

OPEN ACCESS

Full open access to this and thousands of other papers at <http://www.la-press.com>.

Glycosylation of Capsaicin Derivatives and Phenylpropanoid Derivatives Using Cultured Plant Cells

Hisashi Katsuragi¹, Kei Shimoda², Ryohei Yamamoto³, Kohji Ishihara³ and Hiroki Hamada³

¹Sunny Health Co. Ltd, Nakajima Bldg. 8-8, Kabuto-cho, Nihonbashi, Chuo-ku, Tokyo 103-0026, Japan. ²Department of Chemistry, Faculty of Medicine, Oita University, 1-1, Hasama-machi, Oita 879-5593, Japan. ³Department of Life Science, Faculty of Science, Okayama University of Science, 1-1, Ridai-cho, Kita-ku, Okayama 700-0005, Japan.

Corresponding author email: hamada@dls.ous.ac.jp

Abstract: Biotransformations of capsaicinoids such as capsaicin and 8-nordihydrocapsaicin and phenylpropanoids such as cinnamic acid, *p*-coumaric acid, caffeic acid, and ferulic acid have been investigated using cultured plant cells. Capsaicin and 8-nordihydrocapsaicin were converted into the corresponding glycosides which are three glycosides respectively using the cultured cells of *Catharanthus roseus*. In a time-course study under sterile conditions, the changes in amounts of their reaction products were determined. Furthermore phenylpropanoid, such as cinnamic acid, *p*-coumaric acid, caffeic acid and ferulic acid have been biotransformed using the cultured cells of the *Eucalyptus perriniana*, and then cinnamic acid was converted into two glycosides. In addition, *p*-coumaric acid, caffeic acid and ferulic acid were converted into four, four and three glycosides respectively. Then in time-course study under sterile conditions, the change in amounts of their reaction products were determined. Finally it was found that the cultured plant cells have the ability to glycosylate the phenolic group of capsaicinoids and phenylpropanoids regioselectively.

Keywords: biotransformation, capsaicinoid, glycosylation, phenylpropanoid, plant cultured cells, *Catharanthus roseus*, *Eucalyptus perriniana*

Biochemistry Insights 2011:4 1–12

doi: [10.4137/BCI.S6682](https://doi.org/10.4137/BCI.S6682)

This article is available from <http://www.la-press.com>.

© the author(s), publisher and licensee Libertas Academica Ltd.

This is an open access article. Unrestricted non-commercial use is permitted provided the original work is properly cited.



Introduction

The synthesis of the biologically active compounds using living cells by biotransformation of various organic compounds has been investigated using mammalian cells, bacteria, enzymes and plant cells in the biotechnology application. As plant cells have glycosyltransferases in their cells, glycosylation is a characteristic biotransformation reaction using cultured plant cells.

Plants of the *Capsicum* species have been important sources of food, spices and medicines worldwide for centuries. The chemical constituents in the fruits of *Capsicum* plants, such as hot chilli pepper fruits, which are responsible for the sensory effects associated with pungency, are a series of branched or straight-chain alkylvanillylamides, capsaicinoids.

Capsaicin is the most pungent principle among naturally occurring capsaicinoids.¹ Capsaicin has also been reported to decrease adipose tissue weight and serum triacylglycerol content in rats by enhancing energy metabolism.² It has also shown a wide range of pharmacological properties, such as body heat generation,³ lipid metabolism,^{4,5} antioxidant activity,^{6–8} antibacterial activity,⁹ anodyne effect,¹⁰ and perspiration effect.¹¹ However capsaicinoids possess extensive neurological toxicity and direct irritant effects on skin and mucous membrane.¹²

Furthermore, capsaicinoids are almost insoluble in water and poorly absorbed after oral administration. These disadvantages prevent capsaicinoids from being used as food additives and medicines.

Recently, it has been reported that capsaicin was glucosylated by cultured plant cells to its monoglucoside, capsaicin- β -D-glucopyranoside. Its pungency was approximately 1/100 of that of capsaicin and its water solubility was enhanced.¹³

We report here the biotransformations of capsaicin and 8-nordihydrocapsaicin, both of which are naturally occurring capsaicinoids, into the corresponding monoglucosides and disaccharides, β -primeverosides which are more soluble in water. The *Catharanthus roseus* cell cultures were used.

Phenylpropanoids such as cinnamic acid, *p*-coumaric acid, caffeic acid and ferulic acid are naturally occurring anti-oxidants which act as effective scavengers of free radicals.^{14–16} It is well known that shikimic acid is metabolized, in plant cells, to cinnamic

acid and *p*-coumaric acid, which is further converted into caffeic acid and ferulic acid.

We report here the biotransformations of cinnamic acid, *p*-coumaric acid, caffeic acid, and ferulic acid into the corresponding monoglucosides and disaccharides, which are more soluble in water, using cultured plant cells of the *Eucalyptus perriniana*.

Experimental Procedures

Substrates

Capsaicin and 8-nordihydrocapsaicin were purchased from Tokyo Kasei Kogyo Co. Ltd.. Cinnamic acid, *p*-coumaric acid, caffeic acid, and ferulic acid, which were used as substrates, were purchased from Aldrich Chemical Co.

Cell suspension culture and culture conditions

The cultured cells of *C. roseus* were prepared as described previously.¹⁷ A cell suspension culture was started by transferring static cultured callus (fr. wt 40 g (0.40 g fr. wt of cells per ml of medium) to 100 ml of Schenk and Hidebrandt (SH) medium (pH 5.7) in a 300-ml conical flask. The cells were incubated for 2 weeks on an illuminated (4000 lx) rotary shaker (120 rpm) at 25 °C.

The cultured cells of *E. perriniana* were subcultured at 4-week intervals on solid Murashige and Skoog (MS) medium (100 ml in a 300-ml conical flask) containing 3% sucrose, 10 mM 2,4-dichlorophenoxyacetic acid, and 1% agar (adjusted to pH 5.7) at 25 °C in the dark. A cell suspension culture was started by transferring subcultured cells (fr. wt 40 g) to 100 ml of MS liquid medium (pH 5.7) in a 300-ml conical flask. The cells were incubated for 2 weeks in a rotary shaker (120 rpm) at 25 °C in the dark.

Biotransformation and purification of products

Biotransformation experiments of capsaicin and 8-nordihydrocapsaicin were performed by adding 0.1 mmol of substrate to each of eight 300-ml conical flasks containing 70 ml of SH medium and 30 g of cultured cells suspension of *C. roseus*. The cultures were incubated for a 6 h interval to 48 h on an illuminated (4000 lx) rotary shaker (120 rpm) at 25 °C. After incubation, the products were extracted

and purified according to the previously reported method.¹⁸ Concerning cinamic acid, *p*-coumaric acid, caffeic acid and ferulic acid, time course experiments were performed by adding 0.1 mmol of each substrate to six 300-ml conical flasks containing 70 ml of MS medium and 30 g of cultured cells suspension of *E. perriniana*. The cultures were incubated for a 12 h interval to 72 h on rotary shaker at 25 °C in the dark. After incubation period, the cells and medium were separated by filtration with suction. Extraction and purification procedures of biotransformation products were same as described above. The yield of the products was determined on the basis of the peak area from HPLC and expressed as a relative percentage to the total amount of the whole reaction products extracted.

Analysis of the products

HPLC was carried out with a YMC-Pack R&D ODS column (150 × 30 mm) using MeOH-H₂O (9:11, v/v) as the eluent [detection: UV (280 nm); flow rate: 1.0 ml/min]. ¹H and ¹³C NMR, H-H COSY, C-H COSY, NOE, and HMBC spectra were measured in CD₃OD on a Varian XL-400 spectrometer and the chemical shifts were expressed in δ (ppm) referring to TMS. HRFABMS spectra were taken on a JEOL MStation JMS-700 spectrometer. The structures of the products were determined by HRFABMS, ESIMS, ¹H and ¹³C NMR, H-H COSY, C-H COSY, NOE, and HMBC spectra. As regarding capsaicin and 8-nordihydrocapsaicin, the absolute configuration of the sugar moieties in disaccharide products was determined as follows. Each disaccharides, 3, 4, 7, and 8 was added to a vial containing 100 μl of 4.0 M HCl, heated to 80 °C for 2 h, and then cooled to room temperature. The solvent was removed in a stream of N₂ and each hydrolysate was converted into the corresponding pentafluoropropionate with pentafluoropropionic anhydride (400 μl) in 400 μl CH₂Cl₂ in a sealed tube at 120 °C for 2 h. Excess reagents were removed under a stream of N₂ and the derivatives were analyzed by chiral GLC (Shimadzu GC-17A with FID) on CP cyclodextrin β236M-19 (Chrompack, column temp: 100 °C, injector temp: 200 °C, detector temp: 200 °C, split ratio: 50, carrier gas: N₂, 100 kPa). The peaks of the derivatives from primeverosides 3 and 7 were assigned to those of

D-xylose and D-glucose, and the peaks of derivatives from vicianosides 4 and 8 were assigned to those of L-arabinose and D-glucose.

Result and Discussion

Glycosylation of capsaicin

A time-course experiment was carried out to investigate the ability of the cultured cells of *C. roseus* to biotransform capsaicin. Three glycoside compounds (named products 2–4) were detected in this experiment. The product 2 was identified as capsaicin 4-*O*-β-D-glucopyranoside by comparison of its ¹H and ¹³C NMR spectroscopic data with previously reported data.^{13,19,20}

Structures of products 3 and 4 were determined on the basis of their HRFABMS, ¹H and ¹³C NMR spectra, and chiral-GLC as capsaicin 4-*O*-(6-*O*-β-D-xylopyranosyl)-β-D-glucopyranoside and capsaicin 4-*O*-(6-*O*-α-L-arabinopyranosyl)-β-D-glucopyranoside. The ¹H and ¹³C NMR spectra of sugar moiety of 3 and 4 indicated that they were β-primeverose and β-vicianose, respectively.^{21,22}

Capsaicin 4-*O*-(6-*O*-β-D-xylopyranosyl)-β-D-glucopyranoside: HRFABMAS: *m/z* 622.2850 [M+Na]⁺ (calcd for C₂₉H₄₅NO₁₂Na, 622.2839), ¹H NMR (400 MHz, CD₃OD): δ 0.95 (6H, *d*, *J* = 6.8 Hz, H-16, 17), 1.37 (2H, *q*, *J* = 7.6 Hz, H-11), 1.60 (2H, *q*, *J* = 7.6 Hz, H-10), 1.98 (2H, *m*, H-12), 2.20 (3H, *m*, H-9,15), 3.15 (1H, *dd*, *J* = 11.6, 9.6 Hz, H-5a''), 3.28–3.60 (7H, *m*, H-2', 2'', 3', 3'', 4', 4'', 5'), 3.75–3.81 (2H, *m*, H-5b'', 6a'), 3.83 (3H, *s*, OCH₃), 4.05 (1H, *dd*, *J* = 11.6, 1.6 Hz, H-6b'), 4.28 (2H, *s*, H-7), 4.32 (1H, *d*, *J* = 7.6 Hz, H-1''), 4.91 (1H, *d*, *J* = 7.2 Hz, H-1'), 5.30–5.40 (2H, *m*, H-13, 14), 6.80 (1H, *dd*, *J* = 8.4, 1.6 Hz, H-6), 6.92 (1H, *d*, *J* = 1.6 Hz, H-2), 7.10 (1H, *d*, *J* = 8.4 Hz, H-5); ¹³C NMR (100 MHz, CD₃OD): δ 23.1 (C-16, 17), 26.5 (C-10), 30.3 (C-11), 32.3 (C-15), 33.2 (C-12), 36.8 (C-9), 43.7 (C-7), 56.6 (OCH₃), 67.1 (C-5''), 69.4 (C-6'), 71.4 (C-4''), 71.6 (C-4'), 74.7 (C-2''), 75.2 (C-2'), 77.8 (C-3''), 77.9 (C-3', 5'), 102.5 (C-1'), 105.0 (C-1''), 113 (C-2), 118.0 (C-5), 121.2 (C-6), 127.9 (C-13), 135 (C-1), 139.0 (C-14), 147.1 (C-4), 150.7 (C-3), 175.9 (C-8).

Capsaicin 4-*O*-(6-*O*-α-L-arabinopyranosyl)-β-D-glucopyranoside (4): HRFABMAS: *m/z* 622.2841 [M+Na]⁺ (calcd for C₂₉H₄₅NO₁₂Na, 622.2839), ¹H NMR (400 MHz, CD₃OD): δ 0.95 (6H, *d*, *J* = 6.8 Hz,

H-16, 17), 1.36 (2H, *q*, $J = 7.6$ Hz, H-11), 1.60 (2H, *q*, $J = 7.6$ Hz, H-10), 1.98 (2H, *m*, H-12), 2.20 (3H, *m*, H-9, 15), 3.28 (1H, *dd*, $J = 12.2, 1.8$ Hz, H-5a''), 3.30–3.60 (7H, *m*, H-2', 2'', 3', 3'', 4', 4'', 5'), 3.73–3.81 (2H, *m*, H-5b'', 6a'), 3.83 (3H, *s*, OCH₃), 4.05 (1H, *dd*, $J = 11.6, 1.6$ Hz, H-6b'), 4.28 (2H, *s*, H-7), 4.30 (1H, *d*, $J = 6.4$ Hz, H-1'), 4.91 (1H, *d*, $J = 7.2$ Hz, H-1''), 5.30–5.39 (2H, *m*, H-13, 14), 6.80 (1H, *dd*, $J = 8.4, 1.6$ Hz, H-6), 6.92 (1H, *d*, $J = 1.6$ Hz, H-2), 7.10 (1H, *d*, $J = 8.4$ Hz, H-5); ¹³C NMR (100 MHz, CD₃OD): δ 23.1 (C-16, 17), 26.5 (C-10), 30.3 (C-11), 32.2 (C-15), 33.2 (C-12), 36.9 (C-9), 43.7 (C-7), 56.6 (OCH₃), 67.0 (C-5''), 69.3 (C-6'), 70.0 (C-4''), 71.8 (C-4'), 72.2 (C-2''), 74.0 (C-3''), 75.2 (C-2'), 78.0 (C-3', 5'), 102.6 (C-1'), 104.6 (C-1''), 113 (C-2), 117.9 (C-5), 121.2 (C-6), 127.9 (C-13), 134.9 (C-1), 139.0 (C-14), 147.0 (C-4), 150.8 (C-3), 175.9 (C-8).

Figure 1 showed that capsaicin was converted into product 2 at early stage incubation and products 3 and 4 were accumulated after 12 h of incubation. At 48 h reaction, the amounts of products 2, 3, and 4 were 36.2%, 18.8%, and 15.0%, respectively, relative to the total amount of reaction products on a molar basis. This result indicated that product 1 was first converted to monoglucoside 2 and further product 2 was converted to disaccharides 3 and 4, as shown in Scheme 1.

Glycosylation of 8-nordihydrocapsaicin

In a similar way to capsaicin, a time-course experiment was carried out biotransformation of

8-nordihydrocapsaicin. Three glycoside compounds (named products 6–8) were reproduced by this experiment. The product 6 was identified as 8-nordihydrocapsaicin 4-*O*- β -D-glucopyranoside by comparison of its ¹H and ¹³C NMR spectroscopic data with previously reported data.¹⁹

Structures of products 7 and 8 were identified on the basis of their HRFABMS, ¹H and ¹³C NMR spectra, and chiral-GLC as 8-nordihydrocapsaicin 4-*O*-(6-*O*- β -D-xylopyranosyl)- β -D-glucopyranoside and 8-nordihydrocapsaicin 4-*O*-(6-*O*- α -L-arabinopyranosyl)- β -D-glucopyranoside. The ¹H and ¹³C NMR spectra of sugar moiety of 3 and 4 were shown that they were β -primeverose and β -vicianose, respectively.^{21,22}

8-nordihydrocapsaicin 4-*O*-(6-*O*- β -D-xylopyranosyl)- β -D-glucopyranoside: HRFABMAS: m/z 610.2833 [M+Na]⁺ (calcd for C₂₈H₄₅NO₁₂Na, 610.2839), ¹H NMR (400 MHz, CD₃OD): δ 0.90 (3H, *t*, $J = 6.8$ Hz, H-16), 1.19–1.26 (10H, *m*, H-11, 12, 13, 14, 15), 1.62 (2H, *q*, $J = 7.6$ Hz, H-10), 2.22 (2H, *t*, $J = 7.6$ Hz, H-19), 3.16 (1H, *dd*, $J = 11.6, 9.6$ Hz, H-5a''), 3.32–3.60 (7H, *m*, H-2', 2'', 3', 3'', 4', 4'', 5'), 3.75–3.81 (2H, *m*, H-5b'', 6a'), 3.85 (3H, *s*, OCH₃), 4.01 (1H, *dd*, $J = 11.6, 1.6$ Hz, H-6b'), 4.30 (2H, *m*, H-7), 4.33 (1H, *d*, $J = 7.6$ Hz, H-1'), 4.91 (1H, *d*, $J = 7.2$ Hz, H-1''), 6.85 (1H, *dd*, $J = 8.4, 1.6$ Hz, H-6), 6.94 (1H, *d*, $J = 1.6$ Hz, H-2), 7.15 (1H, *d*, $J = 8.4$ Hz, H-5); ¹³C NMR (100 MHz, CD₃OD): δ 14.5 (C-16), 23.7 (C-15), 27.1 (C-10), 30.5 (C-11, 12, 13), 33.0 (C-14),

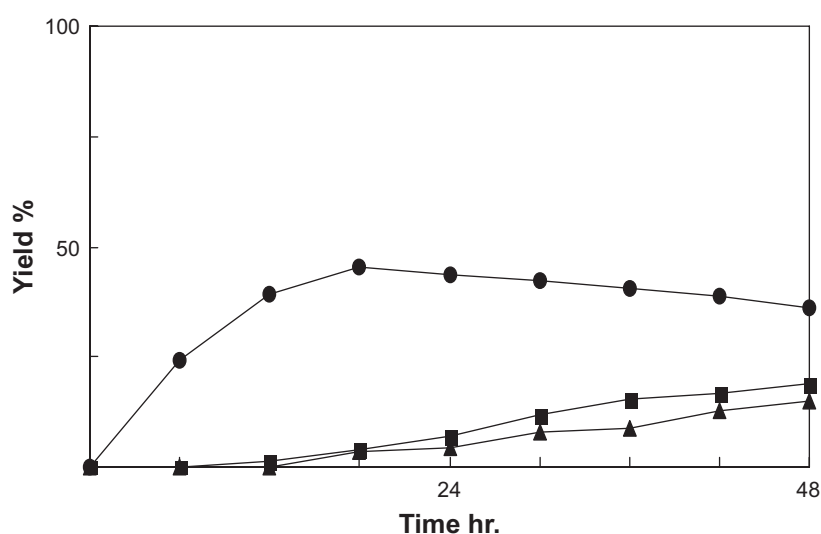
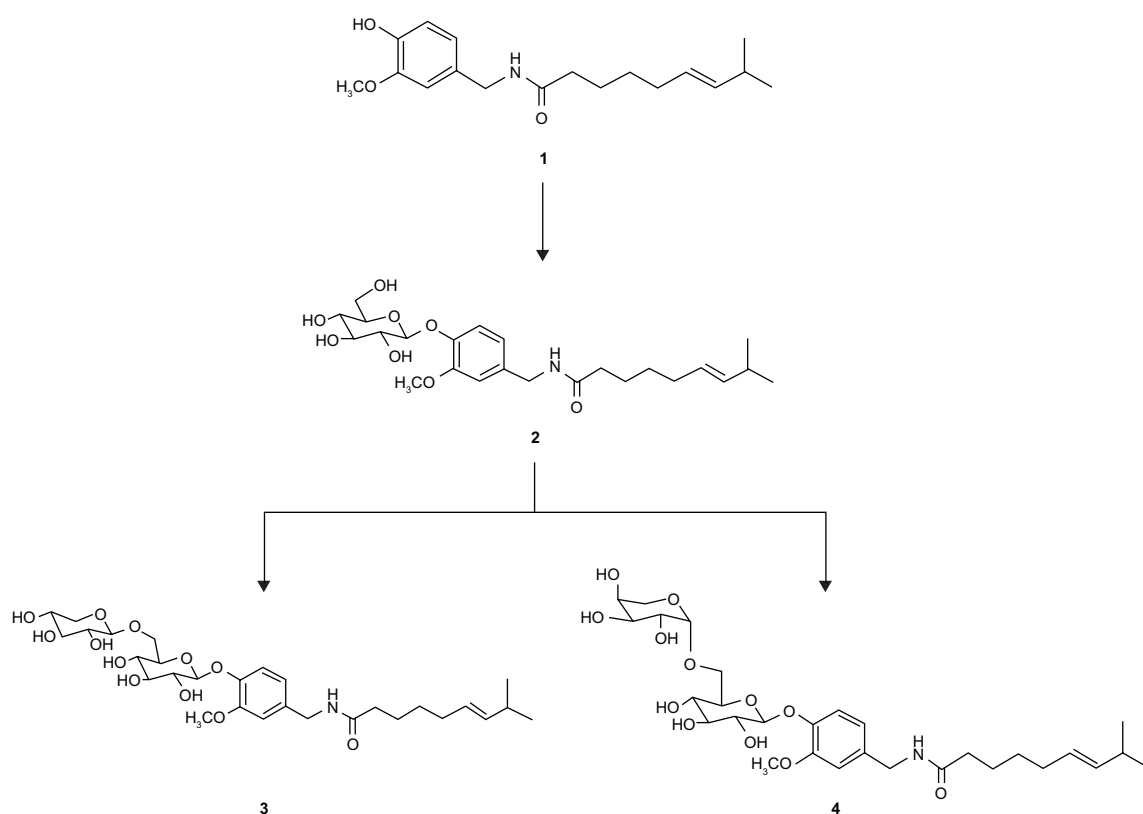


Figure 1. Time-course of the biotransformation of capsaicin by cultured cells of *C. roseus*. Yield is expressed as a percentage relative to the total amount of reaction products on a molar basis. Yields of 2(●), 3(■), and 4(▲) are plotted.



Scheme 1. Glycosylation of capsaicin by cultured cells of *C. roseus*.

37.2 (C-9), 43.8 (C-7), 67.2 (C-5''), 69.7 (C-6'), 71.4 (C-4''), 71.6 (C-4'), 74.7 (C-2''), 75.1 (C-2'), 78.0 (C-3'), 77.9 (C-3'', 5'), 102.6 (C-1'), 105.1 (C-1''), 113.2 (C-2), 118.4 (C-5), 121.5 (C-6), 135.2 (C-1), 146.9 (C-4), 151.0 (C-3), 176.2 (C-8).

8-nordihydrocapsaicin 4-*O*-(6-*O*- α -L-arabinopyranosyl)- β -D-glucopyranoside: HRFABMAS: m/z 610.2838 [M+Na]⁺ (calcd for C₂₈H₄₅NO₁₂Na, 610.2839), ¹H NMR (400 MHz, CD₃OD): δ 0.89 (3H, *t*, *J* = 6.8 Hz, H-16), 1.18–1.32 (10H, *m*, H-11, 12, 13, 14, 15), 1.63 (2H, *q*, *J* = 7.6 Hz, H-10), 2.24 (2H, *t*, *J* = 7.6 Hz, H-9), 3.29 (1H, *dd*, *J* = 12.0, 2.0 Hz, H-5a''), 3.32–3.61 (7H, *m*, H-2', 2'', 3', 3'', 4', 4'', 5'), 3.74–3.82 (2H, *m*, H-5b'', 6a'), 3.85 (3H, *s*, OCH₃), 4.05 (1H, *dd*, *J* = 11.6, 1.6 Hz, H-6b'), 4.30 (2H, *m*, H-7), 4.31 (1H, *d*, *J* = 6.4 Hz, H-1''), 4.92 (1H, *d*, *J* = 7.6 Hz, H-1'), 6.85 (1H, *dd*, *J* = 8.4, 1.6 Hz, H-6), 6.94 (1H, *d*, *J* = 1.6 Hz, H-2), 7.15 (1H, *d*, *J* = 8.4 Hz, H-5); ¹³C NMR (100 MHz, CD₃OD): δ 14.4 (C-16), 23.7 (C-15), 27.1 (C-10), 30.3 (C-11, 12, 13), 32.9 (C-14), 37.1 (C-9), 43.8 (C-7), 67.1 (C-5''), 69.5 (C-6'), 70.0 (C-4''), 71.7 (C-4'), 72.2 (C-2''), 74.1 (C-3''), 75.3 (C-2'), 78.0 (C-3', 5'), 102.6 (C-1'),

104.6 (C-1''), 113.1 (C-2), 118.3 (C-5), 121.4 (C-6), 135.1 (C-1), 146.7 (C-4), 150.8 (C-3), 176.0 (C-8).

Figure 2 showed that 8-nordihydrocapsaicin was converted into product 6 at early stage of incubation and products 7 and 8 were accumulated after 12 h of incubation. At 48 h reaction, the amounts of products 6, 7, and 8 were 45.5%, 27.9%, and 19.4%, respectively, relative to the total amount of reaction products on a molar basis. The same as capsaicin, 8-nordihydrocapsaicin was first converted to monoglucoside 6 and further product 6 was converted to disaccharides 7 and 8, as shown in Scheme 2.

Glycosylation of cinnamic acid

In order to examine the ability of the cultured cells of *E. perriniana* to biotransform cinnamic acid (9), the time course in the conversion of 9 was followed. Three compounds (named products 10–12) were obtained and the structure of the product 11 was identified as *p*-coumaric acid by HPLC spectra. Structures of products 10 and 12 were determined on the basis of their ESIMS, ¹H and ¹³C NMR spectra as cinnamoyl-*O*- β -D-glucoside, and *p*-coumaroyl-*O*- β -D-glucoside, respectively.

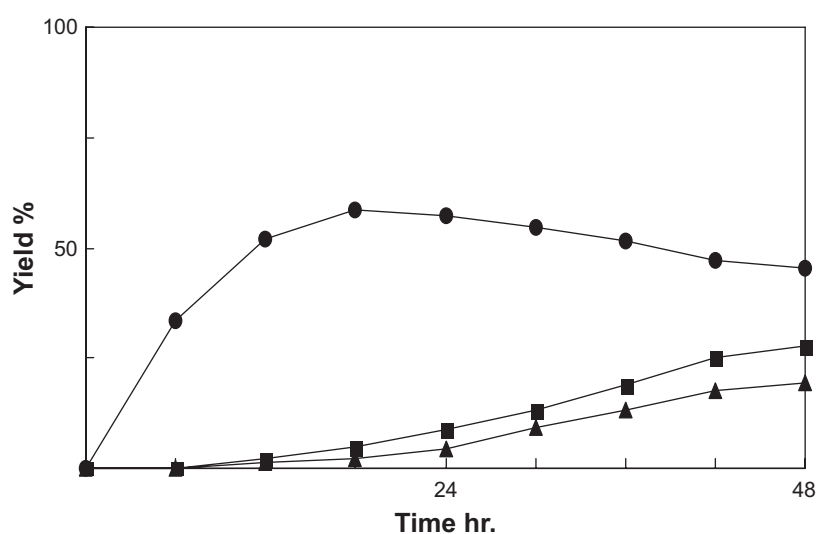
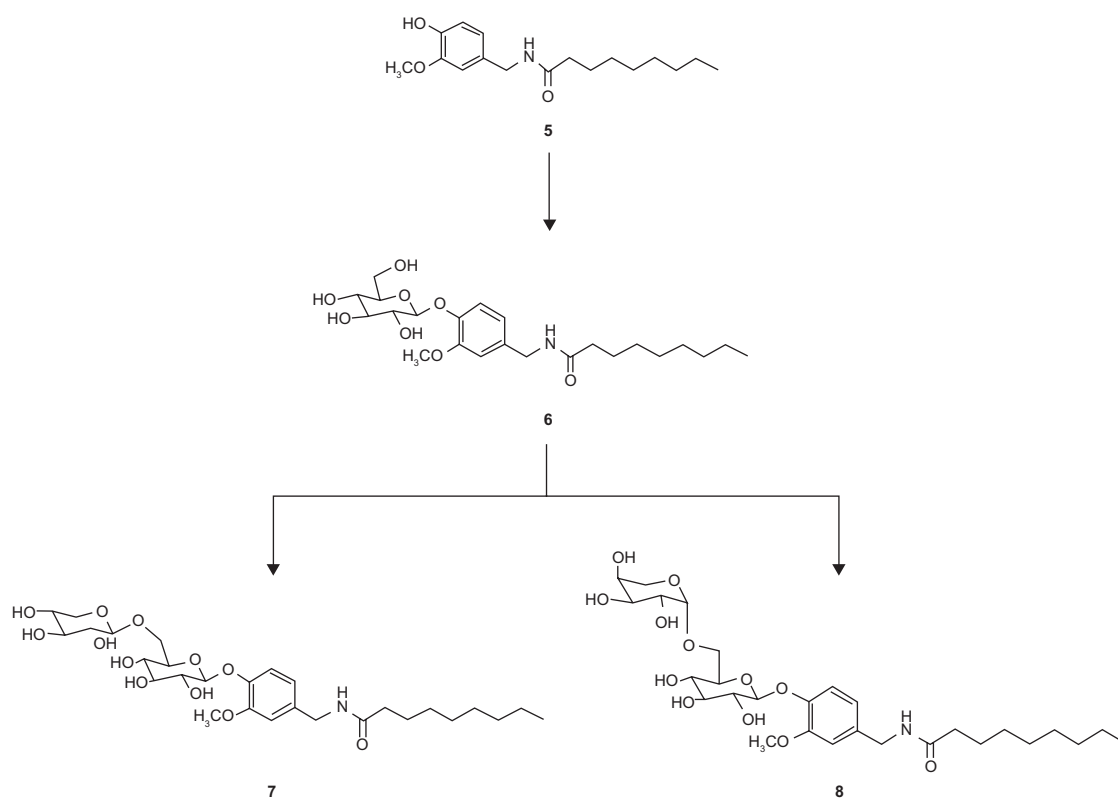


Figure 2. Time-course of the biotransformation of 8-nordihydrocapsaicin by cultured cells of *C. roseus*. Yield is expressed as a percentage relative to the total amount of reaction products on a molar basis. Yields of 6(●), 7(■), and 8(▲) are plotted.

Cinnamoyl-*O*- β -D-glucoside (10): ESIMS: m/z 333 $[M+Na]^+$, 1H NMR (400 MHz, CD_3OD): δ 5.61 (1H, *d*, $J = 7.6$ Hz, H-1'), 6.57 (1H, *d*, $J = 16.0$ Hz, H-8), 7.85 (1H, *d*, $J = 16.0$ Hz, H-7); ^{13}C NMR (100 MHz, CD_3OD): δ 62.2 (C-6'), 70.9–78.7 (C-2', 3', 4', 5'), 95.7 (C-1'), 118.0 (C-8), 129.1 (C-2, 6),

129.8 (C-3, 5), 131.6 (C-4), 135.3 (C-1), 147.4 (C-7), 166.9 (C-9).

p-Coumaroyl-*O*- β -D-glucoside (12): ESIMS: m/z 349 $[M+Na]^+$, 1H NMR (400 MHz, CD_3OD): δ 5.57 (1H, *d*, $J = 8.0$ Hz, H-1'), 6.37 (1H, *d*, $J = 16.0$ Hz, H-8), 6.81 (2H, *d*, $J = 8.8$ Hz, H-3, 5), 7.48 (2H, *d*,



Scheme 2. Glycosylation of 8-nordihydrocapsaicin by cultured cells of *C. roseus*.

$J = 8.4$ Hz, H-2, 6), 7.72 (1H, d , $J = 16.0$ Hz, H-7); ^{13}C NMR (100 MHz, CD_3OD): δ 62.2 (C-6'), 71.1–78.7 (C-2', 3', 4', 5'), 95.7 (C-1'), 114.3 (C-8), 116.8 (C-3, 5), 126.9 (C-1), 131.2 (C-2, 6), 147.8 (C-7), 161.4 (C-4), 167.5 (C-9).

Figure 3 showed that cinnamic acid (9) was converted into 10 at early stage of incubation and the products 11 and 12 were produced at a low level. At 72 h reaction, the amounts of products 10, 11, and 12 were 28.8%, 5.2%, and 2.6%, respectively, relative to the total amount of reaction products on a molar basis. This result indicated that glucosylation at carboxyl group of cinnamic acid is the predominant modification compared to hydroxylation at 4-position of cinnamic acid to *p*-coumaric acid. Then, it was suggested that hydroxylation at 4-position of cinnamoyl-*O*- β -D-glucoside, or glucosylation at carboxyl group of *p*-coumaric acid was occurred. Biotransformation pathway of cinnamic acid is shown in Scheme 3.

Glycosylation of *p*-coumaric acid (11)

Five compounds (named products 12–16) were produced by incubation of the cultured cells of *E. perriniana* with *p*-coumaric acid (11). The structure of the product 14 was identified as caffeic acid by HPLC spectra. Structures of products 13, 15, and 16 were determined on the basis of their ESI, ^1H and ^{13}C NMR spectra as 4-*O*- β -D-glucosyl coumarate, 3-*O*- β -D-glucosyl caffeate, and (3-*O*- β -D-glucosyl)-caffeoyl-*O*- β -D-glucoside, respectively.

4-*O*- β -D-Glucosyl coumarate (13): ESIMS: m/z 349 $[\text{M}+\text{Na}]^+$, ^1H NMR (400 MHz, CD_3OD): δ 4.96 (1H, d , $J = 7.6$ Hz, H-1'), 6.38 (1H, d , $J = 16.4$ Hz, H-8), 7.11 (2H, d , $J = 8.8$ Hz, H-3, 5); ^{13}C NMR (100 MHz, CD_3OD): δ 62.4 (C-6'), 71.2–78.1 (C-2', 3', 4', 5'), 101.7 (C-1'), 117.8 (C-3, 5, 8), 130.3 (C-1, 2, 6), 146.5 (C-7), 160.3 (C-4), 170.8 (C-9).

3-*O*- β -D-Glucosyl caffeate (15): ESIMS: m/z 365 $[\text{M}+\text{Na}]^+$, ^1H NMR (400 MHz, CD_3OD): δ 4.82 (1H, d , $J = 7.2$ Hz, H-1'), 6.32 (1H, d , $J = 15.6$ Hz, H-8), 6.86 (1H, d , $J = 8.8$ Hz, H-5), 7.17 (1H, dd , $J = 8.0$, 2.0 Hz, H-6); ^{13}C NMR (100 MHz, CD_3OD): δ 62.4 (C-6'), 71.4–78.4 (C-2', 3', 4', 5'), 104.2 (C-1'), 117.1 (C-8), 117.3 (C-2), 117.9 (C-5), 125.7 (C-6), 128.1 (C-1), 145.7 (C-3), 146.9 (C-7), 150.7 (C-4), 171.2 (C-9).

(3-*O*- β -D-Glucosyl)-caffeoyl-*O*- β -D-glucoside (16): ESIMS: m/z 527 $[\text{M}+\text{Na}]^+$, ^1H NMR (400 MHz, CD_3OD): δ 4.82 (1H, d , $J = 7.2$ Hz, H-1'), 5.56 (1H, d , $J = 8.4$ Hz, H-1'), 6.41 (1H, d , $J = 16.0$ Hz, H-8), 6.87 (1H, d , $J = 8.4$ Hz, H-5), 7.20 (1H, dd , $J = 8.0$, 2.0 Hz, H-6), 7.56 (1H, d , $J = 1.6$ Hz, H-2), 7.61 (1H, d , $J = 16.0$ Hz, H-7); ^{13}C NMR (100 MHz, CD_3OD): δ 62.3 (C-6'), 62.5 (C-6''), 71.1–78.5 (C-2'-5''), 71.4–78.7 (C-2', 3', 4', 5'), 95.7 (C-1'), 104.2 (C-1''), 115.3 (C-8), 117.4 (C-2), 118.1 (C-5), 126.3 (C-6), 127.7 (C-1), 146.9 (C-3), 147.6 (C-4), 151.2 (C-7), 167.4 (C-9).

Figure 4 showed that *p*-coumaric acid (11) was converted into 13 at early stage of incubation,

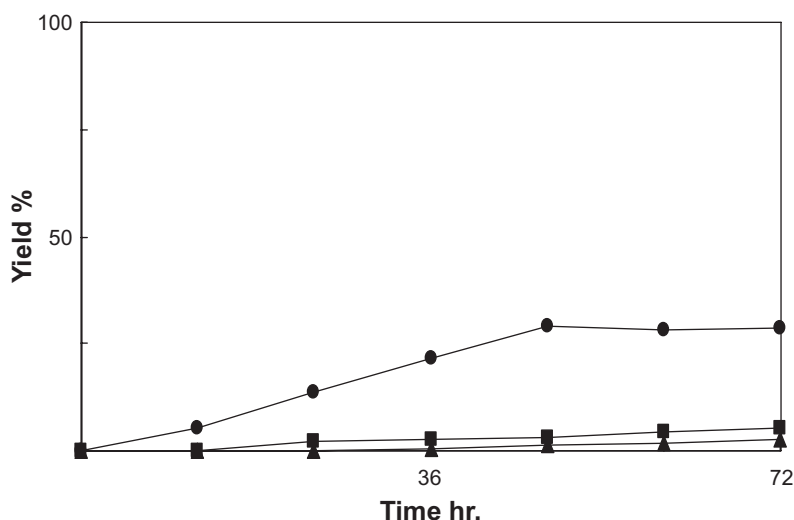
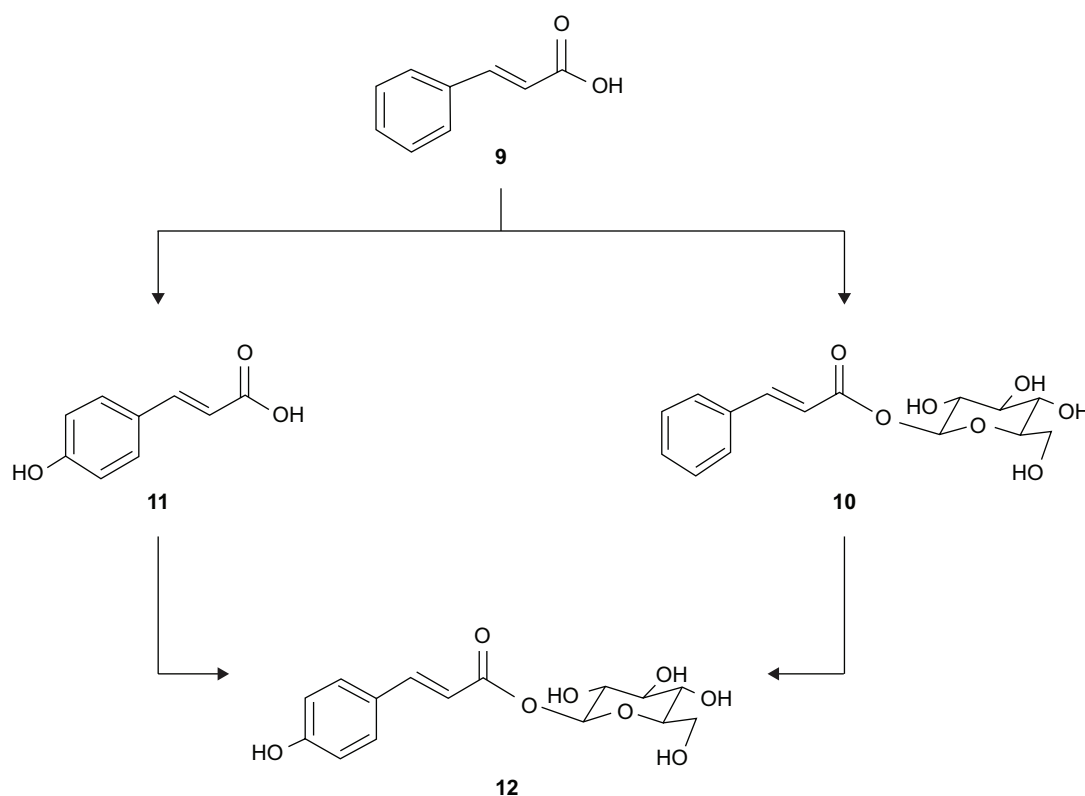


Figure 3. Time-course of the biotransformation of cinnamic acid by cultured cells of *E. perriniana*. Yield is expressed as a percentage relative to the total amount of reaction products on a molar basis. Yields of 10(●), 11(■), and 12(▲) are plotted.



Scheme 3. Biotransformation of cinnamic acid by cultured cells of *E. perriniana*.

and products 12 and 15 were accumulated after 12 h of incubation, and products 14 and 16 were slightly produced. At 72 h reaction, the amounts of products 12–16 were 11.0%, 12.8%, 1.2%, 9.5%, and 2.3%, respectively, relative to the total amount of reaction products on a molar basis. This result indicated that

glucosylation at phenolic hydroxyl group and carboxyl group of *p*-coumaric acid preferentially occurred. And the cultured cells of *E. perriniana* regioselectively hydroxylated at 3-position of *p*-coumaric acid (11) to produce caffeic acid (14). Subsequently, produced caffeic acid (14) was predominantly glucosylated

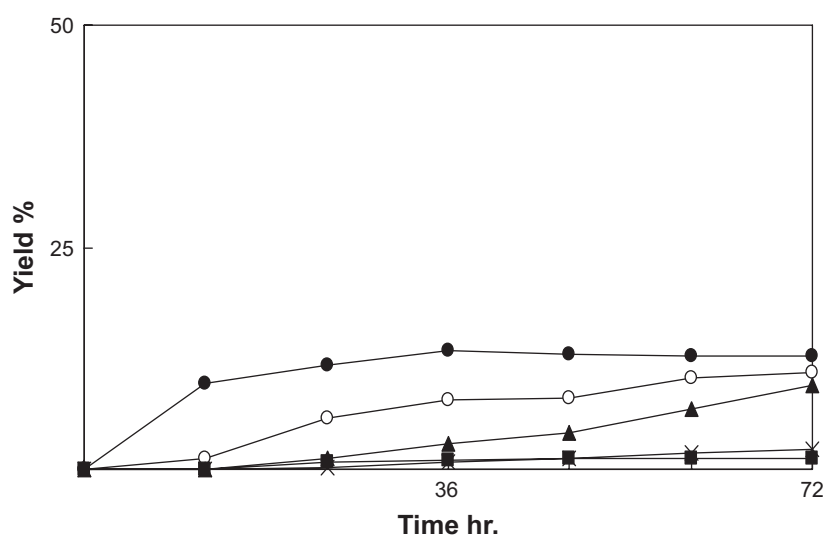
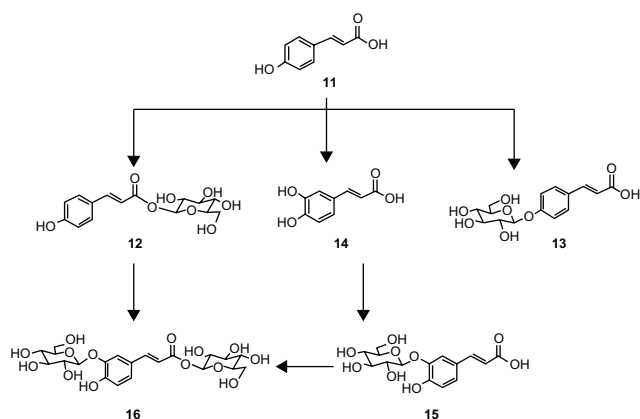


Figure 4. Time-course of the biotransformation of *p*-coumaric acid by cultured cells of *E. perriniana*. Yield is expressed as a percentage relative to the total amount of reaction products on a molar basis. Yields of 12(○), 13(●), 14(■), 15(▲), and 16(×) are plotted.



Scheme 4. Biotransformation of *p*-coumaric acid by cultured cells of *E. perriniana*.

at 3-position of phenolic hydroxyl group. It was considered that disaccharide product 16 was subsequently biotransformed from *p*-coumaroyl-*O*- β -D-glucoside (12) and. 3-*O*- β -D-glucosyl caffeate (15) Biotransformation pathway of *p*-coumaric acid is shown in Scheme 4.

Glycosylation of caffeic acid (14)

Four compounds 15–18 were produced by incubation of the cultured cells of *E. perriniana* with caffeic acid (14). Structures of products 17 and 18 were determined on the basis of their ESIMS, ^1H and ^{13}C NMR spectra as 4-*O*- β -D-glucosyl ferulate and (4-*O*- β -D-glucosyl)-feruloyl-*O*- β -D-glucoside, respectively.

4-*O*- β -D-Glucosyl ferulate (17): ESIMS: m/z 379 $[\text{M}+\text{Na}]^+$, ^1H NMR (400 MHz, CD_3OD): δ

3.89(3H, *s*, OCH_3), 4.98 (1H, *d*, $J = 7.2$ Hz, H-1'), 6.39 (1H, *dd*, $J = 16.2, 1.2$ Hz, H-6), 7.61 (1H, *d*, $J = 15.6$ Hz, H-7); ^{13}C NMR (100 MHz, CD_3OD): δ 56.6 (OCH_3), 62.4 (C-6'), 71.1–78.1 (C-2', 3', 4', 5'), 102.0 (C-1'), 112.1 (C-2), 117.1 (C-5), 117.6 (C-8), 123.3 (C-6), 130.4 (C-1), 145.9 (C-7), 149.8 (C-3), 150.7 (C-4), 170.4 (C-9).

(4-*O*- β -D-Glucosyl)-feruloyl-*O*- β -D-glucoside (18): ESIMS: m/z 541 $[\text{M}+\text{Na}]^+$, ^1H NMR (400 MHz, CD_3OD): δ 3.89 (3H, *s*, OCH_3), 4.98 (1H, *d*, $J = 7.2$ Hz, H-1'), 5.69 (1H, *d*, $J = 8.0$ Hz, H-1''), 6.48 (1H, *d*, $J = 16.4$ Hz, H-8), 7.73 (1H, *d*, $J = 16.0$ Hz, H-7); ^{13}C NMR (100 MHz, CD_3OD): δ 56.7 (OCH_3), 62.4 (C-6''), 62.3 (C-6'), 71.0–78.7 (C-2', 3', 4', 5'), 71.2–78.2 (C-2'', 3'', 4'', 5''), 95.8 (C-1'), 102.0 (C-1''), 112.4 (C-2), 116.5 (C-8), 117.2 (C-5), 123.6 (C-6), 130.1 (C-1), 147.3 (C-7), 150.1 (C-3), 150.8 (C-4), 167.2 (C-9).

Time course experiment showed that caffeic acid (14) was converted into 15 at early stage of incubation, and subsequently product 16 was accumulated after 12 h of incubation (Figure 5). It was confirmed that products 17 and 18 were slightly produced. At 72 h reaction, the amounts of products 15–18 were 11.6%, 9.3%, 1.2% and 4.2%, respectively, relative to the total amount of reaction products on a molar basis. This result indicated that glucosylation at phenolic hydroxyl group of caffeic acid (14) occurred. It showed that no glucosylation at carboxyl group of caffeic acid (14) occurred. And the cultured

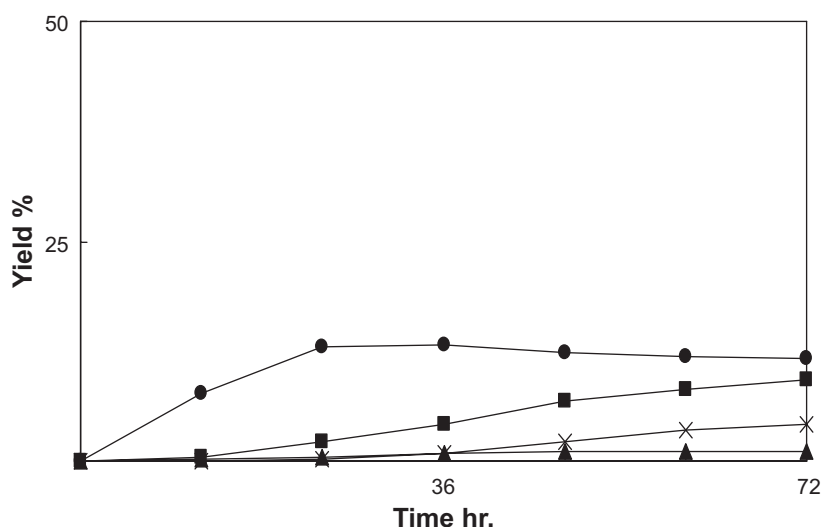
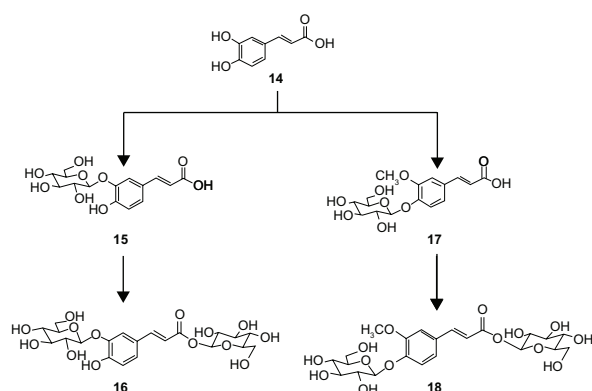


Figure 5. Time-course of the biotransformation of caffeic acid by cultured cells of *E. perriniana*. Yield is expressed as a percentage relative to the total amount of reaction products on a molar basis. Yields of 15(●), 16(■), 17(▲), and 18(×) are plotted.



Scheme 5. Biotransformation of caffeic acid by cultured cells of *E. perriniana*.

cells of *E. perriniana* regioselectively methylated at 3-position of caffeic acid (14). However, in this experiment ferulic acid which is methylated at 3-position of caffeic acid was not confirmed, probable due to low yield of it. Subsequently, produced 3-*O*- β -D-glucosyl caffeate (15) was preferentially glucosylated at 3-position of phenolic hydroxyl group. Biotransformation pathway of caffeic acid is shown in Scheme 5.

Glycosylation of ferulic acid (19)

Three compounds (named products 17, 18, and 20) were produced by incubation of the cultured cells of *E. perriniana* with ferulic acid (19). The structure of product 20 was determined on the basis of its ESIMS, ^1H and ^{13}C NMR spectra as feruloyl-*O*- β -D-glucoside.

Feruloyl-*O*- β -D-glucoside (20): ESIMS: m/z 379 $[\text{M}+\text{Na}]^+$, ^1H NMR (400 MHz, CD_3OD): δ 3.89 (3H, s, OCH_3), 5.58 (1H, d, $J = 7.6$ Hz, H-1'), 6.40 (1H, d, $J = 15.6$ Hz, H-8), 6.81 (1H, d, $J = 8.0$ Hz, H-5), 7.09 (1H, dd, $J = 8.4, 2.0$ Hz, H-6), 7.20 (1H, d, $J = 2.0$ Hz, H-2), 7.72 (1H, d, $J = 15.6$ Hz, H-7); ^{13}C NMR (100 MHz, CD_3OD): δ 56.4 (OCH_3), 62.3 (C-6'), 71.0–78.7 (C-2', 3', 4', 5'), 95.7 (C-1'), 111.6 (C-2), 114.6 (C-8), 116.4 (C-5), 124.2 (C-6), 127.4 (C-1), 148.1 (C-7), 149.2 (C-3), 150.7 (C-4), 167.5 (C-9).

Time course experiment showed that ferulic acid (19) was converted into 17 in short time incubation, and 18 was accumulated following to the formations of 17 and 20 (Figure 6). At 72 h reaction, the amounts of products 17, 18, and 20 were 20.3%, 3.4% and 2.5%, respectively, relative to the total amount of reaction products on a molar basis. This result indicated that glucosylation of ferulic acid predominantly occurred at 4-position of phenolic hydroxyl group rather than at carboxyl group. Biotransformation pathway of ferulic acid is shown in Scheme 6.

The result of this study established that plant cultured cells of *C. roseus* and *E. perriniana* can glycosylate capsaicinoids such as capsaicin and 8-nordihydrocapsaicin, and phenylpropanoids such as cinnamic acid, *p*-coumaric acid, caffeic acid, and ferulic acid. The cultured cells of *C. roseus* preferentially glycosylated 8-nordihydrocapsaicin rather than capsaicin, probably due to the difference in the structure of their side chain. It was found that

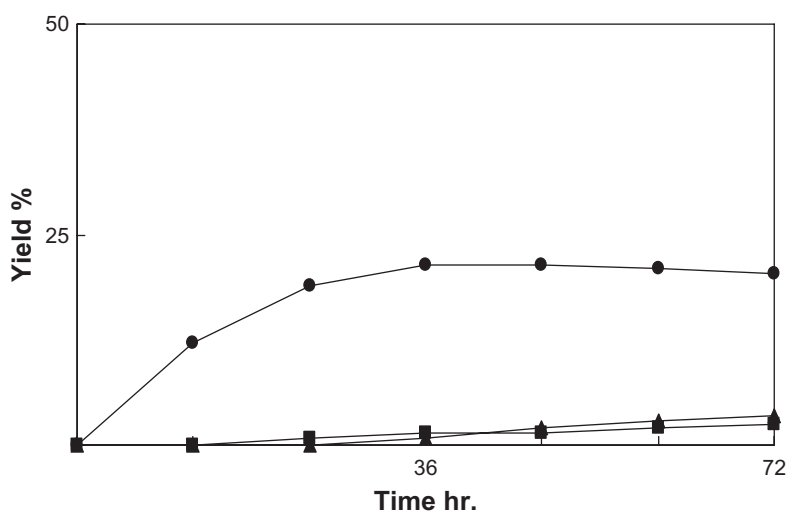
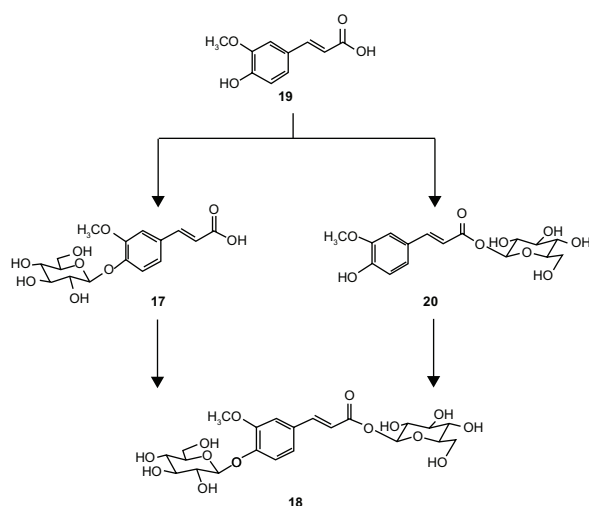


Figure 6. Time-course of the biotransformation of ferulic acid by cultured cells of *E. perriniana*. Yield is expressed as a percentage relative to the total amount of reaction products on a molar basis. Yields of 17(●), 20(■), and 18(▲) are plotted.



Scheme 6. Biotransformation of ferulic acid by cultured cells of *E. perriniana*.

the cultured cells of *C. roseus* convert capsaicin and 8-nordihydrocapsaicin into their mono- and disaccharides. From this study, the glycosylation of capsaicinoids by the cultured cells of *C. roseus* was attempted to reduce their pungency and enhance their solubility in water. It is well known that glycosylated drugs reduce their toxicity and enhance their oral absorption. Therefore, glycosylated capsaicinoids may be useful as drugs, functional foods, and cosmetics. A chemically synthesized capsaicinoid glucoside, vanillylnonamide β -D-glucopyranoside,²³ has been reported to have a remarkable in vivo effect on lipid metabolism in rats.²⁴ Studies on the physiological activities of glycosylated capsaicin and 8-nordihydrocapsaicin are now in progress.

From this study, it was found that both phenolic hydroxyl group and carboxyl group of phenylpropanoids such as cinnamic acid, *p*-coumaric acid, caffeic acid, and ferulic acid were glycosylated using the cultured cells of *E. perriniana* to give the corresponding mono- and disaccharides. Furthermore, the cultured cells of *E. perriniana* were able to catalyze hydroxylation and methylation as well as glucosylation on cinnamic acid, *p*-coumaric acid, and caffeic acid. Phenylpropanoids such as caffeic acid and ferulic acid has been reported to have a good effect as topical photoprotective agents.²⁵ But physiological studies of glycosylated phenylpropanoids have not been reported yet. On the other hand, from this study, the glycosylation of phenylpropanoids using the cultured cells of *E. perriniana*

was attempted to enhance their solubility in water and enlarge effects. Further studies on the physiological properties of glycosylated phenylpropanoids are now in progress. Also, this biotransformation method is of considerable interest in green chemistry. Studies of biotransformation of another foreign substrate using the cultured cells of *C. roseus* and *E. perriniana* are now in progress.

Abbreviations

COSY, correlation spectroscopy; ESIMS, electrospray ionization mass spectrometry; GLC, gas-liquid chromatography; HMBC, heteronuclear multiple-bond correlation; HPLC, high performance liquid chromatography; HRFABMS, high resolution fast atom bombardment mass spectrometry; NMR, nuclear magnetic resonance; TMS, tetramethylsilane.

Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

References

1. Tylor VE, Brady LR, Robberts JE. Capsicum in pharmacognosy, 8th ed. Lea and Febiger. Philadelphia. 1981:155.
2. Kawada T, Hagihara K, Iwai K. Effects of capsaicin on lipid metabolism in rats fed a high fat diet. *J Nutr*. 1985;116:1272–8.
3. Kobayashi A, Osaka T, Namba Y, Inoue S, Lee TH, Kimura S. Capsaicin activates heat loss and heat production simultaneously and independently in rats. *Am J Physiol*. 1998;275:R92–8.
4. Kawada T, Watanabe T, Takashi T, Tanaka T, Iwai K. Capsaicin-induced β -adrenergic action on energy metabolism in rats. Influence of capsaicin on oxygen consumption, the respiratory quotient, and substrate utilization. *Proc Soc Exp Biol Med*. 1987;83:250–6.
5. Watanabe T, Kawada T, Iwai K. Enhancement by capsaicin of energy metabolism in rats through secretion of catecholamine from adrenal medulla. *Agric Biol Chem*. 1987;51:75–9.
6. Henderson DE, Slickman AM, Henderson SK. Quantitative HPLC determination of the antioxidant activity of capsaicin on the formation of lipid hydroperoxides of linoleic acid: a comparative study against BHT and melatonin. *J Agric Food Chem*. 1999;47:2563–70.
7. Perucka I, Materska M. Antioxidant activity and content of capsaicinoids isolated from paprika fruits. *Pol J Food Nutr Sci*. 2005;12:15–8.
8. Materska M, Perucka I. Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annum* L.). *J Agric Food Chem*. 2005;53:1750–6.
9. Gal IE. Antibacterial activity of the spice, paprika. Testing of capsaicin and capsaicin activity. *Z Lebensm-Unters Forsch*. 1968;138:86–92.
10. Hue JP, Dong EM. Pharmacologic management of chronic pain. *Korean J Pain*. 2010;23(2):99–108.



11. Lee TS. Physiological gustatory sweating in a warm climate. *J Physiol.* 1954;124:528–42.
12. Watanabe T, Kawada T, Yamamoto M, Iwai K. Capsaicin, a pungent principle of hot red pepper, evokes catecholamine secretion from the adrenal medulla of anesthetized rats. *Biochem Biophys Res Commun.* 1987;142:259–64.
13. Kometani T, Tanimoto H, Nishimura T, Kanabara I, Okada S. Glucosylation of capsaicin by cell suspension cultures of *Coffea Arabica*. *Biosci Biotech Biochem.* 1993;57(12):2192–3.
14. Srinivasan M, Sudheer AR, Menon VP. Ferulic acid: therapeutic potential through its antioxidant property. *J Clin Biochem Nutr.* 2007;40:92–100.
15. Feng Y, Lu YW, Xu PH, Long Y, Wu WM, Wang R. Caffeic acid phenethyl ester and its related compounds limit the functional alterations of the isolated mouse brain and liver mitochondria submitted to in vitro anoxiareoxygenation: relationship to their antioxidant activities. *Biochim Biophys Acta.* 2008;1780:659–72.
16. Ivanauskas L, Jakstas V, Radusiene J, Lukosius A, Baranauskas A. Evaluation of phenolic acids and phenylpropanoids in the crude drugs. *Medicina.* 2008;44:48–55.
17. Hamada H, Fuchikami Y, Ikematsu Y, Hirata T, Williams H, Scott AI. Hydroxylation of piperitone by cell suspension cultures of *Catharanthus roseus*. *Phytochemistry.* 1994;37:1037–8.
18. Shimoda K, Kondo Y, Nishida T, Hamada H, Nakajima N, Hamada H. Biotransformation of thymol, carvacrol, and eugenol by cultured cells *Eucalyptus perriniana*. *Phytochemistry.* 2006;67:2256–61.
19. Hamada H, Ohiwa S, Nishida T, et al. One-step glucosylation of capsaicinoids by cultured cells of *Phytolacca Americana*. *Plant Biotech.* 2003;20:253–5.
20. Higashiguchi F, Nakamura H, Hayashi H, Kometani T. Purification and structure determination of glucosides of capsaicin and dihydrocapsaicin from various capsicum fruits. *J Agric Food Chem.* 2006;54:5948–53.
21. Li B, Zhang DM, Luo YM, Chen XG. Tree new and antitumor anthraquinone glycosides from *Lasianthus acuminatissimus* MERR. *Chem Pharm Bull.* 2006;54:297–300.
22. Kawahara E, Fujii M, Ida Y, Akita H. Chemoenzymatic synthesis of sacranosides A and B. *Chem Pharm Bull.* 2006;54:387–90.
23. Mihara S, Hiraoka K, Kameda W. Synthesis of *N*-[4-(*R*-D-glucopyranosyloxy)-3-methoxybenzyl] nonanamide and its β -anomer. *J Agric Food Chem.* 1992;40:2057–9.
24. Tani Y, Fujioka T, Hamada H, Kunimatsu M, Furuichi Y. Favorable effects of vanillylnonanamide-[β]-D-glucoside on serum lipids in hyperlipidemic rats. *J Jpn Soc Nutr Food Sci.* 2003;56:181–7.
25. Antonella S, Antonio T, Domenico T, et al. In vitro and in vivo evaluation of caffeic and ferulic acids as topical photoprotective agents. *Inter J Pharm.* 2000;199:39–47.

Publish with Libertas Academica and every scientist working in your field can read your article

"I would like to say that this is the most author-friendly editing process I have experienced in over 150 publications. Thank you most sincerely."

"The communication between your staff and me has been terrific. Whenever progress is made with the manuscript, I receive notice. Quite honestly, I've never had such complete communication with a journal."

"LA is different, and hopefully represents a kind of scientific publication machinery that removes the hurdles from free flow of scientific thought."

Your paper will be:

- Available to your entire community free of charge
- Fairly and quickly peer reviewed
- Yours! You retain copyright

<http://www.la-press.com>