)-1-phenylethanol with cultured cells of *Catharanthus roseus* gave its vicianoside [α-L-arabinopyranosyl-(1-6)-β-D-glucopyranoside], primeveroside [β-D-xylopyranosyl-(1-6)-β-D-glucopyranoside] and gentiobioside [β-D-glucopyranosyl-(1-6)-β-D-glucopyranoside] via the corresponding glucoside. It was found that primeveroside and gentiobioside were enantioselectively composed of only (*R*)-1-phenylethanol as its aglycone moiety.

A glucosylation reaction of alcohols with biocatalysts is useful for the preparation of pharmacologically important substances. Although there are many reports of the glycosylation of alcohols with cultured plant cells,[1–10] little attention has been paid to the enantioselectivity in the glycosylation reaction.[6] We recently found that cultured cells of *Catharanthus roseus* are able to transform an exogenous secondary alcohol to a mono-glucoside having the (*R*)-configuration at its aglycone moiety.[11,12] During the course of a study on enantioselective glycosylation by cultured plant cells, we took into consideration the diastereoselective formation of a disaccharide, and report here that the cultured cells of *C. roseus* were capable diastereoselectively of transforming 1-phenylethanol into disaccharides, such as vicianoside, primeveroside and gentiobioside, via the corresponding mono-glucoside.

Results and Discussion

(*RS*)-, (*R*)-, and (*S*)-1-phenylethanol (**1**, **1a**, and **1b**) were separately administered to a cultured cell suspension of *C. roseus*; the cultures were then incubated at 25 °C for 5–10 days. The products were isolated by preparative TLC and HPLC to give disaccharides, such as vicianoside **2**, primeveroside **3** and gentiobioside **4** (Chart 1, Table 1), as well as 1-phenylethyl β-D-glucopyranoside (**5**), which was reported in our previous paper.[11] The structures of the products were determined by means of FABMS and ^{1}H- and ^{13}C NMR measurements. The vicianoside **2** obtained from incubating (*RS*)-1-phenylethanol (**1**) was found to be a mixture of diastereomers, **2a** composed of the *R*-alcohol and **2b** composed of the *S*-alcohol. The primeveroside **3** and gentiobioside **4** were composed of only the aglycone with the *R*-configuration (each 100% e.e.). On the other hand, 1-phenylethyl β-D-glucopyranoside (**5**) obtained from the (*RS*)-alcohol was preferentially composed of the (*S*)-alcohol (12% e.e.), although we previously found that glucosylation with the cultured cells of *C. roseus* occurred enantioselectively to preferentially give the mono-glucoside of the (*R*)-alcohol.[11]

In order to clarify the enantioselectivity in the glycosylation reaction, the time courses for the biotransformation of the (*RS*)-alcohol **1** were examined; the results are given in Table 2. From an early stage of the incubation, **1** was transformed to the corresponding mono-glucoside **5** and disaccharides **2**–**4**. Further transformation products were not found during the time-course period, despite careful analysis of the products by HPLC. The yield of the mono-glucoside **5** was at its maximum in the 5-days incubation, and then decreased. The diastereomeric excess (d.e.) of **5** was over 60% (*R*-enantioselective), but gradually decreased with lapse of the incubation time. during 10 days of incubation, **5** was composed preferentially of the (*S*)-alcohol. On the other hand, the yields of the disaccharides, **2**–**4**, increased during the time course period, especially after 5–7 days of incubation. Interestingly, the configurations of the aglycone moiety of the primeveroside **3** and gentiobioside **4** were *R* (100% e.e.). In contrast, a diastereomeric excess of the vicianoside **2** decreased with decrease in the diastereomeric excess of the mono-glucoside **5**. These observations indicate that the (*RS*)-alcohol **1** was first transformed *R*-enantioselectively to the mono-glucoside **5** (about 60% d.e.), and then the diastereomer **5a** was subsequently transformed to disaccharides, such as vicianoside **2a**, primeveroside **3** and gentiobioside **4**, whereas **5b** was converted to only vicianoside **2b**. Such a difference of the diastereoselectivity in the formation of disaccharides may reflect decrease in the diastereomeric purity of the remaining **5**.

It was thus demonstrated that the cultured cells of *C. roseus* were able to glycosylate 1-phenylethanol stepwise to give the corresponding vicianoside, primeveroside and gentiobioside. The xylosylation and glucosylation of 1-phenylethyl glucoside (**5**) occurred diastereospecifically to give the disaccharides

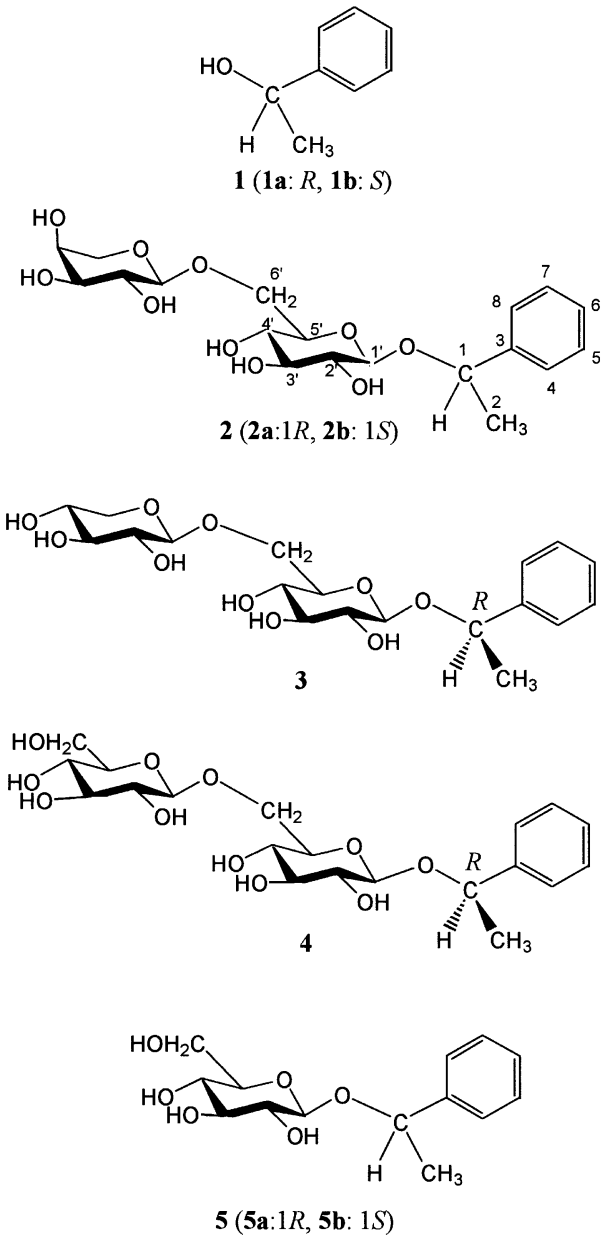

Chart 1.

Table 1. Biotransformation of (*RS*)-, (*R*)-, and (*S*)-1-Phenylethanol (**1**, **1a**, and **1b**) by the Cultured Cells of *C. roseus*

Substrate	Incubation time /d	Product	Yield /%	d.e. /%	Configuration[a)]
1	10	**2**	7.8	0	*RS*
		3	5.3	100	*R*
		4	2.6	100	*R*
		5	4.1	14	*S*
1a	5	**2**	1.5	100	*R*
		3	2.1	100	*R*
		4	0.9	100	*R*
		5	9.5	100	*R*
1b	5	**2**	1.0	100	*S*
		5	3.7	100	*S*

a) Preferred configuration of the aglycone.

Table 2. Time Courses in the Biotransformation of (*RS*)-1-Phenylethanol (**1**) by the cultured Cells of *C. roseus*

Incubation time /d	Product	Yield/%	d.e./%	Configuration[a)]
1	**2**	0.8	51	*R*
	3	tr.[b)]	—	—
	4	tr.	—	—
	5	1.8	63	*R*
3	**2**	2.0	48	*R*
	3	0.7	100	*R*
	4	0.6	100	*R*
	5	5.2	62	*R*
5	**2**	2.8	29	*R*
	3	1.1	100	*R*
	4	1.0	100	*R*
	5	15.0	49	*R*
7	**2**	4.5	25	*R*
	3	2.5	100	*R*
	4	2.1	100	*R*
	5	13.2	35	*R*
10	**2**	8.0	0	*RS*
	3	6.1	100	*R*
	4	3.8	100	*R*
	5	3.5	12	*S*

a) Preferred configuration of the aglycone. b) Trace amount.

with only (*R*)-1-phenylethanol, but the arabinosylation was not high in diastereoselectivity. To our knowledge, there are only a few enzymatic studies on enantioselective glycosylation. Matsumura et al. recently reported that β-galactosidase from *E. coli* are able to catalyze *R*-enantioselective galactosylation of 1-phenylethanol by its reverse hydrolytic reaction.[13] It is worth noting that the enantioselective glycosylation with biocatalysts may be useful for preparing of diastereomeric pure glycosides and olygosaccharides. For the practical use of those enzymes, further investigations at the enzymatic level are necessary, and are now in progress in our laboratory.

Experimental

General. Analytical and prep. TLC was carried out on glass sheets (0.25 mm and 0.5 mm) coated with silica gel (Merck silica gel 60; GF_{254}). NMR spectra were measured in MeOH-d_4 on a JEOL GSX500 [500 MHz (^{1}H) and 125.8 MHz (^{13}C)] NMR spectrometer. FABMS were taken on a JEOL SX102A mass spectrometer. GLC was carried out with FID and an Alltech Chirasil-Val capillary column (0.25 mm × 25 m) using He as carrier gas (1 cm^3 min^{-1}) at column temp: 50–100 °C (1 °C min^{-1}) and HPLC with Puresil C18 (Waters) column using MeOH:H_2O (1:3 v/v) as eluent. (*RS*)-1-Phenylethanol (**1**), (*R*)-1-phenylethanol (**1a**), and (*S*)-1-phenylethanol (**1b**) were purchased from Wako Pure Chemical Industries. Ltd.

Incubation of (*RS*)-1-Phenylethanol (1) with the Cultured Cells of *C. roseus*. Suspension cells of *Catharanthus roseus*[14] were cultivated at 25 °C for 14 days in 1000 cm^3 conical flasks containing 600 cm^3 of SH medium[15] supplemented with 3% of sucrose and 10 mM (1 M = 1 mol dm^{-3}) of 2,4-dichlorophenoxyacetic acid under illumination (4000 lux) on a rotary shaker (75 rpm).

Table 3. ^{13}C Chemical Shifts of the Products, **2a**, **2b**, **3** and **4**, in the Biotransformation of 1-Phenylethanol (**1**) by the Cultured Cells of *C. roseus*

Position	2a	2b	3	4
C-1	78.3	77.0	78.3	78.2
C-2	23.1	25.5	23.0	23.0
C-3	146.0	144.9	145.9	145.9
C-4	128.2	128.8	128.3	128.3
C-5	129.9	130.2	129.9	129.9
C-6	129.0	129.4	129.0	129.0
C-7	129.9	130.2	129.9	129.9
C-8	128.2	128.8	128.3	128.3
C-1′	103.4	102.0	103.3	103.2
C-2′	76.0	76.0	76.0	76.0
C-3′	78.8[a)]	78.6[b)]	78.9[c)]	78.7[d)]
C-4′	72.5	72.5	72.3	72.4
C-5′	78.0[a)]	77.6[b)]	78.0[c)]	78.1[d)]
C-6′	70.0	70.3	70.4	70.5
C-1″	105.8	106.0	106.2	105.6
C-2″	73.2	73.2	75.7	76.0
C-3″	74.9	75.1	78.3[c)]	78.9[d)]
C-4″	70.3	70.3	72.0	72.4
C-5″	67.4	67.5	67.6	78.7[d)]
C-6″	—	—	—	63.5

a)–d) Assignments may be interchanged.

(*RS*)-1-phenylethanol (**1**) (20 mg) in MeOH (0.35 cm^3) and glucose (1.3 g) were administered to the cultured suspension cells (50 g), and the cultures were incubated at 25 °C for 10 days on a rotary shaker (75 rpm). After incubation, the cells and medium were separated by filtration with suction. The cells were extracted with MeOH and the extract was concentrated by evaporation in vacuo. After the methanolic fraction was partitioned between H_2O and EtOAc, the aqueous layer was further extracted with BuOH. The filtered medium was extracted with BuOH. The BuOH extracts from the cells and the culture medium were combined and purified by preparative TLC with MeOH:EtOAc (1:3) and HPLC with a Puresil C18 column using MeOH:H_2O (1:3 v/v) to give vicianoside **2**, primeveroside **3**, gentiobioside **4**, and 1-phenylethyl β-D-glucopyranoside **5**.[12] The structures of the products were determined by means of FABMS, ^{1}H and ^{13}C NMR, H–H COSY, C–H COSY, and HMBC measurements. The diastereomeric excess of **2** was determined by the intensities of the anomeric proton signals of the arabinose moiety in its ^{1}H NMR spectra.

1-*O*-[β-D-xylopyranosyl-(1-6)-β-D-glucopyranosyl]-(*R*)-1-phenylethanol (**3**) (primeveroside): FABMS *m/z* 439 [M+Na]$^+$; ^{1}H NMR (CD_3OD) δ 4.96 (1H, q, *J* = 6.4 Hz, H-1), 1.45 (3H, d, *J* = 6.4 Hz, H-2), 7.41 (2H, d, *J* = 7.3 Hz, H-4,8), 7.30 (2H, t, *J* = 7.3 Hz, H-5,7), 7.21 (1H, t, *J* = 7.3 Hz, H-6), 4.46 (1H, d, *J* = 8.3 Hz, H-1′), 3.25 (1H, m, H-2′), 3.38 (1H, m, H-3′), 3.32 (1H, m, H-4′), 3.40 (1H, m, H-5′), 3.67 (1H, dd, *J* = 11.6, 5.8 Hz, H-6a′), 3.97 (1H, dd, *J* = 11.6, 1.8 Hz, H-6b′), 4.26 (1H, d, *J* = 7.3 Hz, H-1″), 3.16 (1H, dd, *J* = 8.8, 7.6 Hz, H-2″), 3.28 (1H, m, H-3″), 3.44 (1H, m, H-4″), 3.08 (1H, dd, *J* = 11.6, 10.1 Hz, H-5a″), 3.79 (1H, dd, *J* = 11.6, 3.4 Hz, H-5b″); ^{13}C NMR (CD_3OD) see Table 3.

1-*O*-[β-D-glucopyranosyl-(1-6)-β-D-glucopyranosyl]-(*R*)-1-phenylethanol (**4**) (gentiobioside): FABMS *m/z* 447 [M+H]$^+$; ^{1}H NMR (CD_3OD) δ 4.97 (1H, q, *J* = 6.4 Hz, H-1), 1.47 (3H, d, *J* = 6.4 Hz, H-2), 7.42 (2H, d, *J* = 7.3 Hz, H-4,8), 7.30 (2H, t, *J* = 7.3 Hz, H-5,7), 7.22 (1H, t, *J* = 7.3 Hz, H-6), 4.47 (1H, d, *J* = 7.8 Hz, H-1′), 3.23 (1H, dd, *J* = 9.3, 7.8 Hz, H-2′), 3.31 (1H, m, H-3′), 3.37 (1H, t, *J* = 7.8 Hz, H-4′), 3.41 (1H, ddd, *J* = 7.8, 5.9, 2.0 Hz, H-5′), 3.73 (1H, dd, *J* = 11.7, 5.9 Hz, H-6a′), 4.03 (1H, dd, *J* = 11.7, 2.0 Hz, H-6b′), 4.31 (1H, d, *J* = 7.8 Hz, H-1″), 3.16 (1H, dd, *J* = 8.3, 7.8 Hz, H-2″), 3.30 (1H, m, H-3″), 3.26 (1H, t, *J* = 8.8 Hz, H-4″), 3.15 (1H, m, H-5″), 3.63 (1H, dd, *J* = 11.7, 5.9 Hz, H-6a″), 3.82 (1H, dd, *J* = 11.7, 2.4 Hz, H-6b″); ^{13}C NMR (CD_3OD) see Table 3.

Incubation of (*R*)-1-Phenylethanol (1a) and (*S*)-1-Phenylethanol (1b) with the Cultured Cells of *C. roseus*. According to the same procedure as described above, **1a** and **1b** were transformed into the corresponding glucosides. Samples of **1a** and **1b** (60 mg) in MeOH (0.6 cm^3) were administered to flask containing the suspended cells (150 g), and the cultures were incubated at 25 °C for 5 days on a rotary shaker (75 rpm). The products were extracted and purified in the same way as for the biotransformation of **1**. Purification of the products with preparative TLC and HPLC gave the glycosides, **2a** (3 mg), **3** (4 mg), **4** (2 mg) and **5a** (14 mg) from **1a**, while the glycosides, **2b** (2 mg) and **5b** (5 mg) from **1b**.

1-*O*-[α-L-arabinopyranosyl-(1-6)-β-D-glucopyranosyl]-(*R*)-1-phenylethanol (**2a**): FABMS *m/z* 439 [M+Na]$^+$; ^{1}H NMR (CD_3OD) δ 4.96 (1H, q, *J* = 6.4 Hz, H-1), 1.48 (3H, d, *J* = 6.4 Hz, H-2), 7.41 (2H, d, *J* = 7.3 Hz, H-4,8), 7.30 (2H, t, *J* = 7.3 Hz, H-5,7), 7.21 (1H, t, *J* = 7.3 Hz, H-6), 4.46 (1H, d, *J* = 7.3 Hz, H-1′), 3.23 (1H, dd, *J* = 7.3, 8.7 Hz, H-2′), 3.32 (1H, m, H-3′), 3.27 (1H, m, H-4′), 3.36 (1H, m, H-5′), 3.67 (1H, dd, *J* = 11.6, 6.7 Hz, H-6a′), 3.97 (1H, dd, *J* = 11.6, 2.1 Hz, H-6b′), 4.24 (1H, d, *J* = 7.0 Hz, H-1″), 3.53 (1H, dd, *J* = 8.8, 6.7 Hz, H-2″), 3.41 (1H, dd, *J* = 8.8, 3.4 Hz, H-3″), 3.73 (1H, m, H-4″), 3.30 (1H, m, H-5a″), 3.77 (1H, dd, *J* = 12.5, 3.4 Hz, H-5b″); ^{13}C NMR (CD_3OD) see Table 3.

1-*O*-[α-L-arabinopyranosyl-(1-6)-β-D-glucopyranosyl]-(*S*)-1-phenylethanol (**2b**): FABMS *m/z* 439 [M+Na]$^+$; ^{1}H NMR (CD_3OD) δ 5.01 (1H, q, *J* = 7.3 Hz, H-1), 1.45 (3H, d, *J* = 7.3 Hz, H-2), 7.42 (2H, d, *J* = 7.3 Hz, H-4,8), 7.31 (2H, t, *J* = 7.3 Hz, H-5,7), 7.24 (1H, t, *J* = 7.3 Hz, H-6), 4.06 (1H, d, *J* = 7.3 Hz, H-1′), 3.23 (1H, dd, *J* = 7.3,8.5 Hz, H-2′), 3.20 (1H, t, *J* = 8.5 Hz, H-3′), 3.32 (1H, dd, *J* = 8.5, 10.1 Hz, H-4′), 3.28 (1H, m, H-5′), 3.73 (1H, dd, *J* = 11.0, 6.1 Hz, H-6a′), 4.06 (1H, dd, *J* = 11.0, 1.8 Hz, H-6b′), 4.34 (1H, d, *J* = 6.4 Hz, H-1″), 3.62 (1H, dd, *J* = 8.2, 6.4 Hz, H-2″), 3.55 (1H, dd, *J* = 8.2, 1.8 Hz, H-3″), 3.81 (1H, m, H-4″), 3.55 (1H, dd, *J* = 12.2, 1.8 Hz, H-5a″), 3.87 (1H, dd, *J* = 12.2, 2.4 Hz, H-5b″); ^{13}C NMR (CD_3OD) see Table 3.

Determination of Absolute Configuration of Sugar Moieties. Each of the disaccharides, **2a**, **2b**, **3**, and **4**, was added to the vial in 4.0 M HCl (100 mm^3) and heated to 80 °C for 2 h and then cooled. After removing of the solvent in a stream of N_2, each hydrolysate was converted into a pentafluoropropionate with pentafluoropropionic anhydride (400 mm^3) in CH_2Cl_2 (400 μL) in a sealed tube at 120 °C for 2 h. Excess reagents were removed under a stream of N_2 and the derivatives were analyzed by chiral GC on Chirasil-Val. The peaks of the derivatives from vicianosides **2** and **2b** were assigned to those of L-arabinose and D-glucose, peaks of the derivatives from primeveroside **3** to those of D-xylose and D-glucose, and peaks of the derivatives from gentiobioside **4** to that of D-glucose.

Time Course Experiments in the Glycosylation of (*RS*)-1-Phenylethanol (1). Each 50 g of the suspension cells of *C. roseus* was portioned to 5 flasks containing 100 ml of the SH medium. (*RS*)-1-Phenylethanol (**1**) (21 mg) and glucose (1.3 g) were administered to the flasks and the mixtures were incubated on a rotary shaker (75 rpm) at 25 °C. At a regular time interval, 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 combined with the medium and the

mixture was extracted with EtOAc. The aqueous phase was further extracted with *n*-BuOH. The butanolic fraction was subjected to preparative TLC on silica gel with MeOH:EtOAc (1:3 v/v) to give the glucoside and disaccharide fractions. The glucoside fraction was further subjected to preparative HPLC with Puresil C18 column using MeOH:H_2O (1:3 v/v) to give glucoside **5**, of which the diasteromeric excess was determined by the peak intensities of the anomeric proton signals for **5a** and **5b** in its ^{1}H NMR spectrum. On the other hand, the disaccharide fraction was subjected to reverse phase TLC with MeOH:H_2O (1:1 v/v) and then prep. HPLC with a Puresil C18 column using MeOH:H_2O (1:3 v/v) to give disaccharides **2**–**4**. The diasteromeric excesses of the disaccharides **2**–**4** were determined by the ratio of the peak intensities of their corresponding anomeric proton signals in their ^{1}H NMR spectra.

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