Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron Letters
Tetrahedron Letters 47 (2006) 2695–2698

Formation of water-soluble vitamin derivatives from lipophilic vitamins by cultured plant cells

Kei Shimoda,[a] Yoko Kondo,[b] Koichi Abe,[c] Hatsuyuki Hamada[d] and Hiroki Hamada[e,*]

[a]*Department of Pharmacology and Therapeutics, Faculty of Medicine, Oita University, 1-1 Hasama-machi, Oita 879-5593, Japan*
[b]*Department of Applied Science, Faculty of Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan*
[c]*Eisai Co. Ltd, 4-20-22 Koishikawa, Bunkyo-ku, Tokyo 112-8088, Japan*
[d]*National Institute of Fitness and Sports in Kanoya, 1 Shiromizu-cho, Kagoshima 891-2390, Japan*
[e]*Department of Life Science, Faculty of Science, Okayama University of Science, 1-1 Ridai-cho, Okayama 700-0005, Japan*

Received 17 January 2006; revised 13 February 2006; accepted 16 February 2006
Available online 3 March 2006

Abstract—Glycosylation of vitamin E, its homologues, and vitamin A by cultured plant cells of *Phytolacca americana* and *Catharanthus roseus* was investigated to produce water-soluble vitamin derivatives. Two new compounds, that is, 2,5,7,8-tetramethyl-2-(4-methylpentyl)chroman-6-yl β-D-glucopyranoside and 2,5,7,8-tetramethyl-2-(4,8-dimethylnonyl)chroman-6-yl β-D-glucopyranoside, together with 2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl β-D-glucopyranoside were isolated from the cultured cells of *P. americana* following administration of vitamin E and its homologues, that is, 2,5,7,8-tetramethyl-2-(4-methylpentyl)-6-chromanol, 2,5,7,8-tetramethyl-2-(4,8-dimethylnonyl)-6-chromanol and 2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol (vitamin E). On the other hand, glycosylation by *C. roseus* gave two new compounds, that is, 2,5,7,8-tetramethyl-2-(4-methylpentyl)chroman-6-yl 6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside and 2,5,7,8-tetramethyl-2-(4,8-dimethylnonyl)chroman-6-yl 6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside, as well. Furthermore, conversion of vitamin A (retinol) by these cultured cells afforded retinyl β-D-glucopyranoside.
© 2006 Elsevier Ltd. All rights reserved.

Vitamins control nutrition in humans and are involved in various physiological phenomena in the living body. Since the 1970s, vitamin E has attracted clinical attention because of its potential to be a very useful medicine having effects on gynecological internal secretion control against sterility, heart circulation, liver diseases, aging, atherosclerosis, thrombosis, and carcinogenesis.[1] On the other hand, clinical information of vitamin A includes its effects on night blindness, coronary heart diseases, certain kinds of cancer, and age-related macular degeneration.[2] Despite such specific physiological and pharmacological activities, water-insolubility, instability, and light decomposition of these vitamins have been problems responsible for the poor absorption following oral administration and for the limit of their use as medicines. Recently, several attempts have been made to increase the bioavailability of vitamin E and vitamin A, that is, their amphiphilic glycosides such as β-glucoside and β-galactoside have been synthesized by chemical glycosylation.[3–6] These glycosides would act as prodrugs of vitamin E and vitamin A, which are expected to be hydrolyzed by glycosidases in the living body to display the physiological activities of the corresponding vitamins.[7] On the other hand, glycosylation with plant cells has been the subject of increasing attention,[8,9] because one-step enzymatic glycosylation is useful for preparation of glycosides rather than chemical glycosylation which requires long protection–deprotection procedure. However, there are no reports on the enzymatic glycosylation of vitamin E and vitamin A with cultured plant cells. We report, herein, the enzymatic glycosylation of vitamin E, its homologues, and vitamin A into the corresponding glycosides, water-soluble vitamin derivatives, by cultured plant cells of *Phytolacca americana* and *Catharanthus roseus*.

Each callus strains, *P. americana*[10] and *C. roseus*,[11] were prepared as described previously. Just prior to use for this work, 50 g of cultured cells was transplanted to a 300 mL conical flask containing 100 mL of freshly prepared SH medium (pH 5.7) containing 3% sucrose and grown with continuous shaking for 1 week at 25 °C

* Corresponding author. Tel.: +81 86 256 9473; fax: +81 86 256 8468; e-mail: hamada@das.ous.ac.jp

0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.02.082

under illumination (4000 lux). A total of 90 mg of each vitamin substrates was administered to six flasks (15 mg/flask) containing the suspension cultured cells and the cultures were incubated at 25 °C for 7 days on a rotary shaker (120 rpm). After incubation, the cells were harvested and extracted (×3) by homogenization with MeOH. The 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 MeOH extract was concentrated and 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) to give products. No products were observed in the medium. The structures of the products were identified using HRFABMS, ^1H and ^{13}C NMR, H–H COSY, and C–H COSY. On administration of 2,5,7,8-tetramethyl-2-(4-methylpentyl)-6-chromanol (**1**)[12,13] to the cultured cells of *P. americana*, a product **5** (63%) was obtained (Fig. 1). The product **5** was identified as 2,5,7,8-tetramethyl-2-(4-methylpentyl)chroman-6-yl β-D-glucopyranoside, which was a new compound.[14] Next, 2,5,7,8-tetramethyl-2-(4,8-dimethylnonyl)-6-chromanol (**2**)[12,13] and 2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)-6-chromanol (vitamin E, **3**)[12] with longer side chains were tested for the same biotransformation system. The structures of the isolated products **6** (35%) and **7** (7%) were determined as β-glucosides, that is, 2,5,7,8-tetramethyl-2-(4,8-dimethylnonyl)chroman-6-yl β-D-glucopyranoside and 2,5,7,8-tetramethyl-2-(4,8,12-trimethyltridecyl)chroman-6-yl β-D-glucopyranoside.[4–6] The product **6** was a new compound.[14] These suggest that the long side chains of the substrates drastically decrease the yield of the products.

On the other hand, two products, **5** (56%) and **8** (14%), were isolated from the cultured cells of *C. roseus* following administration of **1**. The structure of **8** was determined to be 2,5,7,8-tetramethyl-2-(4-methylpentyl)chroman-6-yl 6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside (β-gentiobioside), which was a new compound.[14] On administration of **2**, two products were also isolated and identified as β-glucoside **6** (32%) and β-gentiobioside **9** (5%), that is, 2,5,7,8-tetramethyl-2-(4,8-dimethylnonyl)chroman-6-yl 6-*O*-β-D-glucopyranosyl-β-D-glucopyranoside. The product **9** was a new compound.[14] When **3** was used as the substrate, only β-glucoside product **7** (8%) was obtained, suggesting that no further glycosylation products such as β-gentiobioside were produced due to low yield of the product **7**.

These demonstrate that the cultured plant cells of *P. americana* are able to convert vitamin E and its homologues into the corresponding β-glucosides, whereas *C. roseus* cells are capable of further glucosylation to give β-gentiobiosides as well.

Furthermore, retinol (vitamin A, **4**) was subjected to these glycosylation systems. After incubation with the cultured cells of *P. americana*, β-glucoside product, retinyl β-D-glucopyranoside (**10**, 22%),[3] was isolated. Interestingly, conversion by the cultured cells of *C. roseus* gave only **10** (31%) and no further glycosylation products such as β-gentiobioside were obtained, suggesting that the enzymes responsible for β-gentiobioside production from phenolic compounds are not efficient for the formation of β-gentiobioside of primary alcohol. These demonstrate that both of these cultured cells are able to catalyze mono-glucosylation of vitamin A to give the corresponding β-glucoside.

Glycosylation of organic compounds often improves their bio- and pharmacological properties, for example, glycosides of terpene alcohols have been widely used in folk medicines.[15] Therefore, vitamin glycosides are expected to possess new physiological activities which can be of pharmacological interest. The suppressive action of the glycosides **5**–**10** on IgE antibody formation

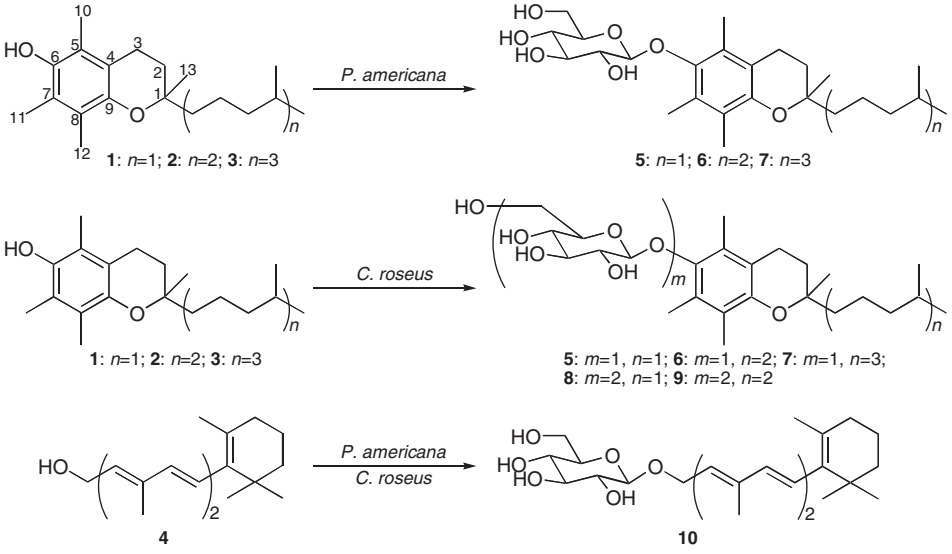

Figure 1. Glycosylation of lipophilic vitamins **1**–**4** by the cultured cells of *P. americana* and *C. roseus*.

was examined according to the reported procedure.[16,17] As a result, **6** exerted the strongest action among the glycosides tested, whereas no actions were observed in the cases of **8**–**10**.[18] This shows that the β-glucosides of vitamin E and its homologues would be useful antiallergic drugs.

Thus, the formation of water-soluble vitamin derivatives from lipophilic vitamins has been achieved by glycosylation with cultured plant cells of *P. americana* and *C. roseus*. It should be emphasized that the glycosides of vitamin E and vitamin A have been produced, for the first time, by whole cell-mediated process. Further studies on pharmacological activities and therapeutic effects of the glycosides are currently in progress.

Acknowledgements

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. Chiswick, M. L.; Wynn, J.; Toner, N. *Ann. N. Y. Acad. Sci.* **1982**, *393*, 109.
2. Omenn, G. S.; Goodman, G. E.; Thornquist, M. D.; Balmes, J.; Cullen, M. R.; Glass, A.; Koegh, J. P.; Meyskens, F. L.; Valanis, B.; Williams, J. H.; Barnhart, S.; Hammar, S. *N. Engl. J. Med.* **1996**, *334*, 1150.
3. Miyakoshi, T.; Numata, A. *Yukagaku* **1994**, *43*, 31.
4. Lahmann, M.; Thiem, J. *Carbohydr. Res.* **1997**, *299*, 23.
5. Uhrig, R. K.; Picard, M. A.; Beyreuther, K.; Wiessler, M. *Carbohydr. Res.* **2000**, *325*, 72.
6. Witkowski, S.; Walejko, P. *Z. Naturforsch.* **2002**, *57b*, 571.
7. Barua, A. B.; Olson, J. A. *Int. J. Vitam. Nutr. Res.* **1992**, *62*, 298.
8. Hamada, H.; Tomi, R.; Asada, Y.; Furuya, T. *Tetrahedron Lett.* **2002**, *43*, 4087.
9. Kondo, Y.; Shimoda, K.; Takimura, J.; Hamada, H.; Hamada, H. *Chem. Lett.* **2006**, *35*, 324.
10. Hamada, H.; Nishida, K.; Furuya, T.; Ishihara, K.; Nakajima, N. *J. Mol. Catal. B: Enzymatic* **2001**, *16*, 115.
11. Hamada, H.; Fuchikami, Y.; Ikematsu, Y.; Hirata, T.; Williams, H.; Scott, A. I. *Phytochemistry* **1994**, *37*, 1037.
12. Substrate **1** (all racemic form) was prepared from trimethylhydroquinone and 3-methenyl-7-methylocta-1,6-diene, and **2** (all racemic form) was from trimethylhydroquinone and 3-methenyl-7,11-dimethyldodeca-1,6-diene, according to the reported procedure.[13] Substrate **3** (*RRR* form) was purchased from Sigma–Aldrich Co.
13. Matsui, M.; Yamamoto, T. Jpn. Patent 292495, 1994; *Chem. Abstr.* **1996**, *125*(13), 168389g.
14. Spectral data for selected products; product **5**: HRFABMS: *m/z* 475.1924 $[M+Na]^+$; ^1H NMR (400 MHz, CD_3OD, δ in ppm): δ 0.88 (6H, d, $J = 6.8$ Hz, H-18, 19), 1.17 (2H, m, H-15), 1.21 (3H, s, H-13), 1.41–1.58 (5H, m, H-14, 16, 17), 1.78 (2H, t, $J = 6.8$ Hz, H-2), 2.04 (3H, s, H-10), 2.19 (3H, s, H-12), 2.22 (3H, s, H-11), 2.58 (2H, t, $J = 6.8$ Hz, H-3), 3.10–3.51 (4H, m, H-2′, 3′, 4′, 5′), 3.63 (1H, dd, $J = 12.0$, 5.2 Hz, H-6a′), 3.76 (1H, dd, $J = 12.0$, 2.4 Hz, H-6b′), 4.52 (1H, d, $J = 7.6$ Hz, H-1′); ^{13}C NMR (100 MHz, CD_3OD, δ in ppm): δ 12.0 (C-10), 13.2 (C-12), 14.1 (C-11), 21.6 (C-3), 22.4 (C-16), 22.9 (C-18, C-19), 24.1 (C-13), 29.0 (C-17), 32.6 (C-2), 40.6 (C-14), 40.7 (C-15), 62.8 (C-6′), 71.7 (C-4′), 75.6 (C-1), 75.7 (C-2′), 77.6 (C-5′), 77.8 (C-3′), 105.8 (C-1′), 118.3 (C-4), 123.2 (C-5), 127.7 (C-7), 129.6 (C-8), 147.1 (C-6), 149.1 (C-9). Product **6**: HRFABMS: *m/z* 545.2085 $[M+Na]^+$; ^1H NMR (CD_3OD): δ 0.88 (9H, apparent d, $J = 6.8$ Hz, H-22, 23, 24), 1.07–1.55 (14H, m, H-14, 15, 16, 17, 18, 19, 20, 21), 1.22 (3H, s, H-13), 1.77 (2H, t, $J = 7.2$ Hz, H-2), 2.04 (3H, s, H-10), 2.19 (3H, s, H-12), 2.22 (3H, s, H-11), 2.57 (2H, t, $J = 6.8$ Hz, H-3), 3.06–3.53 (4H, m, H-2′, 3′, 4′, 5′), 3.64 (1H, dd, $J = 11.6$, 5.2 Hz, H-6a′), 3.75 (1H, dd, $J = 12.0$, 2.4 Hz, H-6b′), 4.52 (1H, d, $J = 7.6$ Hz, H-1′); ^{13}C NMR (CD_3OD): δ 12.1 (C-10), 13.2 (C-12), 14.1 (C-11), 20.1 (C-23), 21.6 (C-3), 22.0 (C-16), 23.1 (C-22, C-24), 24.1 (C-13), 25.8 (C-21), 29.1 (C-17), 32.6 (C-2), 33.8, 38.2, 38.6 (C-14, C-18, C-19, C-20), 40.5 (C-15), 62.8 (C-6′), 71.7 (C-4′), 75.6 (C-1), 75.7 (C-2′), 77.6 (C-5′), 77.9 (C-3′), 105.8 (C-1′), 118.3 (C-4), 123.2 (C-5), 127.7 (C-7), 131.0 (C-8), 147.1 (C-6), 149.1 (C-9). Product **8**: HRFABMS: *m/z* 637.2171 $[M+Na]^+$; ^1H NMR (CD_3OD): δ 0.88 (6H, d, $J = 6.8$ Hz, H-18, 19), 1.17 (2H, m, H-15), 1.20 (3H, s, H-13), 1.40–1.58 (5H, m, H-14, 16, 17), 1.79 (2H, t, $J = 6.8$ Hz, H-2), 2.04 (3H, s, H-10), 2.18 (3H, s, H-12), 2.21 (3H, s, H-11), 2.57 (2H, t, $J = 6.8$ Hz, H-3), 3.10–3.53 (8H, m, H-2′, 2″, 3′, 3″, 4′, 4″, 5′, 5″), 3.65 (1H, dd, $J = 11.6$, 5.6 Hz, H-6a″), 3.73 (1H, dd, $J = 12.0$, 5.2 Hz, H-6a′), 3.85 (1H, dd, $J = 11.6$, 2.0 Hz, H-6b″), 4.04 (1H, dd, $J = 11.6$, 2.4 Hz, H-6b′), 4.24 (1H, d, $J = 7.6$ Hz, H-1″), 4.52 (1H, d, $J = 7.6$ Hz, H-1′); ^{13}C NMR (CD_3OD): δ 12.1 (C-10), 13.2 (C-12), 14.1 (C-11), 21.6 (C-3), 22.3 (C-16), 22.9 (C-18, C-19), 24.0 (C-13), 28.9 (C-17), 32.6 (C-2), 40.5 (C-14), 40.7 (C-15), 62.6 (C-6″), 70.0 (C-6′), 71.4 (C-4″), 71.5 (C-4′), 74.9 (C-2″), 75.6 (C-1, C-2′), 76.6, 77.6, 77.7 (C-3′, C-3″, C-5′, C-5″), 104.4 (C-1″), 105.7 (C-1′), 118.3 (C-4), 123.2 (C-5), 127.8 (C-7), 129.5 (C-8), 147.0 (C-6), 149.0 (C-9). Product **9**: HRFABMS: *m/z* 707.2206 $[M+Na]^+$; ^1H NMR (CD_3OD): δ 0.87 (9H, apparent d, $J = 6.8$ Hz, H-22, 23, 24), 1.09–1.61 (14H, m, H-14, 15, 16, 17, 18, 19, 20, 21), 1.29 (3H, s, H-13), 1.78 (2H, t, $J = 6.8$ Hz, H-2), 2.05 (3H, s, H-10), 2.18 (3H, s, H-12), 2.21 (3H, s, H-11), 2.59 (2H, t, $J = 6.8$ Hz, H-3), 3.12–3.54 (8H, m, H-2′, 2″, 3′, 3″, 4′, 4″, 5′, 5″), 3.65 (1H, dd, $J = 11.6$, 5.2 Hz, H-6a″), 3.73 (1H, dd, $J = 11.6$, 4.4 Hz, H-6a′), 3.85 (1H, dd, $J = 11.6$, 2.0 Hz, H-6b″), 4.04 (1H, dd, $J = 10.8$, 3.2 Hz, H-6b′), 4.24 (1H, d, $J = 8.4$ Hz, H-1″), 4.52 (1H, d, $J = 7.6$ Hz, H-1′); ^{13}C NMR (CD_3OD): δ 12.1 (C-10), 13.2 (C-12), 14.1 (C-11), 20.1 (C-23), 21.6 (C-3), 22.0 (C-16), 23.0, 23.1 (C-22, C-24), 24.1 (C-13), 25.8 (C-21), 29.1 (C-17), 30.7 (C-2), 33.8, 38.2, 38.6, 40.5, 40.8 (C-14, C-15, C-18, C-19, C-20), 62.7 (C-6″), 70.2 (C-6′), 71.5 (C-4″), 71.6 (C-4′), 74.9 (C-2″), 75.7 (C-1, C-2′), 76.8, 77.7, 77.8 (C-3′, C-3″, C-5′, C-5″), 104.4 (C-1″), 105.7 (C-1′), 118.4 (C-4), 123.3 (C-5), 127.7 (C-7), 129.7 (C-8), 147.0 (C-6), 149.1 (C-9).
15. Mastelic, J.; Jerkovic, I.; Vinkovic, M.; Dzolic, Z.; Vikic-Topic, D. *Croat. Chem. Acta* **2004**, *77*, 491.
16. The inhibitory action of the glycosides on IgE production was examined as follows. Ovalbumin 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 to 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)[17] method on the 15th day. The results were expressed as average of plasma IgE level of five rats administered a total of 10 mg/kg of each glycoside.

17. Koda, A.; Miura, T.; Inagaki, N.; Sakamoto, O.; Arimura, A.; Nagai, H.; Mori, H. *Int. Arch. Allergy. Appl. Immunol.* **1990**, *92*, 209.
18. The suppressive action of β-glucosides of vitamin E and its homologues on IgE antibody formation, that is, **5** (184 of average of plasma IgE level), **6** (170), and **7** (195), was stronger than that of hydrocortisone (341). The results for β-gentiobiosides of vitamin E homologues **8** and **9**, and retinyl β-glucoside **10** were 366, 353, and 372, respectively.