Synthesis of Glycosides of Resveratrol, Pinostilbene, and Piceatannol by Bioconversion with *Phytolacca americana*

Kei Shimoda¹, Naoji Kubota¹, Daisuke Uesugi², Yuya Fujitaka², Shouta Doi², Hiroki Hamada², Atsuhito Kuboki², Yuya Kiriake³, Takaumi Iwaki⁴, Tomohiro Saikawa⁵, and Shin-ichi Ozaki⁶

Abstract

Cultured cells of *Phytolacca americana*, which had been cultivated in the dark, biotransformed resveratrol to pinostilbene and pterostilbene. Pinostilbene was converted into its 3-O-β-D-glucoside and pterostilbene. In addition, the cells transformed piceatannol to its 4′-O-β-D-glucoside, isorhapontigenin, andisorhaptogentenin 3-O-β-D-glucoside.

Keywords

*Phytolacca americana* cultivated in the dark, resveratrol, pinostilbene, piceatannol, glycosylation, methylation

Received: January 2nd, 2019; Accepted: June 25th, 2019.

Stilbenoids, such as resveratrol, pinostilbene, and piceatannol, are important plant polyphenols and have attracted considerable pharmaceutical interest because of their diverse biological activities. However, the water insolubility of stilbenoids limits further pharmacological exploitation. Recently, several attempts have been made to synthesize water-soluble glycosides of resveratrol by chemical methods, including tedious protection-deprotection procedures, which resulted in low yields. Plant cell cultures would be useful for practical preparation of matric glucosylation. However, little attention has been paid to the effects of cultivation conditions with or without light on the biochemical potential of plant cell cultures to convert exogenous compounds. We report, herein, the biocatalytic glycosylation and methylation of resveratrol, pinostilbene, and piceatannol by cultured *Phytolacca americana* cells and the effects of light on the biotransformation reactions.

Resveratrol (1) was used as a substrate for the biotransformation system using cultured cells of *P. americana* that had been cultivated in the dark for over 1 year. The biotransformation was carried out as follows. Substrate 1 was administered to conical flasks containing suspension cell cultures of *P. americana*, which were incubated at 25°C for 2 days on a rotary shaker. The cells and medium were then divided. Compounds 2 to 4 were purified by HPLC from the extracts of the cells with MeOH. The chemical structures of the products were determined on the basis of ESIMS and 1H and 13C NMR spectra.

Products 2 to 4 were identified as resveratrol 3-O-β-D-glucoside (2), resveratrol 4′-O-β-D-glucoside (3), and pterostilbene 4′-O-β-D-glucoside (4) (Figure 1). On the other hand, the methylated product 5 of resveratrol (1) was isolated by HPLC from extracts of the medium with ethyl acetate. The chemical structure of 5 was determined as pinostilbene on the basis of its spectroscopic data. Next, cultured *P. americana* cells cultivated under light were used as the biocatalysts. The cells converted resveratrol (1) into resveratrol 3-O-β-D-glucoside (2) and resveratrol 4′-O-β-D-glucoside (3). No other products were detected, despite careful HPLC analyses.

Pinostilbene (5) was subjected to the same biotransformation system as described above using cultured *P. americana* cells.

1Department of Biomedical Chemistry, Faculty of Medicine, Oita University, Oita-shi, Japan
2Department of Life Science, Faculty of Science, Okayama University of Science, Kita-ku, Japan
3Faculty of Medicine and Health Sciences, Yamaguchi University, Ube-shi, Yamaguchi, Japan
4Department of Biophysics, Faculty of Medicine, Oita University, Oita-shi, Japan
5Department of Nursing, Junshin Gakuen University, Fukuoka, Japan
6Department of Biological Sciences, Faculty of Agriculture, Yamaguchi University, Ube-shi, Yamaguchi, Japan

Corresponding Author:
Hiroki Hamada, Department of Life Science, Faculty of Science, Okayama University of Science, 1-1 Dalai-cho, Kita-ku, Okayama 700-0005, Japan.
Email: hamada@dls.ous.ac.jp
that had been cultivated in the dark over 1 year. After 2 days of incubation, compound 6 was purified by HPLC from the extracts of the cells with MeOH. The chemical structure of 6 was determined as pinostilbene 3-O-\(\beta\)-d-glucoside (6) (Figure 2) on the basis of ESIMS and \(^1\)H and \(^{13}\)C NMR spectra. In addition, the methylated product (7) of pinostilbene (5) was purified by HPLC from the extracts of the medium with ethylacetate. Product 7 was determined as pterostilbene on the basis of its spectroscopic data. On the other hand, cultured P. americana cells cultivated under light transformed pinostilbene (5) to pinostilbene 3-O-\(\beta\)-d-glucoside (6) and pinostilbene 4′-O-\(\beta\)-d-glucoside (8).

Piceatannol (9) was then transformed using cultured P. americana cells that had been cultivated in the dark over 1 year, as described above. After the incubation period, compounds 10 and 11 were purified by HPLC from the MeOH extracts of the cells, and, on the basis of ESIMS and \(^1\)H and \(^{13}\)C NMR spectra, were identified as piceatannol 4′-O-\(\beta\)-d-glucoside (10) and isorhapontigenin 3-O-\(\beta\)-d-glucoside (11) (Figure 3). The methylated product (12) of piceatannol (9) was obtained by HPLC from the extracts of the medium with ethylacetate. Product 12 was identified on the basis of its spectroscopic data as isorhapontigenin. On the other hand, cultured P. americana cells

Experimental

General

HPLC was carried out with a YMC-Pack R&D ODS column (150 × 30 mm) (detection: UV, 280 nm; flow rate: 1.0 mL/min). Resveratrol, pinostilbene, and piceatannol used as substrates were purchased from Wako Pure Chemical Ind. The cultured plant cells were subcultured at 4-week intervals for 2 months on solid MS medium containing 2% glucose, 1 ppm 2,4-dichlorophenoxyacetic acid, and 1% agar (adjusted to pH 5.7). A suspension culture was started by transferring 20 g of the cultured cells to 100 mL of liquid MS medium in a 300 mL conical flask.

Biotransformation Procedure

The suspension plant cells were incubated in 300 mL conical flasks for 3 weeks. The cultured cells in the stationary growth
phase have been used for the experiments. After the cultivation period, 10 mg of substrate was added to a flask containing 20 g of suspension cell cultures. In all, 100 mg of substrate was administered to 10 flasks. The transformation was performed by incubating the mixture at 25°C on a rotary shaker (70 rpm) for 2 days. The culture medium was extracted with EtOAc, and the cells by homogenization with MeOH (×3). The various EtOAc and MeOH layers were combined, concentrated, and analyzed by HPLC. The MeOH fraction was partitioned between H$_2$O and EtOAc. The EtOAc fractions were combined, concentrated, and analyzed by HPLC. The H$_2$O fraction was applied to a Diaion HP-20 column and the column was washed with H$_2$O followed by elution with MeOH. The MeOH eluate was subjected to HPLC to obtain the glycosylated product. The yield of product was determined by HPLC analyses using calibration curves prepared with authentic samples and expressed as a percentage of the amount of administered substrate.

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Funding**

The author(s) received no financial support for the research, authorship, and/or publication of this article.

**References**

1. Stewart JR, Artme MC, O’Brian CA, Jubilee RS, Marlene CA, Catherine AO. Resveratrol: a candidate nutritional substance for prostate cancer prevention. *J Nutr*. 2003;133(7 Suppl):2440S-2443.

2. Orsini F, Pelizzoni F, Bellini B, Miglierini G. Synthesis of biologically active polyphenolic glycosides (combretastatin and resveratrol series. *Carbohydr Res*. 1997;301(3-4):95-109.

3. Shimoda K, Kubota N, Hamada M, et al. Glycosylation of quercetin with cultured plant cells and cyclodextrin glucanotransferase. *Nat Prod Commun*. 2014;9(5):647-648.

4. Shimoda K, Kubota N, Uesugi D, Hamadab H. Glycosylation of artemisinin with cultured plant cells of *Phytolacca americana*. *Nat Prod Commun*. 2014;9(5):683-685.

5. Shimoda K, Kubota N, Uesugi D, et al. Regioselective glycosylation of 3-, 5-, 6-, and 7-Hydroxyflavones by cultured plant cells. *Nat Prod Commun*. 2015;10(6):923-924.

6. Shimoda K, Kubota N, Hamada H. Synthesis of resveratrol glycosides by plant glucosyltransferase and cyclodextrin glucanotransferase and their neuroprotective activity. *Nat Prod Commun*. 2015;10(6):995-996.

7. Hamada H, Hamada H, Shimoda K. Synthesis of ε-viniferin glycosides by glucosyltransferase from *Phytolacca americana* and their inhibitory activity on histamine release from rat peritoneal mast cells. *Nat Prod Commun*. 2015;10(6):1017-1018.