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Note

Glycosylation of *trans*-Resveratrol by Plant-Cultured Cells

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Plant-cultured cells of *Catharanthus roseus* converted *trans*-resveratrol into its 3-*O*-*β*-D-glucopyranoside, 4′-*O*-*β*-D-glucopyranoside, 3-*O*-(6-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranoside, and 3-*O*-(6-*O*-*α*-L-arabinopyranosyl)-*β*-D-glucopyranoside. The 3-*O*-(6-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranoside and 3-*O*-(6-*O*-*α*-L-arabinopyranosyl)-*β*-D-glucopyranoside compounds of *trans*-resveratrol are both new. Incubation of plant-cultured cells of *Ipomoea batatas* and *Strophanthus gratus* with *trans*-resveratrol gave *trans*-resveratrol 3-*O*-*β*-D-glucopyranoside and *trans*-resveratrol 4′-*O*-*β*-D-glucopyranoside.

Key words: biotransformation; *trans*-resveratrol; plant-cultured cell

trans-Resveratrol is one of the most important plant polyphenols and has attracted considerable pharmaceutical interest because of its diverse biological activities.[1)] However, the water-insoluble nature of *trans*-resveratrol limits its further pharmacological exploitation. However, its water-soluble derivatives, *i.e.*, *trans*-resveratrol 3-*O*-*β*-D-glucoside and *trans*-resveratrol 4′-*O*-*β*-D-glucoside, have been reported to show such pharmaceutical properties as cancer prevention,[2,3)] anti-oxidative activity,[4,5)] and estrogenic activity.[6,7)] Several attempts have recently been made to synthesize resveratrol glycosides by chemical methods involving tedious protection-deprotection procedures, and resulted in low yields.[8,9)] Plant cell cultures would be useful for the practical preparation of glycosides, due to the high potential of plant glycosyltransferases to diastereoselectively produce glycosides by one-step enzymatic glycosylation.

We report here the biotransformation of *trans*-resveratrol by plant-cultured cells to *trans*-resveratrol 3-*O*-(6-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranoside and *trans*-resveratrol 3-*O*-(6-*O*-*α*-L-arabinopyranosyl)-*β*-D-glucopyranoside which are both new compounds.

The substrate, *trans*-resveratrol (**1**), was biotransformed by using plant-cultured cells as biocatalysts. To a 500-mL flask containing 200 mL of the culture medium and suspension-cultured cells (100 g) was added 15 mg of the substrate. The culture was incubated at 25 °C for 5 d on a rotary shaker (120 rpm). After the incubation period, the cells and medium were separated by filtration with suction. The cells were extracted (×3) by homogenization with MeOH, and the resulting extract was concentrated. The residue was partitioned between H_2O and EtOAc. The H_2O layer was applied to a Diaion HP-20 column, and the column was washed with H_2O and then eluted with MeOH. The MeOH eluate was subjected to HPLC in a 150 × 20 mm column to give the products. No products were apparent in the medium. A control experiment using cells which had not been treated with the substrate, resulted in no detection of the substrate or products. The yield of the products was determined on the basis of the peak area from HPLC, and is expressed as a percentage relative to the total amount of whole reaction products extracted and the substrate. Large-scale biotransformation of a total of 500 mg of the substrate in ten 1-L flasks each containing 500 mL of the medium prepared the products for NMR analyses.

After a 5 d incubation period, products **2** (37%), **3** (15%), **4** (2%), and **5** (1%) were obtained from the MeOH extract of the *C. roseus* cells treated with **1**. The structures of products **2** and **3** were determined to be those of *trans*-resveratrol 3-*O*-*β*-D-glucopyranoside and *trans*-resveratrol 4′-*O*-*β*-D-glucopyranoside on the basis of their HRFABMS, ^{1}H and ^{13}C NMR, H-H COSY, C-H COSY, HMBC, and NOE spectra. The ^{1}H- and ^{13}C-NMR, H-H COSY, C-H COSY, and HMBC spectra were recorded in a DMSO-d_6 solution by using a Varian XL-400 spectrometer and the chemical shifts are expressed in δ (ppm), referring to TMS. Products **4** and **5** were identified as *trans*-resveratrol 3-*O*-(6-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranoside and *trans*-resveratrol 3-*O*-(6-*O*-*α*-L-arabinopyranosyl)-*β*-D-glucopyranoside. These disaccharide products **4** and **5** have not been identified before. The yield of products **4** and **5** was lower than that of previously reported 3-*O*-(6-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranosides and 3-*O*-(6-*O*-*α*-L-arabinopyranosyl)-*β*-D-glucopyranosides of capsaicinoids, which had been produced by incubating of *C. roseus* cells with capsaicinoids,[10)] probably due to the substrate specificity of the enzymes participating in the formation of the 3-*O*-(6-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranosides and 3-*O*-(6-*O*-*α*-L-arabinopyranosyl)-*β*-D-glucopyranosides. The spectral data for products **4** and **5** follow.

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trans-*Resveratrol 3*-O-*(6*-O-*β*-D-*xylopyranosyl)*-β*-D-*glucopyranoside (**4**).* HRFABMS m/z $(M + Na)^+$: calcd. for $C_{25}H_{30}O_{12}Na$ 545.1519; found, 545.1522. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 3.12–3.70 (11H, m, H-2″, 2‴, 3″, 3‴, 4″, 4‴, 5″, 5‴, 6″), 4.80 (1H, d, J = 8.0 Hz, H-1‴), 5.10 (1H, d, J = 7.6 Hz, H-1″), 6.33 (1H, m, H-4), 6.55 (1H, d, J = 1.8 Hz, H-6), 6.73 (1H, d, J = 1.8 Hz, H-2), 6.76 (2H, d, J = 8.8 Hz, H-3′, 5′), 6.86 (1H, d, J = 16.0 Hz, H-7), 7.01 (1H, d, J = 16.0 Hz, H-8), 7.39 (2H, d, J = 8.8 Hz, H-2′, 6′). ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: 60.6 (C-5‴), 68.8 (C-6″), 69.8, 69.9 (C-4″, C-4‴), 72.5 (C-2‴), 73.3 (C-2″), 76.4, 76.7 (C-3″, C-3‴), 77.0 (C-5″), 100.2 (C-1″), 102.0 (C-1‴), 102.7 (C-2), 104.7 (C-4), 107.0 (C-6), 115.5 (C-3′, C-5′), 125.1 (C-7), 127.9 (C-2′, C-6′), 128.5 (C-8), 129.9 (C-1′), 139.3 (C-1), 157.2 (C-4′), 158.2 (C-3), 158.8 (C-5).

trans-*Resveratrol 3*-O-*(6*-O-*α*-L-*arabinopyranosyl)*-β*-D-*glucopyranoside (**5**).* HRFABMS m/z $(M + Na)^+$: calcd. for $C_{25}H_{30}O_{12}Na$, 545.1519; found, 545.1520. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 3.09–3.78 (11H, m, H-2″, 2‴, 3″, 3‴, 4″, 4‴, 5″, 5‴, 6″), 4.77 (1H, d, J = 6.0 Hz, H-1‴), 5.09 (1H, d, J = 7.6 Hz, H-1″), 6.32 (1H, m, H-4), 6.55 (1H, d, J = 1.8 Hz, H-6), 6.73 (1H, d, J = 1.8 Hz, H-2), 6.76 (2H, d, J = 8.8 Hz, H-3′, 5′), 6.86 (1H, d, J = 16.0 Hz, H-7), 7.00 (1H, d, J = 16.0 Hz, H-8), 7.39 (2H, d, J = 8.8 Hz, H-2′, 6′). ^{13}C-NMR (100 MHz, DMSO-d_6) δ_C: 59.5 (C-5‴), 67.0 (C-4‴), 68.5 (C-6″), 69.8 (C-4″), 72.5 (C-2‴), 72.9 (C-3‴), 73.2 (C-2″), 76.7 (C-3″), 77.0 (C-5″), 100.1 (C-1″), 101.5 (C-1‴), 102.8 (C-2), 104.9 (C-4), 107.0 (C-6), 115.5 (C-3′, C-5′), 125.1 (C-7), 127.7 (C-2′, C-6′), 128.5 (C-8), 129.9 (C-1′), 139.3 (C-1), 157.2 (C-4′), 158.5 (C-3), 158.8 (C-5).

A time-course experiment was used to investigate the biotransformation pathway for **1** by the cultured cells of *C. roseus*. Figure 1A shows that mono-glucoside products **2** and **3** were produced at an early stage of incubation, whereas disaccharides **4** and **5** were accumulated after 3 d of incubation. These findings indicate that the disaccharide products were formed from the corresponding mono-glucosides, as shown in Fig. 2. The entire experiments were conducted three times with essentially the same result, and one representative data set is shown in Fig. 1.

On the other hand, the biotransformation of *trans*-resveratrol by cultured cells of *I. batatas* and *S. gratus*

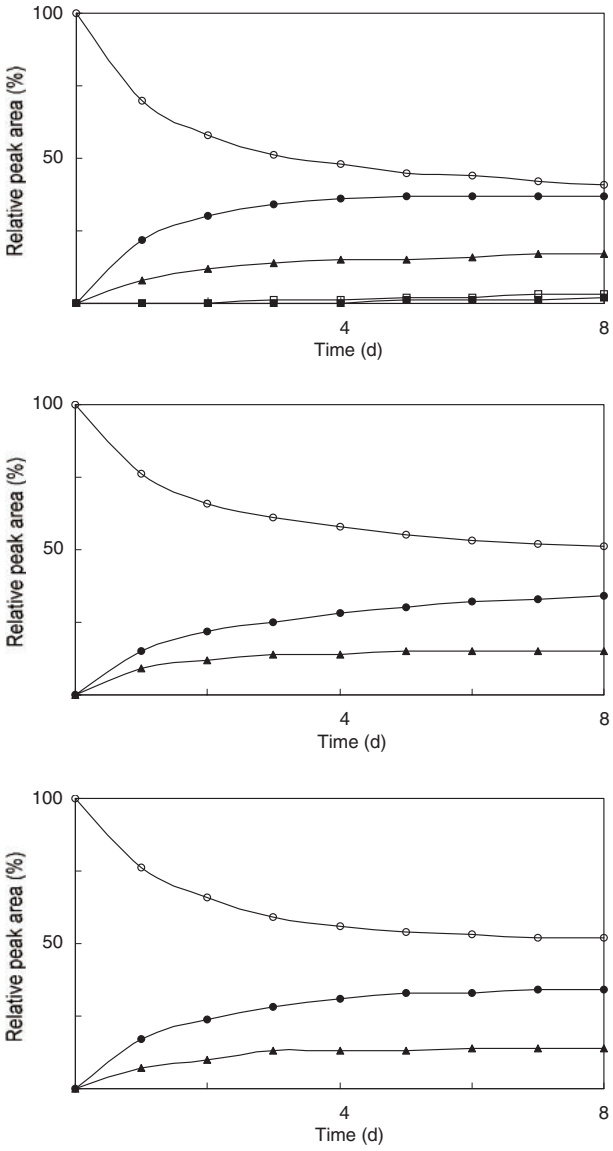

Fig. 1. Time-Course Characteristics for the Biotransformation of *trans*-Resveratrol (**1**) by Cultured Cells of (A) *C. roseus*, (B) *I. batatas*, and (C) *S. gratus*.

The substrate, *trans*-resveratrol (**1**, 15 mg), was incubated at 25 °C with 100 g of the suspension cell culture on a rotary shaker (120 rpm). The relative peak areas by HPLC of substrate **1** (○) and product **2** (●), **3** (▲), **4** (□) and **5** (■) are plotted.

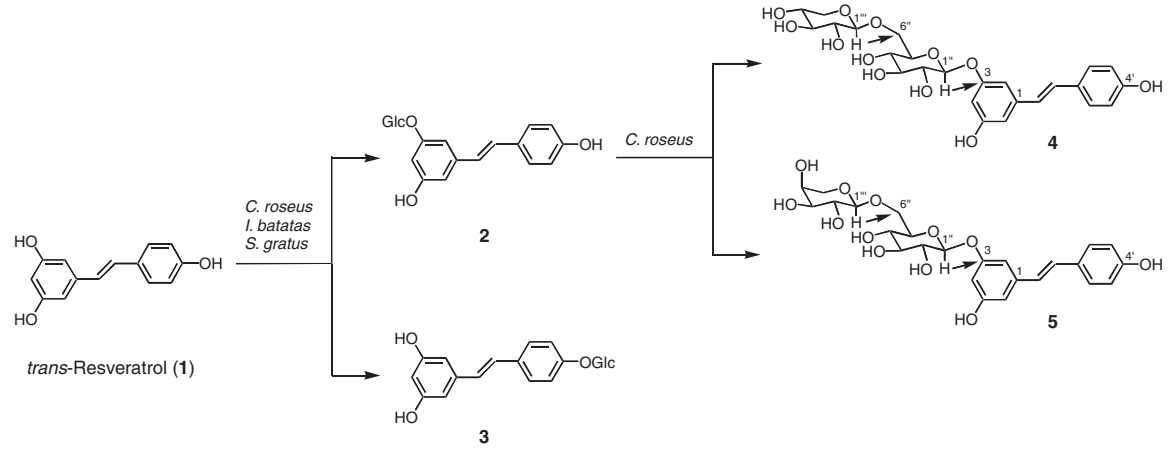

Fig. 2. Biotransformation Pathway for *trans*-Resveratrol (**1**) by Plant-Cultured Cells.
Arrows indicate the HMBC correlations.

resulted in the formation of its 3-*O*-*β*-D-glucopyranoside and 4′-*O*-*β*-D-glucopyranoside (Fig. 1B and C).

The results of this experiment show that plant-cultured cells of *I. batatas* and *S. gratus* converted *trans*-resveratrol into its 3-*O*-*β*-D-glucopyranoside and 4′-*O*-*β*-D-glucopyranoside and that cultured *C. roseus* cells glycosylated *trans*-resveratrol to its 3-*O*-(6-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranoside (primeveroside) and 3-*O*-(6-*O*-*α*-L-arabinopyranosyl)-*β*-D-glucopyranoside (vicianoside), which are both new compounds, together with *trans*-resveratrol 3-*O*-*β*-D-glucopyranoside and *trans*-resveratrol 4′-*O*-*β*-D-glucopyranoside. The biotransformation pathways were different between these plant cells. It has recently been reported that cultured cells of *C. roseus* transformed capsaicinoids to the corresponding primeverosides and vicianosides.[10] The cultured cells of *C. roseus* are useful biocatalysts to prepare primeveroside and vicianoside derivatives. Further studies on the pharmacological activity of *trans*-resveratrol glycosides are now in progress.

Acknowledgments

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