Synthesis of β-Maltooligosaccharides of α-Tocopherol Derivatives by *Xanthomonas campestris* and Cyclodextrin Glucanotransferase and Their Anti-allergic Activity

Kei Shimoda,*[1] Daisuke Sato,[2] and Hiroki Hamada*[2]
[1]*Department of Chemistry, Faculty of Medicine, Oita University, 1-1 Hasama-machi, Oita 879-5593*
[2]*Department of Life Science, Faculty of Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005*

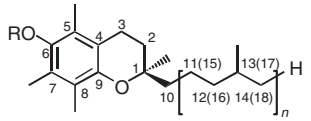
(Received June 18, 2009; CL-090579; E-mail: shimoda@med.oita-u.ac.jp, hamada@dls.ous.ac.jp)

Synthesis of β-glucosides, β-maltosides, and β-maltotriosides of two α-tocopherol derivatives, i.e., 2,5,7,8-tetramethyl-2-(4-methylpentyl)-6-chromanol and 2,5,7,8-tetramethyl-2-(4,8-dimethylnonyl)-6-chromanol, was achieved by bacterial glucosylation with *Xanthomonas campestris* followed by cyclodextrin glucanotransferase-catalyzed glucooligosaccharide formation. The β-glucosides and β-maltosides of α-tocopherol derivatives showed inhibitory effects on IgE antibody formation and on histamine release from rat peritoneal mast cells.

α-Tocopherol is known to serve as a chain-breaking antioxidant to prevent the propagation of free radical reactions.[1] Recently, α-tocopherol has attracted much attention clinically because of its potential to be a very useful medicine which has effects on gynecological internal secretion control against sterility, heart circulation, liver diseases, aging, atherosclerosis, thrombosis, and carcinogenesis.[2] α-Tocopherol is a lipophilic compound that is poorly absorbed after oral administration. These disadvantages prevent α-tocopherol from being used as a food additive and medicine. Glycosylation is a characteristic reaction which converts water-insoluble and unstable aromatic compounds into the corresponding water-soluble and stable derivatives to improve their bioavailability and pharmacological properties.[3] A considerable effort has been made to synthesize α-tocopheryl glycosides by chemical methods so far.[4] However, little attention has been paid to bacterial glycosylation of α-tocopherol.

Some bacteria such as *Xanthomonas campestris*, *Klebsiella* spp., and *Pseudomonas* spp. are known to produce exopolysaccharides which are excreted into the culture medium.[5] These bacteria are expected to convert exogenous lipophilic food ingredients to their water-soluble glycosides. On the other hand, cyclodextrin glucanotransferase (CGTase) serves as a convenient biocatalyst to produce maltooligosaccharides.[6] We report, herein, the synthesis of β-maltooligosaccharides of α-tocopherol derivatives by glucosylation with *X. campestris* followed by CGTase-catalyzed glycosylation, and their anti-allergic activity.

The cultures of *X. campestris*[7] were grown in 1-L conical flasks containing 500 mL of growth medium, that consisted of 10 g of maltose, 6 g of peptone, 0.8 g of yeast extract, 0.4 g of $MgSO_4$, at 30 °C. Prior to use for the experiments, the cells (ca. 2 g in 500 mL medium) were harvested by centrifugation at 8000 g for 10 min. The β-glucosides of α-tocopherol derivatives were prepared as follows. A total of 5 mmol of 2,5,7,8-tetramethyl-2-(4-methylpentyl)-6-chromanol (**1**) (Figure 1) was added to ten 1-L conical flasks (0.5 mmol/flask) containing 500 mL of culture medium, 3.5 mmol of glucose, and 5 g of *X. campestris* cells. The mixture was incubated for 48 h at 30 °C. The reaction mixture was centrifuged at 8000 g for 10 min to remove the cells and the supernatant was extracted with *n*-butanol. The *n*-butanol fraction was purified by preparative HPLC [column: YMC-Pack R&D ODS column (150 × 30 mm); solvent: MeOH–H_2O (9:11, v/v); detection: UV (280 nm); flow rate: 1.0 mL min^{-1}] to give 2,5,7,8-tetramethyl-2-(4-methylpentyl)chroman-6-yl β-glucoside (**2**) in 12% yield. Substrate, 2,5,7,8-tetramethyl-2-(4,8-dimethylnonyl)-6-chromanol (**5**), was subjected to the same bacterial glucosylation system, and was converted into 2,5,7,8-tetramethyl-2-(4,8-dimethylnonyl)chroman-6-yl β-glucoside (**6**) in 10% yield.



1: $n = 1$, R = H; **2**: $n = 1$, R = β-Glc1; **3**: $n = 1$, R = α-Glc(1→4)β-Glc1; **4**: $n = 1$, R = $[\alpha\text{-Glc}(1\rightarrow4)]_2\beta$-Glc1; **5**: $n = 2$, R = H; **6**: $n = 2$, R = β-Glc1; **7**: $n = 2$, R = α-Glc(1→4)β-Glc1; **8**: $n = 2$, R = $[\alpha\text{-Glc}(1\rightarrow4)]_2\beta$-Glc1.

Figure 1. Structures of substrates **1** and **5** and glycoside products **2**–**4** and **6**–**8**. Numbering in parenthesis is for the carbons in **5**–**8**.

A typical procedure for CGTase-catalyzed glycosylation is as follows. To a solution containing 0.5 mmol of substrate, 2,5,7,8-tetramethyl-2-(4-methylpentyl)chroman-6-yl β-glucoside (**2**), and 5 g of starch in 25 mM of sodium phosphate buffer (pH 7.0) was added 100 U of CGTase (Amano Pharmaceutical Co., Ltd.). After stirring the reaction mixture at 40 °C for 24 h, the mixture was centrifuged at 3000 g for 10 min. The supernatant was passed through a Sephadex G-25 column equilibrated with water to remove CGTase. The fractions containing glycosides were purified by preparative HPLC to give 2,5,7,8-tetramethyl-2-(4-methylpentyl)chroman-6-yl β-maltoside (**3**, 58%) and 2,5,7,8-tetramethyl-2-(4-methylpentyl)chroman-6-yl β-maltotrioside (**4**, 29%), which were two new compounds. 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. Similarly, 2,5,7,8-tetramethyl-2-(4,8-dimethylnonyl)chroman-6-yl β-glucoside (**6**) was converted into 2,5,7,8-tetramethyl-2-(4,8-dimethylnonyl)chroman-6-yl β-maltoside (**7**, 51%) and 2,5,7,8-tetramethyl-2-(4,8-dimethylnonyl)chroman-6-yl β-maltotrioside (**8**, 41%), which were two new compounds, by CGTase.

Recently, α-tocopheryl glycosides have been reported to show anti-allergic activity.[4] The suppressive action of the β-glycosides **2**–**4** and **6**–**8** on IgE antibody formation was examined according to a reported procedure.[8,9] As shown in Table 1, a β-glucoside **6** exhibited the strongest inhibitory activity among the six β-glycosides, whereas weak action was observed in the cases of β-maltotriosides **4** and **8**. Next, effects of β-glycosides **2**–**4** and **6**–**8** on histamine release from rat peritoneal mast cells

Table 1. Suppressive action of glycosides of α-tocopherol derivatives **2**–**4** and **6**–**8** on IgE antibody formation

Compound	IgE level[a]
2	174
3	201
4	293
6	165
7	183
8	274
Hydrocortisone	329

[a]The results were expressed as average of plasma IgE level of 7 rats administered a total of 10 mg kg^{-1} of each test compound.

Table 2. Effects of glycosides of α-tocopherol derivatives **2**–**4** and **6**–**8** on histamine release from rat peritoneal mast cells

Compound	Histamine release/%[a]
None	38
2	15
3	29
4	41
6	12
7	22
8	39

[a]Compound 48/80 (0.35 μg mL^{-1})-induced histamine release from rat peritoneal mast cells after treatment with or without test sample.

were examined.[10,11] As a result, β-glucosides, **2** and **6**, and β-maltosides, **3** and **7**, showed inhibitory effects on histamine release (Table 2). These findings suggested that the β-glucosides and β-maltosides of α-tocopherol derivatives would be useful anti-allergic agents.

In summary, the production of glycosides of α-tocopherol derivatives,[12] which are more soluble in water, has been achieved by glucosylation with *X. campestris* followed by CGTase-catalyzed glycosylation. It should be emphasized that the present two-step glycosylation system by two biocatalysts is useful for practical preparation of β-maltooligosaccharides. Further studies on physiological activities and therapeutic values of the glycosides of α-tocopherol derivatives are now in progress.

This work was supported by a grant from the Elizabeth Arnold Fuji Foundation, Japan.

References and Notes

1 L. Packer, *Sci. Am. Sci. Med.* **1994**, *1*, 54; A. Kamal-Eldin, L. A. Appelqvist, *Lipids* **1996**, *31*, 671.
2 E. B. Rimm, M. J. Stampfer, A. Ascherio, E. Giovannucci, G. A. Colditz, W. C. Willett, *N. Engl. J. Med.* **1993**, *328*, 1450; M. Stampfer, C. Hennekens, J. Manson, G. Colditz, B. Rosner, W. Willett, *N. Engl. J. Med.* **1993**, *328*, 1444; R. Brigelius-Flohe, M. G. Traber, *FASEB J.* **1999**, *13*, 1145.
3 A. B. Barua, J. A. Olson, *Int. J. Vitam. Nutr. Res.* **1992**, *62*, 298; Y. Kondo, K. Shimoda, J. Takimura, H. Hamada, H. Hamada, *Chem. Lett.* **2006**, *35*, 324; K. Shimoda, T. Otsuka, Y. Morimoto, H. Hamada, H. Hamada, *Chem. Lett.* **2007**, *36*, 1292; K. Shimoda, K. Mikuni, K. Nakajima, H. Hamada, H. Hamada, *Chem. Lett.* **2008**, *37*, 362.
4 T. Satoh, H. Miyataka, K. Yamamoto, T. Hirano, *Chem. Pharm. Bull.* **2001**, *49*, 948; R. K. Uhrig, M. A. Picard, K. Beyreuther, M. Wiessler, *Carbohydr. Res.* **2000**, *325*, 72.
5 I. W. Sutherland, *Adv. Microb. Physiol.* **1972**, *8*, 143; I. W. Sutherland, *Adv. Microb. Physiol.* **1982**, *23*, 79; I. W. Sutherland, *Camb. Stud. Biotechnol.* **1990**, *9*, 1; S. Faria, P. A. Vieira, M. M. Resende, F. P. Franca, V. L. Cardoso, *Appl. Biochem. Biotechnol.* **2009**, *156*, 475.
6 K. Shimoda, H. Hamada, H. Hamada, *Tetrahedron Lett.* **2008**, *49*, 601.
7 H.-J. Hsieh, R. Giridhar, W.-T. Wu, *Enzyme Microb. Technol.* **2007**, *40*, 324.
8 The inhibitory action of the glycosides on IgE production was examined as follows. Glutenin was used as the antigen (1 mg/rat), and $Al(OH)_3$ and pertussis vaccine were used as the adjuvants (20 mg and 0.6 mL/rat, respectively). Sensitization was made by injection of a mixture (0.6 mL) of the antigen and the adjuvant into the paws of each rat (male, ca. 200 g). Paw edema was measured 24 h after injection and the treated rats were divided in groups with an equal average swelling volume. Hydrocortisone was used as the positive control. Each test glycoside was dissolved in physiological saline containing 10% Nikkol and the solution was injected daily into the rat for 11 d starting on the day of grouping. The amount of IgE was measured by the passive cutaneous anaphylaxis (PCA)[9] method on the 15th day. The results were expressed as average of plasma IgE level of 7 rats administered a total of 10 mg kg^{-1} of each glycoside.
9 A. Koda, T. Miura, N. Inagaki, O. Sakamoto, A. Arimura, H. Nagai, H. Mori, *Int. Arch. Allergy. Appl. Immunol.* **1990**, *92*, 209.
10 Peritoneal mast cells were collected from the abdominal cavity of rats (Male Wistar rats, Nippon SLC) and purified to a level higher than 95% according to a method previously described.[11] The purified mast cells were suspended in a physiological buffered solution (PBS) containing 145 mM NaCl, 2.7 mM KCl, 1.0 mM $CaCl_2$, 5.6 mM glucose, and 20 mM HEPES (pH 7.4) to give approximately 10^4 mast cells/mL. Cell viability was always greater than 90% as judged by the trypan blue exclusion test. Mast cells were preincubated with the test compound (1 μM) for 15 min at 37 °C, and subsequently exposed to compound 48/80 at 0.35 μg mL^{-1}. Histamine release was determined by a fluorometric assay according to the previously reported method, and was expressed as a percentage of total histamine.[11]
11 M. Akagi, Y. Katakuse, N. Fukuishi, T. Kan, R. Akagi, *Biol. Pharm. Bull.* **1994**, *17*, 732.
12 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

Published on the web (Advance View) September 5, 2009; doi:10.1246/cl.2009.930