Notes

Enzymatic Preparation of Phenolic Glucosides by *Streptococcus mutans*

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Trans-resveratrol(3,4',5-trihydroxystilbene) was isolated from grapevines (*Vitis vinifera*) in 1976 by Lankcake and Pryce.[1] The fungal infection by *Botrytis cinerea* or exposure to ultraviolet light of leaf tissues induces the synthesis of *trans*-resveratrol. *Trans*-resveratrol shows a strong antioxidant activity *in vitro*, inhibition of arachidonate metabolism, and antiinflammatory activity.[2] Plant [*Erythrophleum lasianthum* Corbishley (Caesalpinoideae, Leguminosae)] produces glucosides (piceid=resveratrol 3-*O*-β-glucoside). The resveratrol 3-O-β-D-glucopyranoside showed antiplatelet aggregation activity,[3] coronary vasodilator action,[4] antileukemic,[5] antifungal[6] and protein-tyrosine kinase inhibitory action.[7]

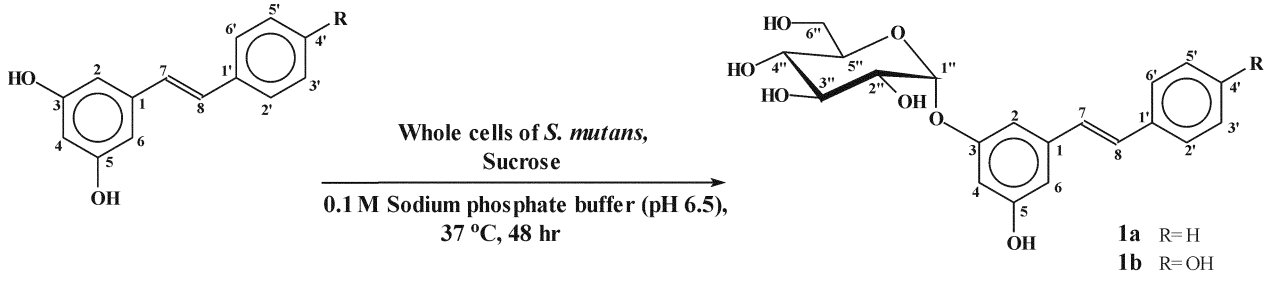
Most studies of transglycosylation with glucosyltransferase have been focusing on the formation of oligosaccharides. One of the best model compounds of glucosyltransferase was the flavonoid cathechin. Several glucosyltransferase and different glucosyl donors were applied to the glucosylation of catechin.[8] The compound containing the two adjacent aromatic hydroxy groups is the best glycosyl acceptor in glucosyltransferase reaction. However, the monohydroxy aromatic compound and di- or trihydroxy aromatic compounds were not glycosylated by glucosyltransferase-D in *Streptococcus mutans*.[9]

In the present study we describe the biotransformation of resveratrol to piceid by whole-cell suspensions of *Streptococcus mutans*. The first biotransformation of resveratrol to piceid by *Bacillus cereus* was reported.[10] Glucosyltransferase in *S. mutans* catalyze the formation of α-linked glucose from sucrose and contribute to the dental plaque matrix polysaccharide composition.[11] The whole cells of *S. mutans* are used in the biotransformation of resveratrol and pinosylvin.

The whole cells were obtained by fermenting of *S. mutans* in 200 mL of brain heart infusion broth for 22 hours at 37 °C. The crude GTase was prepared from the culture suspernatant of *S. mutans* as described by Nakahara *et al.*.[12]

The general procedure of glycosylation was followed; (*E*)-resveratrol (Sigma Co., U.S.A) [3,4',5-trihydroxy-*trans*-stilbene] was added to the mixture of 50 mL of 0.1 M sodium phosphate buffer, pH 6.5 and 1.0 g of sucrose. To the reaction mixture was added whole cells, and the mixture was incubated at 37 °C for 48 hours. The enzymatic reaction progress was monitored with TLC. The glycosylated resveratrol was identified as pink color spot (R_f = 0.38, EtOAc : MeOH = 5 : 2) under UV. The reaction mixture was filtered through celite and the glycosylated resveratrol was purified with column chromatography (silicagel, selvent; EtOAc : MeOH = 4 : 1). The yield of the reaction was 18.7%. The biotransformation of pinosylvin using the whole cell suspension of *S. mutans* afforded glycosylated pinosylvin in 11.3% yield. The low yield may be explained by the very low solubility of substrates in buffer solution.

The ^{1}H-NMR spectrum of the product in methanol-d_4 obtained with NMR spectrometer (Bruker, Avance 500 MHz) was compared with that of piceid.[13] The analysis of NMR spectrum showed that the product consists of resveratrol and one glucose unit. The NMR spectrum of glycosylated resveratrol was compared with those reported in the



1a : 3-*O*-α-D-glucopyranosyl-(*E*)-pinosylvin
1b : 3-*O*-α-D-glucopyranosyl-(*E*)- resveratrol

Scheme 1. Biotransformation of phenolic compounds.

Table 1. ^{13}C-NMR spectral data of compounds **1a** and **1b** (α values)

^{13}C-NMR (δ)	(*E*)-piceid		**1a**	**1b**
	acetone-d_6[14], 125 MHz	DMSO[13], 50.3 MHz	CD_3OD, 50.3 MHz	
C-3	160.3	158.9	158.8	159.1
C-5	159.4	158.4	158.5	158.8
C-4'	158.2	157.3	139.7	158.3
C-1	140.9	139.4	137.5	140.2
C-1'	130.0	130.0	128.8	128.7
C-b	129.9	128.6	128.5	128.6
C-2' and C-6'	128.9	128.0	128.4	127.7
C-a	126.5	125.2	127.4	125.2
C-3' and C-5'	116.4	115.6	126.3	115.6
C-6	108.2	107.2	107.5	107.4
C-4	103.9	104.7	106.5	105.8
C-2	106.2	102.8	103.8	103.4
C-1"	102.1	100.7	98.1	98.0
C-5"	77.8	77.2	73.7	73.7
C-3"	78.1	76.2	73.1	73.1
C-2"	74.8	73.3	72.1	72.1
C-4"	71.5	69.8	70.2	70.2
C-6"	62.8	60.7	61.1	61.0

literature for picied.[14]

In the ^{1}H-NMR spectrum, the peak of H-1" in piceid appeared at 4.80 ppm (d, J = 7.0 Hz). However, the peak of H-1" in glycosylated resveratrol obtained from *S. mutans* showed at 5.33 ppm (d, J = 3.5 Hz). This indicates that the coupling constant of the anomeric proton is characteristic of an α-linked glycosidic bond rather than β-linked glycosidic residue in piceid. The ^{1}H-NMR spectrum (an anomeric proton) of α-linked glycosidic bond of mono glycosylated resveratrol was in agreement with ^{1}H-NMR spectrum (an anomeric proton) of α-glycosylated (+)-catechin by the GTase.[12] The product was identified to be 3-*O*-α-D-glucopyranosyl-(*E*)-resveratrol. Studies on the biological activities of the 3-*O*-α-D-glucopyranosyl-(*E*)-resveratrol and 3-*O*-α-D-glucopyranosyl-(*E*)-pinosylvin are currently in progress.

Experimental Section

Fermentation procedures. *Streptococcus mutans* (KCTC 3065; ATCC 25175) was grown at 37 °C on agar slants containing the following medium: brain heart infusion broth, 3.7 g; agar 1.5 g, distilled water, 100 mL, pH 7.4. Under sterile conditions, a part of the spore suspension was transferred to an autoclaved 500 mL baffled Erlenmeyer flask containing 100 mL of medium: brain heart infusion broth 3.7 g; distiled water, 100 mL; pH 7.4. After 24 hours of incubation at 37 °C, 200 rpm, 10 mL of seed cultures was added to each of five production flasks (each 500 mL baffled Erlenmeyer flask contained 200 mL of the same medium) under sterile conditions. After 24 hours the cultures were harvested. The whole cells or crude extract of GTase from supernatant were used for the biotransformation.

Biotransformation of resveratrol. (*E*)-Resveratrol (50 mg, 0.22 mmol) was added to the mixture of 50 mL of 0.1 M sodium phosphate buffer, pH 6.5 and 1.0 g of sucrose. To the reaction mixture was added whole cells (0.8 g wet weight), and the mixture was incubated at 37 °C, 200 rpm for 48 hours. The reaction mixture was filtered through celite. The aqueous layer was evaporated in vacuo and the residue was purified with column chromatography (silicagel, ethyl acetate : methanol = 4 : 1). The 3-O-α-D-glucopyranosyl-(*E*)-resveratrol was obtained 16 mg (18%). The 30 mg of starting material[(*E*)-Resveratrol] was recovered.

(*E*)-Resveratrol 3-*O*-α-D-glucopyranoside: ^{1}H-NMR (DMSO-d_6, 500 MHz) δ 7.38 (d, 2H J = 8.6 Hz, H-2' and H-6'), 6.99 (d, 1H, J = 16.3 Hz, H-8, vinyl), 6.84 (d, 1H, J = 16.3 Hz, H-7, vinyl), 6.74 (d, 2H, J = 8.6 Hz, H-3' and H5'), 6.72 (s, 1H, H-2), 6.57 (s, 1H, H-6), 6.39 (t, 1H, H-4), 5.33 (d, 1H, J = 3.5 Hz, H-1"), 3.63-3.13 (m, 6H, H-2", H-3", H-4", H-5", H-6"a, H-6"b);

^{13}C-NMR (CD_3OD, 50.3 MHz) δ 159.1 (C-3), 158.8 (c-5), 158.3 (C-4'), 140.2 (C-1), 128.7 (C-1'), 128.6 (C-8), 127.7 (C-2' and C-6'), 125.2 (C-7), 115.6 (C-3' and C-5'), 107.4 (C-6), 105.8 (C-4) 103.4 (C-2), 98.0 (C-1"), 73.7 (C-5"), 73.1 (C-3"), 72.1 (C-2"), 70.2 (C-4"), 61.0 (C-6").

Biotransformation of pinosylvin. The pinosylvin was synthesized as described by Bachelor,[15] and 50 mg of pinosylvin was used for the biotransformation. The same procedure was followed as described above. The 3-O-α-D-glucopyranosyl-(*E*)-pinosylvinl was obtained 12 mg (11%). The pinosylvin was recovered in 35 mg.

(*E*)-Pinosylvin 3-*O*-α-D-glucopyranoside: ^{1}H-NMR (CD_3OD, 500 MHz) δ 7.51 (d, 2H J = 7.38 Hz, H-2' and H-6'), 7.32 (t, 2H, H-8, H-7), 7.20 (m, 1H, H-4'), 7.03 (q, 2H, H-3' and H5'), 6.89 (s, 1H, H-2), 6.67 (s, 1H, H-6), 6.54 (t, 1H, H-4), 5.48 (d, 1H, J = 3.6 Hz, H-1"), 3.86-3.44 (m, 6H, H-2", H-3", H-4", H-5", H-6"a, H-6"b).

^{13}C-NMR (CD_3OD, 50.3 MHz) δ 158.8 (C-3), 158.5 (c-5), 139.7 (C-4'), 137.5 (C-1), 128.8 (C-1'), 128.8 (C-8), 128.4 (C-2' and C-6'), 127.4 (C-7), 126.3 (C-3' and C-5'), 107.5 (C-6), 106.5 (C-4) 103.8 (C-2), 98.1 (C-1"), 73.7 (C-5"), 73.1 (C-3"), 72.1 (C-2"), 70.2 (C-4"), 61.1 (C-6").

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