Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 3105–3108
Bioorganic & Medicinal Chemistry Letters

Enzymatic synthesis of arbutin undecylenic acid ester and its inhibitory effect on melanin synthesis

Yutaka Tokiwa,[a,*] Masaru Kitagawa,[b] Takao Raku,[c] Shusaku Yanagitani[b] and Kenji Yoshino[b]

[a]*National Institute of Advanced Industrial Science and Technology (AIST), Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan*
[b]*Toyobo Research Center, Katata 2-1-1, Ohtsu, Shiga 520-0292, Japan*
[c]*Green Product Laboratory Co., Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan*

Received 18 December 2006; revised 9 March 2007; accepted 13 March 2007
Available online 16 March 2007

Abstract—Transesterification of arbutin and undecylenic acid vinyl ester was catalyzed by alkaline protease, Bioprase, in dimethylformamide to get arbutin derivative having undecylenic acid at 6-position of glucose moiety, 6-*O*-undecylenoyl *p*-hydroxyphenyl β-D-glucopyranoside. The reaction rate increased with increase of arbutin concentration, and when its concentration was 0.9 M, the conversion rate was more than 90% under addition of 2 M undecylenic acid vinyl ester. The obtained arbutin ester significantly suppressed melanin production in murine B16 melanoma cells.

Cosmetics containing skin-whitening agents have attracted the attention of women in Asian countries and become best selling skin care products. Hyperpigmentation in the epidermis is caused by excessive melanin synthesis. Tyrosinase is a rate-limiting enzyme, which is used for the ultimate formation of melanin.[1] Therefore tyrosinase inhibitors may have potential for treating abnormal pigmentation disorders and for use as skin-whitening agents in the cosmetic industry.[2] Many researchers have tried to find tyrosinase inhibitors in certain plant extracts[3–5] and naturally occurring compounds.[6–8]

Arbutin, *p*-hydroxyphenyl β-D-glucopyranoside, which has been extracted from plants, is well known as a tyrosinase inhibitor and has been widely used for skin whitening.[9] Arbutin has recently been reported to exert a potent inhibitory effect on hydroxylation reaction of tyrosinase.[10] As one of the arbutin derivatives, α-arbutin has been developed and commercialized in the cosmetic industry.[11] The other arbutin derivatives substituted at 6-position of glucose moiety in arbutin have been investigated. Nakajima et al. reported the lipase-catalyzed transesterification of arbutin and synthesized cinnomoyl arbutin ester,[12] and Kadokawa et al. synthesized hybrid compound consisting of arbutin and kojic acid.[13]

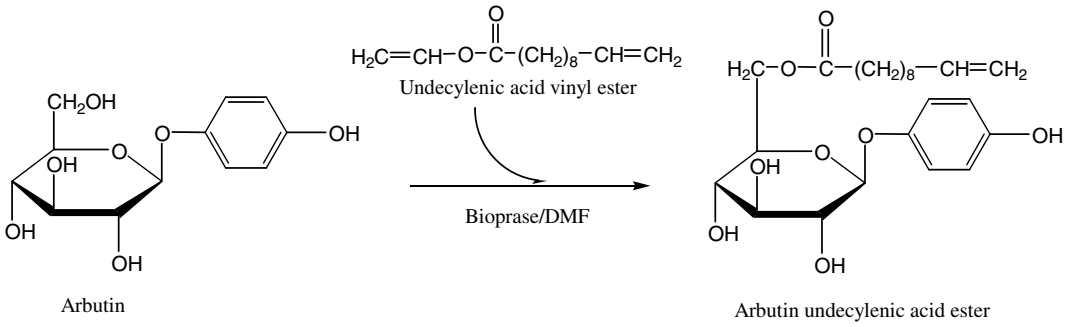
Regioselective acylation of carbohydrate with undecylenic acid was carried out by lipase in hydrophilic organic solvents,[14] and we discussed enzymatic synthesis of an arbutin derivative having undecylenic acid ester, which showed mushroom tyrosinase inhibiting activity in a cell-free experiment.[15] In the previous report, we did not examine the conditions for efficient enzymatic synthesis, that is, preparation yield, concentration and feed rate of raw materials. In the present study, we examined efficient enzymatic synthesis of the arbutin ester and further evaluated the inhibitory effect on melanin synthesis by a cellular experiment using B16 melanoma cells.

First, we investigated efficient synthesis of the arbutin undecylenic acid ester using arbutin and undecylenic acid vinyl ester catalyzed by Bioprase (Scheme 1), because increasing substrate concentration is important for minimizing the size of the reaction vessel and saving time in industrial scale synthesis.

The reaction was initiated in the 20 ml screw-capped flask by adding 10 mg/ml Bioprase conc. (Nagase

Keywords: Arbutin; Tyrosinase; Undecylenic acid; Melanin; Tyrosinase inhibitor.
* Corresponding author. Tel.: +81 29 861 6034; fax: +81 29 856 4898; e-mail: y.tokiwa@aist.go.jp

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.03.039



Scheme 1. Enzymatic synthesis of arbutin undecylenic acid ester in DMF by Bioprase.

Chemtex, Osaka, Japan), protease from *Bacillus subtilis*, to 5 ml dimethylformamide and water (95:5, volume) containing various concentrations of arbutin and undecylenic acid vinyl ester. The suspension was stirred at 130 rpm for 7 days at 40 °C. The reaction was terminated by filtering off the enzyme, and the remaining arbutin concentration was analyzed by HPLC. Arbutin was detected by high performance liquid chromatography (HPLC) with a refractive index detector. A carbohydrate analysis column, TSK gel Amide-80 (TOSOH), was used with a mobile phase of mixture of 90% acetonitrile and 10% water at a flow rate of 1.0 ml/min.

To obtain the product, a scaled-up reaction was carried out by use of 1.5 g Bioprase conc., 11.3 g (0.0415 mol) arbutin, and 35 g (0.167 mol) undecylenic acid vinyl ester in 150 ml of dimethylformamide/water (95:5, volume) at 40 °C. After 7 days reaction, the reaction was terminated by filtering off the enzyme. The solvent was evaporated and the product was separated by silica gel chromatography with an eluent consisting of *n*-hexane/ethyl acetate (1:10, v/v) to give colorless powder of 6-*O*-undecylenoyl *p*-hydroxyphenyl β-D-glucopyranoside (yield 10 g, 55%). Melting point of the product was 153–154 °C. The structure of the products was established by ^{1}H NMR (JEOL:JNM-EX270). DMSO-d_6 was used as a solvent and trimethylsilane was used as an internal reference. ^{1}H NMR (DMSO-d_6): δ 1.237 (br s, 8H, –(C<u>H</u>$_2$)$_4$–), 1.31–1.34 (m, 2H, –C<u>H</u>$_2$–CH$_2$–CH=), 1.49–1.52 (m, 2H, –CO–CH$_2$–C<u>H</u>$_2$–), 1.99(q, 2H, J = 7Hz, J = 12 Hz, –C<u>H</u>$_2$–CH=), 2.28(t, 2H, J = 7 Hz, –CO–C<u>H</u>$_2$–), 3.13–3.26 (m, 3H, H-2,3,4), 3.48–3.52 (m, 1H, H-5), 4.05 (q, 1H, J = 12 Hz, H-6), 4.30 (dd, J = 2 Hz, J = 12 Hz, H-6), 4.66 (d, 1H, J = 7.5 Hz, H-1), 4.91–5.01 (m, 2H, –CH=C<u>H</u>$_2$), 5.15 (br s, 1H, 4-OH), 5.23 (br s, 1H, 3-OH), 5.31 (br s, 1H, 2-OH), 5.75–5.81 (m, 1H, –C<u>H</u>=CH$_2$), 6.65 (d, 2H, J = 9 Hz, aromatic-H), 6.83 (d, 2H, J = 9 Hz, aromatic-H), 9.01(s, 1H, aromatic-OH).

Figure 1 shows the effect of ratio and concentration of arbutin and undecylenic acid vinyl ester on the enzymatic transesterification catalyzed by Bioprase. The conversion rates of these reactions reached a plateau after 4 days' reaction, probably because the enzyme was inactivated in the reaction mixtures. Hence, the reaction should be completed within 4 days. The reaction can be accelerated by increasing the enzyme concentration and/or the substrate concentration. From the practical viewpoint, the latter way is better.

The reaction rates of transesterification increased with increase of the molar ratio of undecylenic acid vinyl ester against arbutin. Excess undecylenic acid vinyl ester is wasted in the reaction from the viewpoint of process economy. At a high concentration of arbutin (0.9 M) near saturated concentration of arbutin in DMF, the reaction proceeded in the presence of even 2 M equivalent of undecylenic acid vinyl ester, and more than 90% conversion rate was obtained at 4 days' reaction.

B16 murine melanoma cells were cultured in Dulbecco's MEM supplemented with 10% heat-inactivated (56 °C, 30 min) fetal bovine serum at 37 °C in a humidified atmosphere containing 5% CO_2. Fifteen microlitres of DMSO solution with or without the arbutin ester was added to the culture medium (15 ml) 24 h after cell seeding (5×10^5 cells/75 cm^2 dish). After 6 days of culture, the cells were harvested and the cell weights were determined. Melanin content was measured using the method of Masamoto with slight modification.[16] Melanoma cells were pelleted by centrifugation at 1500*g* for 5 min and then washed twice with phosphate-buffered saline. After further centrifugation, the supernatant was removed by decanting and the precipitated cells were solubilized with 1 ml of 1 M NaOH for 24 h in a capped test tube. The absorbance was measured at 400 nm, and the melanin content was calculated (A400/g-cell).

Dose-dependent inhibitory effect of the arbutin ester in cultured B16 melanoma cells is shown in Figure 2. The growth rate of B16 melanoma cells was not significantly altered after 6 days of incubation in the 0.1–10 mM concentration range of arbutin and 0.001–0.1 mM of the arbutin ester, although the number of cells that adhered to the plates seemed to decrease at the highest concentration of both samples. After 6 days of incubation with the arbutin ester 0.1 mM, the melanin production decreased to approximately 30% compared with that in control cells, while arbutin 0.1 mM did not affect melanin production.

Tyrosinase catalyzes three distinct reactions in seven step of eumelanin (black type melanin) synthesis: (1) the hydroxylation of tyrosine to 3,4-dihydroxyphenylal-

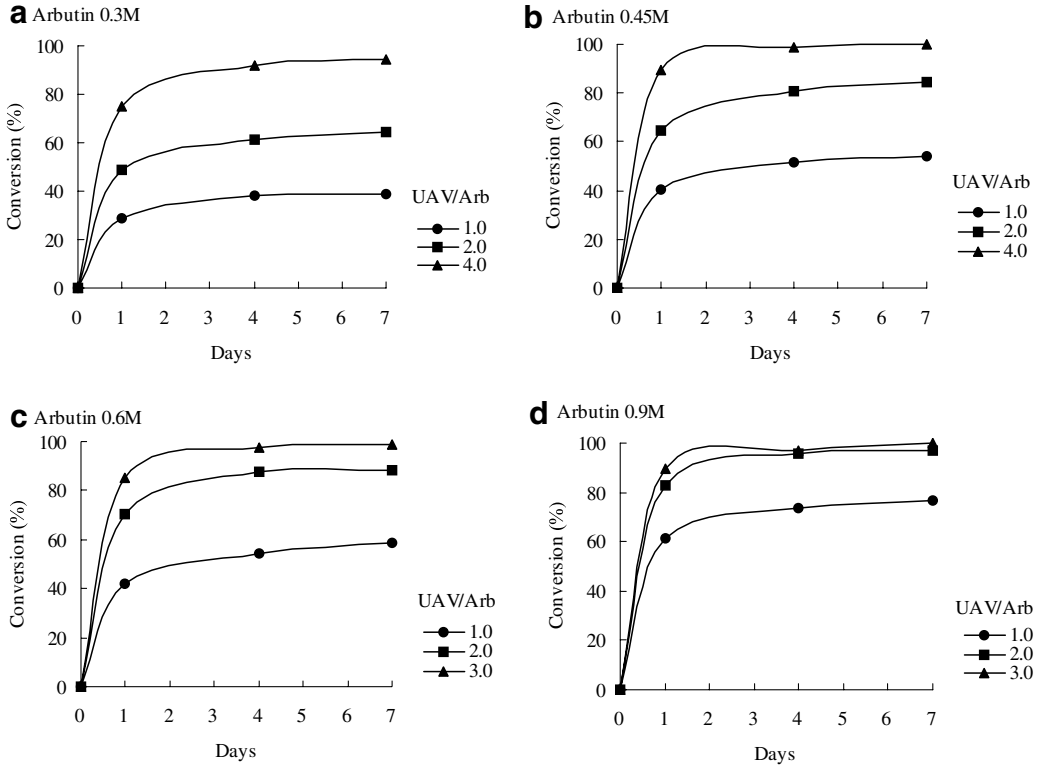


Figure 1. Effect of ratio and concentration of arbutin and undecylenic acid vinyl ester on the enzymatic transesterification catalyzed by Bioprase. Arbutin 0.3–0.9 M, undecylenic acid vinyl ester 0.3–2.7 M, Bioprase conc 10 mg/ml, DMF (5% water).

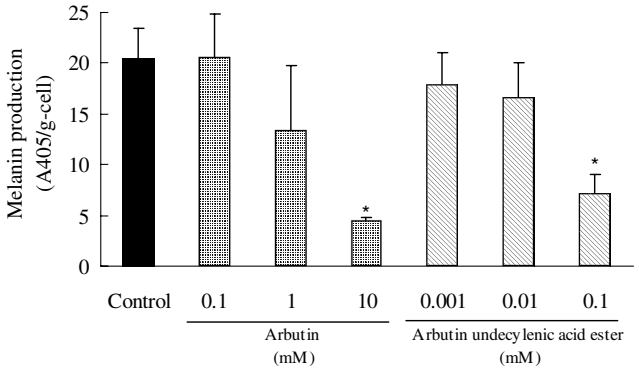


Figure 2. Effect of arbutin and arbutin undecylenic acid ester on the melanin synthesis in a cultured B16 melanoma cell.

anine (DOPA) (monophenolase); (2) the oxidation of DOPA to DOPA quinone (*o*-diphenolase); and (3) the oxidation of 5,6-dihydroxy indole to indole-quinone. The remaining steps can proceed spontaneously at physiological pH.[2]

The cellular experiment involves some additional factors (reaction time, availability of a cofactor, and penetration through the cell membrane) to eliminate the inhibiting activity on melanin synthesis as compared with the cell-free experiment. We previously reported that the arbutin ester exhibited inhibition of oxidation by tyrosinase.[15] In considering the mechanism of the arbutin ester action, it is reasonable to assume that the arbutin ester easily penetrates and hydrolyzes to arbutin in the cells to display antimelanogenic activity.

In conclusion, we could shorten the reaction time and increase the product by increasing the substrate concentration. Further, the arbutin ester decreased melanin production efficiently compared with arbutin. Arbutin has been used as a whitening agent for a long time. The arbutin ester described in this paper would be expected to be one of the second generation of arbutin derivatives having a higher antimelanogenesis activity.

References and notes

1. Marmol, V.; Beermann, F. *FEBS Lett.* **1996**, *381*, 165.
2. Hearing, V. J.; Tsukamoto, K. *FEBS J.* **1991**, *5*, 2902.
3. Masuda, T.; Yamashita, D.; Takeda, Y.; Yonemori, S. *Biosci. Biotechnol. Biochem.* **2005**, *69*, 197.
4. Khanom, F.; Kayaharu, H.; Tadasa, K. *Biosci. Biotechnol. Biochem.* **2000**, *64*, 1967.
5. Jeong, C. H.; Shim, K. H. *Biosci. Biotechnol. Biochem.* **2004**, *68*, 1984.
6. Masamoto, Y.; Ando, H.; Murata, Y.; Shimonishi, Y.; Tada, M.; Takahata, K. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 631.
7. Lee, S. H.; Choi, S. Y.; Kim, H.; Hwang, J. S.; Lee, B. G.; Gao, J. J.; Kim, S. Y. *Biol. Pharm. Bull.* **2002**, *25*, 1045.
8. Sasaki, K.; Yoshizaki, F. *Biol. Pharm. Bull.* **2002**, *25*, 806.
9. Maeda, K.; Fukuda, M. *J. Pharmacol. Exp. Therap.* **1996**, *278*, 765.
10. Hori, I.; Nihei, K.; Kubo, I. *Phytother. Res.* **2004**, *18*, 475.
11. Sugimoto, K.; Nishimura, T.; Nomura, K.; Sugimoto, K.; Kuriki, T. *Biol. Pharm. Bull.* **2004**, *27*, 510.
12. Nakajima, N.; Ishihara, K.; Matsumura, S.; Hamada, H.; Nakamura, K.; Furuya, T. *Biosci. Biotechnol. Biochem.* **1997**, *61*, 1921.

13. Kadokawa, J.; Nishikura, T.; Tagaya, H.; Fukuoka, N. *Nippon Kagaku Kaishi (Japanese)* **2001**, 533.
14. Raku, T.; Kitagawa, M.; Shimakawa, H.; Tokiwa, Y. *Biotechnol. Lett.* **2003**, *16*, 161.
15. Tokiwa, Y.; Kitagawa, M.; Raku, T. *Biotechnol. Lett.* **2007**, *29*, 481.
16. Masamoto, Y.; Murata, Y.; Baba, K.; Shimoishi, Y.; Tada, M.; Takahata, K. *Biol. Pharm. Bull.* **2004**, *27*, 422.