Glycosylation of Vitamin E Homologue by Cultured Plant Cells

Yoko Kondo,[1] Kei Shimoda,[2] Junichi Takimura,[1] Hatsuyuki Hamada,[3] and Hiroki Hamada*[4]
[1]*Department of Applied Science, Faculty of Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005*
[2]*Department of Pharmacology and Therapeutics, Faculty of Medicine, Oita University, 1-1 Idaigaoka, Hasama-machi, Yufu 879-5593*
[3]*National Institute of Fitness and Sports in Kanoya, 1 Shiromizu-cho, Kanoya 891-2393*
[4]*Department of Life Science, Faculty of Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005*

(Received December 9, 2005; CL-051514; E-mail: hamada@das.ous.ac.jp)

Biotransformation of 2,2,5,7,8-pentamethyl-6-chromanol (vitamin E homologue) was investigated with cultured plant cells of *Phytolacca americana*, *Catharanthus roseus*, and *Eucalyptus perriniana*. The cultured cells of *P. americana* converted 2,2,5,7,8-pentamethyl-6-chromanol into its β-glucoside, whereas conversion with *C. roseus* and *E. perriniana* also afforded the hydrolysis product of β-glucoside and a new β-gentiobioside product, i.e., 2,2,5,7,8-pentamethylchroman-6-yl 6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside.

Vitamin E is a very useful inhibitor of free radical-mediated membrane damage acting as free-radical scavenging antioxidant.[1–3] In addition, vitamin E controls nutrition in humans in extremely small amount and is involved in various physiological phenomena in the living body. Recently, vitamin E which has a specific physiological activity has been used as a medicine. Clinical information of vitamin E includes its effects on inhibition of gynecological internal secretion control against sterility, heart circulation, liver diseases, radiation damage, and carcinogenesis.[4–6] Despite of its pharmacological activities, it has some defects as a drug such as low solubility and absorbability. Recently, several attempts to synthesize amphiphilic glycosides of vitamin E (water-soluble vitamin E) by chemical glycosylation have been made.[7–10] On the other hand, glycosylation with plant cells has been the subject of increasing attention,[11,12] because one-step glycosylation can be achieved by enzymatic reaction. However, there are no reports on the enzymatic glycosylation of vitamin E and its homologues with cultured plant cells. We report, herein, the enzymatic glycosylation of the vitamin E homologue, i.e., 2,2,5,7,8-pentamethyl-6-chromanol, by cultured plant cells of *Phytolacca americana*, *Catharanthus roseus*, and *Eucalyptus perriniana*.

Cultured suspension cells of each callus strains, *Phytolacca americana*,[13] *Catharanthus roseus*,[14] and *Eucalyptus perriniana*,[15] were prepared as described previously. Just prior to use for this work, part of the callus tissues (fresh weight 40 g) was transplanted to freshly prepared medium (SH medium for *P. americana* and *C. roseus*, MS medium for *E. perriniana*; 100 mL in a 300-mL conical flask; pH 5.7) containing 3% sucrose and 10 mM 2,4-D, and grown with continuous shaking for 1 week at 25 °C in the dark except that the incubation with *C. roseus* cells was carried out under illumination (4000 lux). A total of 120 mg of 2,2,5,7,8-pentamethyl-6-chromanol (**1**) was administered to the 10 flasks (12 mg/flask, none solvent) containing the suspension cultured cells and the cultures were incubated at 25 °C for 1 day on a rotary shaker (120 rpm).

After incubation of **1** with cultured cells of *P. americana*, the cells were harvested and extracted (×3) by homogenization with MeOH and the 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 followed by elution with MeOH. The MeOH eluate was subjected to HPLC (column: 150 × 20 mm) and product **2** was obtained (Figure 1). No products were observed in the medium. The isolated yield of the products was calculated on the basis of the peak area from HPLC using the calibration curves prepared by the HPLC analyses of authentic glycosides. The structure of the product[16] was identified using HRFABMS, ^{1}H and ^{13}C NMR, H–H COSY, and C–H COSY as 2,2,5,7,8-pentamethylchroman-6-yl β-D-glucopyranoside (**2**, 51%).

On administration of **1** to the cultured cells of *C. roseus*, three products **2** (18%), **3** (57%), and **4** (12%) were obtained. Products **3** and **4** were determined as 4-hydroxy-3-(3-hydroxy-3-methylbutyl)-2,5,6-trimethylphenyl β-D-glucopyranoside and 2,2,5,7,8-pentamethylchroman-6-yl 6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside (β-gentiobioside), respectively. β-Gentiobioside **4** was a new compound. In the case of administration of 1,4-dihydroxy-3-(3-hydroxy-3-methylbutyl)-2,5,6-trimethylbenzene, no formation of **3** was observed during the same incubation period, showing that **3** was the hydrolysis product of **2**. The HRFABMS spectrum of the product **4** showed a pseudomolecular ion $[M + Na]^+$ peak at m/z 567.5724 consistent with a molecular formula $C_{26}H_{40}O_{12}$. The ^{1}H NMR spectrum of **4** showed two anomeric proton signals at δ 4.23 (1H, d, $J = 8.0$ Hz) and 4.47 (1H, d, $J = 7.6$ Hz). The ^{13}C NMR spectrum of **4** exhibited 26 carbon signals including two anomeric carbon signals at δ 104.5 and 105.8. From the coupling pattern of the proton signals and the chemical shifts of the carbon signals due to the sugar moiety, the component sugar in **4** was indicated to be β-D-glucopyranose. The ^{13}C NMR chemical shift of C-6′ comparatively shifted downfield to δ 70.0. In addition, the HMBC correlations were observed between the anomeric proton

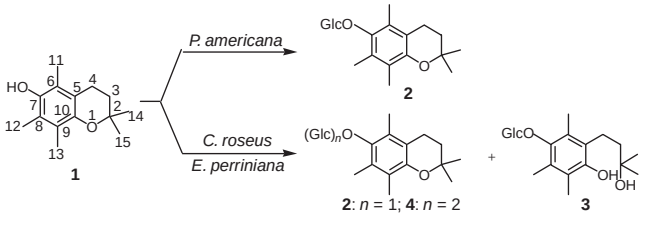

Figure 1. Biotransformation of **1** by *P. americana*, *C. roseus*, and *E. perriniana*.

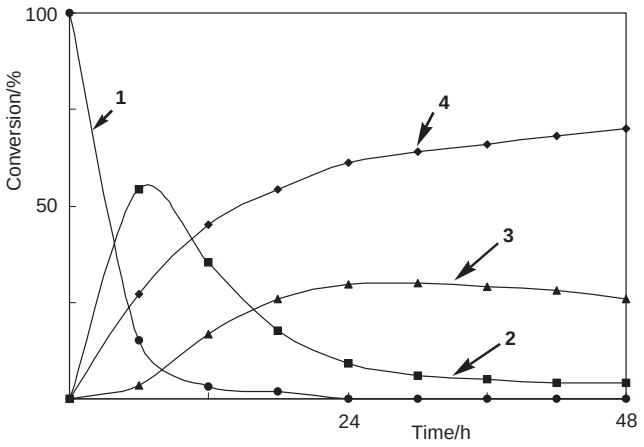

Figure 2. Time course of the biotransformation of **1** by cultured plant cells of *E. perriniana*.

signal at δ 4.47 (H-1′) and the carbon signal at δ 147.1 (C-7) and between the anomeric proton signal at δ 4.23 (H-1″) and the carbon signal at δ 70.0 (C-6′) to establish that the inner glucopyranosyl residue was attached to the phenolic hydroxyl group of **1** and that the pair of β-D-glucopyranosyl residues was 1,6-linked. Thus, the structure of **4** was determined to be 2,2,5,7,8-pentamethylchroman-6-yl 6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside.

Next, **1** was subjected to the biotransformation system using the cultured cells of *E. perriniana*. The cells converted **1** into three products **2** (6%), **3** (27%), and **4** (59%) showing the highest yields among the three callus strains examined. To investigate the biotransformation pathway, the time course in the conversion of **1** was followed. As shown in Figure 2, **1** was glucosylated to **2** at 6 h's incubation, whereas the product **3** and **4** were predominantly accumulated in the cells after 12 hs' incubation with the decrease of the amount of **2**. This also suggested that the β-glucoside **2** was first formed and then hydrolysis of the ether occurred to give **3**.

Thus, it was found that cultured plant cells of *P. americana*, *C. roseus*, and *E. perriniana* were able to convert the vitamin E homologue, i.e., 2,2,5,7,8-pentamethylchromanol, into the corresponding glycosides such as β-glucoside, its hydrolysis product, and β-gentiobioside. The plant enzymes responsible for the glycosylation of vitamin E homologue should be available addition to the group of biocatalysts used for the preparation of water-soluble vitamin E derivatives. Further studies on pharmacological activities of the glycosides of the vitamin E homologue, and characterization of enzymes which catalyze the glycosylation reactions are now in progress.

This work was supported in part by a Grant-in-Aid for Scientific Research (No. 16790014) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

References and Notes

1 G. W. Burton, K. U. Ingold, in *Protective Agents in Cancer*, ed. by D. C. H. McBrien, T. F. Slater, Academic Press, London and New York, **1983**, Vol. 20, p. 7311.
2 L. Valgimigli, K. U. Ingold, J. Lusztyk, *J. Am. Chem. Soc.* **1996**, *118*, 3545.
3 T. Koga, J. Terao, *Biosci. Biotechnol. Biochem.* **1996**, *60*, 1043.
4 M. L. Chiswick, J. Wynn, N. Toner, *Ann. N. Y. Acad. Sci.* **1982**, *393*, 109.
5 T. A. Thompson, G. Wilding, *Mol. Cancer Ther.* **2003**, *2*, 797.
6 R. H. Cichewicz, V. A. Kenyon, S. Whitman, N. M. Morales, J. F. Arguello, T. R. Holman, P. Crews, *J. Am. Chem. Soc.* **2004**, *126*, 14910.
7 M. Lahmann, J. Thiem, *Carbohydr. Res.* **1997**, *299*, 23.
8 R. K. Uhrig, M. A. Picard, K. Beyreuther, M. Wiessler, *Carbohydr. Res.* **2000**, *325*, 72.
9 T. Sato, H. Miyatake, K. Yamamoto, T. Hirano, *Chem. Pharm. Bull.* **2001**, *49*, 948.
10 S. Witkowski, P. Walejko, *Z. Naturforsch., B: Chem. Sci.* **2002**, *57b*, 571.
11 T. Furuya, Y. Asada, Y. Matsuura, S. Mizobata, H. Hamada, *Phytochemistry* **1997**, *46*, 1355.
12 K. Ishihara, H. Hamada, T. Hirata, N. Nakajima, *J. Mol. Catal. B: Enzym.* **2003**, *23*, 145.
13 H. Hamada, K. Nishida, T. Furuya, K. Ishihara, N. Nakajima, *J. Mol. Catal. B: Enzym.* **2001**, *16*, 115.
14 H. Hamada, Y. Fuchikami, Y. Ikematsu, T. Hirata, H. Williams, A. I. Scott, *Phytochemistry* **1994**, *37*, 1037.
15 T. Furuya, Y. Orihara, C. Hayashi, *Phytochemistry* **1987**, *26*, 715.
16 Spectral data for the products; product **2**: HRFABMS: m/z 405.4358 $[M + Na]^+$; ^{1}H NMR (400 MHz, CD_3OD, δ in ppm): δ 1.27 (6H, s, H-14, 15), 1.78 (2H, t, $J = 6.8$ Hz, H-3), 2.01 (3H, s, H-11), 2.19 (3H, s, H-13), 2.22 (3H, s, H-12), 2.60 (2H, t, $J = 6.8$ Hz, H-4), 3.10–3.50 (4H, m, H-2′, 3′, 4′, 5′), 3.63 (1H, dd, $J = 11.6$, 2.4 Hz, H-6a′), 3.75 (1H, dd, $J = 11.6$, 5.2 Hz, H-6b′), 4.52 (1H, d, $J = 8.0$ Hz, H-1′); ^{13}C NMR (100 MHz, CD_3OD, δ in ppm): δ 12.0 (C-11), 13.2 (C-13), 14.1 (C-12), 22.0 (C-4), 27.0 (C-14, C-15), 34.0 (C-3), 62.9 (C-6′), 71.8 (C-4′), 73.7 (C-2), 75.8 (C-2′), 77.7 (C-5′), 78.0 (C-3′), 105.9 (C-1′), 118.2 (C-5), 123.3 (C-6), 127.8 (C-8), 129.6 (C-9), 147.1 (C-7), 149.3 (C-10). Product **3**: HRFABMS: m/z 423.4412 $[M + Na]^+$; ^{1}H NMR (CD_3OD): δ 1.26 (6H, s, H-14, 15), 1.55 (2H, m, H-3), 2.11 (3H, s, H-11), 2.15 (3H, s, H-13), 2.21 (3H, s, H-12), 2.51 (2H, m, H-4), 3.34–3.52 (4H, m, H-2′, 3′, 4′, 5′), 3.66 (1H, dd, $J = 12.0$, 4.4 Hz, H-6a′), 3.75 (1H, dd, $J = 11.6$, 2.4 Hz, H-6b′), 4.60 (1H, d, $J = 7.2$ Hz, H-1′); ^{13}C NMR (CD_3OD): δ 12.7 (C-11), 12.9 (C-13), 14.5 (C-12), 23.3 (C-4), 28.6, 29.5 (C-14, C-15), 44.1 (C-3), 62.4 (C-6′), 71.4 (C-4′), 71.9 (C-2), 75.9 (C-2′), 77.8, 77.9 (C-3′, C-5′), 105.6 (C-1′), 122.5 (C-5), 123.3 (C-6), 128.4 (C-8), 133.7 (C-9), 147.6 (C-7), 150.3 (C-10). Product **4**: HRFABMS: m/z 567.5724 $[M + Na]^+$; ^{1}H NMR (CD_3OD): δ 1.27 (6H, s, H-14, 15), 1.80 (2H, m, H-3), 2.04 (3H, s, H-11), 2.18 (3H, s, H-13), 2.21 (3H, s, H-12), 2.61 (2H, m, H-4), 3.14-4.05 (10H, m, H-2′, 3′, 4′, 5′, 6′, 2″, 3″, 4″, 5″, 6″), 4.23 (1H, d, $J = 8.0$ Hz, H-1″), 4.47 (1H, d, $J = 7.6$ Hz, H-1′); ^{13}C NMR (CD_3OD): δ 12.1 (C-11), 13.3 (C-13), 14.5 (C-12), 22.0 (C-4), 27.0 (C-14, C-15), 34.0 (C-3), 62.7 (C-6″), 70.0 (C-6′), 71.4 (C-4″), 71.6 (C-4′), 73.8 (C-2), 75.0 (C-2″), 75.8 (C-2′), 76.9, 77.7, 77.8 (C-3′, C-5′, C-3″, C-5″), 104.5 (C-1″), 105.8 (C-1′), 118.3 (C-5), 123.3 (C-6), 127.8 (C-8), 130.0 (C-9), 147.1 (C-7), 149.3 (C-10).

Published on the web (Advance View) March 5, 2006; DOI 10.1246/cl.2006.324