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One-step Glucosylation of Capsaicinoids by Cultured Cells of *Phytolacca americana*

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Abstract

The synthesis of capsaisinoid monoglucoside, a key material for the preparation of the capsaicinoid oligosaccharide, using plant cultured cells has been investigated. Among the cultured cells tested, only the cells of *Phytolacca americana* glucosylated capsaicin and 8-nordihydrocapsaicin into the corresponding monoglucoside in good yields. Thus, the one-step glucosylation of capsaicinoids has been achieved using the cells of *P. americana*.

Key words: Capsaicin, glycosylation, 8-nordihydrocapsaicin, monoglucoside, plant cultured cells.

Capsaicin is a fat-soluble phenol compound. It imparts a distinctly pungent taste to water even when diluted to one part in eleven million parts of water (Tyler *et al.*, 1981). It was reported that capsaicin reduced the perirenal adipose tissue weight and serum triglyceride concentration in rats by enhancing the energy metabolism through a β-adrenergic action (Kawada *et al.*, 1986). In humans, it was reported that the ingestion of chili sauce with meals resulted in a marked increase in their energy metabolism (Henry and Emery, 1986). Irrespective of such biological activities, the use of capsaicin and its derivative as food ingredients has been limited, because of their low solubilities.

Glycosylation allows the conversion of water-insoluble organic compounds to the corresponding water-soluble ones in order to improve its bio- and pharmacological properties (Umetani *et al.*, 1982; Suzuki *et al.*, 1996). Recently, we reported the chemoenzymatic (two-step) synthesis of capsaicinoid oligosaccharides which possess a higher water-solubility (Hamada *et al.*, 2001). However, during the first step of the glucosylation of the capsaicinoids, their corresponding monoglucosides were prepared by a chemical method (with tetraacetyl-α-D-glucose fluoride in the presence of $BF_3 \cdot OEt_2$). In this paper, we report the one-step glucosylation of capsaicin and 8-nordihydrocapsaicin using plant cultured cells.

Capsaicin was purchased from Nacalai Tesque, Inc., Kyoto, Japan. 8-Nordihydrocapsaicin was purchased from Tokyo Kasei Kogyo Co., Ltd., Tokyo, Japan. The cultured cells of *Marchantia polymorpha* (liverwort), *Catharanthus roseus*, and *Eucalyptus perriniana* were induced and maintained as previously reported (Hamada *et al.*, 1993, 1997; Furuya *et al.*, 1997). The cultured cells of *Phytolacca americana* were induced from the stalks on Murashige & Skoog's (MS) medium (Murashige and Skoog, 1962) containing 3% sucrose and 0.8% agar. The cultured cells of *P. americana* were subcultured at 4-week intervals on the same medium. All other reagents used were of analytical grade. The ^1H- and ^{13}C-NMR (400 MHz) spectra were recorded on a Bruker AMX-R400 spectrometer in methanol-d_4 using tetramethylsilane (TMS) as the internal standard. The FAB-MS spectrum was measured on a JEOL The MStation JMS-700 spectrometer. The molecular weight was estimated from the *m/z* value of the quasimolecular

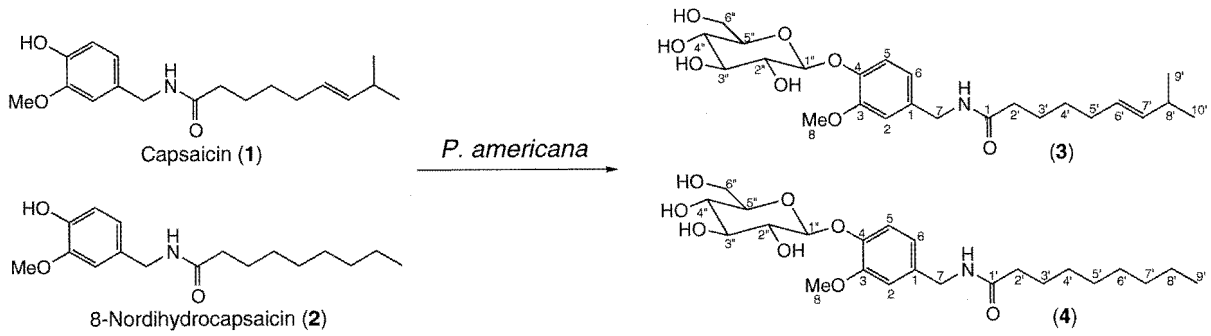

Fig. 1 Synthesis of capsaicin β-D-glucopyranoside (**3**) and 8-nordihydrocapsaicin β-D-glucopyranoside (**4**) from **1** or **2** by the cultured cells of *P. americana*.

ion $[M + Na]^+$ peak.

Because the substrate was an irritant, the experiments were carried out in a small scale as follows. A suspension culture was started by transferring the cultured cells to 100 ml of the liquid medium (without agar) in a 300-ml Erlenmeyer flask. The suspension culture was precultured on a rotary shaker at 120 rpm and with illumination by a white fluorescent light (2,000 lx) for 3 days at 25 ℃. The substrate (10 mg of **1** or **2**, dissolved in 1 ml of MeOH) was administered to the precultured cells (about 30 g) in the flask and the cultures were incubated at 25 ℃ in a rotary shaker (120 rpm) under light (2,000 lx) for 3 days. The reaction was stopped by filtration on a glass filter to separate the used cells and the used medium. The intracellular capsaicinoid glucoside was extracted from the used cells with 10 volumes of MeOH after ultrasonic treatment. The extract (MeOH soln.) was concentrated under reduced pressure. The residue was extracted with AcOEt, and then concentrated. The used medium was extracted with AcOEt. The crude products were separated and purified using an HPLC equipped with a Crestpak C18S (JASCO, φ 4.6x150 mm, UV 275 nm, flow rate: 1.0 ml min^{-1}, mobile phase: CH_3CN : H_2O = 35 : 65, column temp: 40 ℃). The amounts of capsaicinoid glucoside in the cells and the used medium were measured by HPLC under the same conditions mentioned above. The identification of the product was confirmed by ^1H- and ^{13}C-NMR and HPLC analyses based on the comparison with an authentic sample.

Selected NMR data: **3**; ^1H-NMR (400 MHz, CD_3OD): δ = 0.956 (d, 6H), 1.36 (m, 2H), 1.63 (m, 2H), 1.98 (q, 2H), 2.18 (t, 2H), 2.32 (m, 1H), 3.30-3.48 (m, 4H), 3.66 (dd, 1H), 3.69 (dd, 1H), 3.84 (s, 3H), 4.30 (d, 2H), 4.84 (br, 1H), 5.36 (d, 1H, anomeric H: *J* = 6.7 Hz), 5.38 (m, 2H), 6.82 (d, 1H), 6.93 (s, 1H), 7.11 (d, 1H), **4**; ^1H NMR (400 MHz, CD_3OD): δ = 0.895 (t, 3H), 1.30 (m, 10H), 1.63 (m, 2H), 2.21(t, 2H), 3.29-3.51 (m, 4 H), 3.66 (dd, 1H), 3.69 (dd, 1H), 3.84 (s, 3H), 4.29 (d, 2H), 4.64 (br, 1H), 4.86 (d, 1H, anomeric H: *J* = 7.6 Hz), 6.82 (dd, 2H), 6.93 (d, 1H), 7.10 (d, 1H). The FAB-MS spectrum of **3** and **4** gave a molecular ion at *m/z* 490 and 478 $[M + Na]^+$, respectively. The large coupling constant (J = 6.6 and 7.6 Hz) of the anomeric proton in **3** and **4** suggested the β configuration for the anomeric center. Thus, the NMR and FAB-MS spectra data were identical to that for a compound obtained by the chemical method (previously reported, Hamada *et al.*, 2001). Therefore, 3 and 4 could be identified as capsaicin monoglucoside (capsaicin β-D-glucopyraoside) and 8-nordihydrocapsaicin monoglucoside (8-nordihydrocapsaicin β-D-glucopyraoside), respectively (**Fig. 1**).

Table 1 The glucosylation of the capsaicinoids (**1** or **2**) using plant cell cultures

Plant cell culture	**3**, Yield (%)[1)]	**4**, Yield (%)[1)]
Catharanthus roseus	0	0
Marchantia polymorpha	0	0
Eucalyptus perriniana	0	0
Phytolacca americana	50	47

[1)] Isolated yield

Four plant cell cultures were tested for their glucosylation ability toward the capsaicinoids. It was found that the substrates (**1** and **2**) were converted to the corresponding monoglucosides (**3** and **4**) using only cultured *P. americana* cells. These results are summarized in **Table 1**.

In the case of the glucosylation using the cell culture of *P. americana*, the product (**3** or **4**) was not detected in the medium, whereas the substrate (**1** or **2**) was recovered (9% and 11% of inoculum, respectively) in the medium. The monoglucoside was obtained (**3**: 50%, **4**: 47% of inoculum) and the substrate was recovered (**1**: 15%, **2**: 13%) from the used cells. β-Thujaplicin (hinokitiol), one of the phenolic compounds, was glucosylated by the enzyme-fraction(s) from the cultured cells of *Eucalyptus perriana* (Nakajima *et al.*, 1997), however,

the capsaicinoids were not glucosylated by the *Eucalyptus* cultured cells. Neither the cultured cells of *C. roseus* nor the cultured cells of *M. polymorpha* glucosylated the capsaicinoids. Only the cultured cells of *P. americana* glucosylated the capsaicin and 8-nordihydrocapsaicin in good yields. This result seems to be the reason for the differences in the substrate specificity of the related glucosyltransferase among the tested cultured cells.

Thus, the one-step glucosylation of the capsaicinocids was achieved by a biological method using the cultured cells of *P. americana*. Consequently, the large-scale (gram scale) and environment-friendly production of the capsaicin oligosaccharides could be achieved by the combination of the present glucosylation method and the previously reported CGTase-catalyzed additional glucosylation (Hamada *et al.*, 2001). The production of capsaicinoid oligosaccharides under biological conditions in all steps is currently under investigation and will be reported in the future.

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