Production of Curcumin Oligosaccharides by Glycosylation with *Parthenocissus tricuspidata*

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**INTRODUCTION**

Turmeric-derived from the rhizome of *Curcuma longa* Linn. has been widely used as a spice. In addition, it has been used for the treatment of inflammatory disorders such as arthritis, colitis, and hepatitis (Chen et al. 2008). Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadien-3,5-dione] is a representative phenolic antioxidant found in *C. longa* L., and exhibits anti-inflammatory and anticancer properties (Sugimoto et al. 2002; Aggarwal et al. 2003; Salh et al. 2003; Ukil et al. 2003; Jian et al. 2005). Curcumin is insoluble in aqueous solution and is poorly absorbed from the intestine after oral administration (Tønnesen and Karlsen 1985; Tønnesen et al. 1986). These shortcomings restrict its use in medicines. There have been a few reports on the chemical synthesis of curcumin β-glycosides so far (Hergenreder et al. 2002; Mohri et al. 2003). On the other hand, it has been reported that glucosidase-catalyzed glycosylation of curcumin gave curcumin α-glycosides (Vijayakumar and Divakar 2005, 2007).

Plant cell cultures are very useful systems for preparation of glycosides which are produced by plant glycosyltransferases. Many studies have been reported on glycosylation of exogenously added organic compounds by cultured plant cells such as *Eucalyptus perriniana*, *Phytolacca americana*, and *Catharanthus roseus* (Shimoda et al. 2006, 2007a, 2007b, 2007c). There have been no reports on glycosylation of exogenous substrates by *Parthenocissus tricuspidata*. Recently, it has been reported that curcumin prevented alcohol-induced liver disease (Nanj et al. 2003). On the other hand, oligosaccharide conjugates of medicines showed enhanced ability to be targeted to liver (Hashida et al. 1997). Curcumin oligosaccharides are of importance from a pharmacological point of view. We report here the glycosylation of curcumin to the corresponding glucoside, di-glucoside, gentiobioside, and gentiobiosylglucoside by cultured cells of *P. tricuspidata*.

**ABSTRACT**

Curcumin was converted into its oligosaccharides by cultured *Parthenocissus tricuspidata* cells-catalyzed glycosylation. In addition to curcumin 4'-O-β-glucoside (9%) and curcumin 4',4''-O-β-diglucoside (14%), two curcumin oligosaccharides, i.e., curcumin 4'-O-β-gentiobioside (16%) and curcumin 4'-O-β-gentiobiosyl-4''-O-β-glucoside (3%), were isolated from *P. tricuspidata* cells treated with curcumin.

**Keywords:** β-glucoside, β-diglucoside, β-gentiobioside, β-gentiobiosyl-β-glucoside, plant cell culture

**Abbreviations:** COSY, correlation spectroscopy; HMBC, heterobinuclear multiple-bond correlation; HPLC, high performance liquid chromatography; HRFABMS, high resolution fast atom bombardment mass spectrometry; NMR, nuclear magnetic resonance; TMS, tetramethylsilane

**MATERIALS AND METHODS**

**General experimental procedures**

The substrate curcumin (1) and Diaion HP-20 were purchased from Sigma-Aldrich Co. CD₃OD was purchased from Tokyo Kasei Kogyo Co. Ltd. YMC-Pack R&D ODS column was from YMC Co. Ltd. H NMR (400 MHz), 13C NMR (100 MHz), H-H COSY, H-C COSY, and HMBC spectra were recorded using a Varian XL-400 spectrometer in CD₃OD and the chemical shifts (δ ppm) are reported relative to TMS. The HRFABMS spectra were measured using a JEOL MStation JMS-700 spectrometer. HPLC was carried out on a YMC-Pack R&D ODS column (150 × 30 mm) at 25°C [solvent: methanol-water (9:11, v/v); detection: UV (280 nm); flow rate: 1.0 ml/min]. The extracted suspension cells of *P. tricuspidata* were cultured in 300 ml conical flasks containing Murashige and Skoog’s medium (Murashige and Skoog 1962) (100 ml, pH 5.7) and grown with continuous shaking on a rotary shaker (120 rpm) at 25°C in the dark.

**Biotransformation experiments**

Biotransformation experiments were performed by the addition of substrate (0.1 mmol/flask) into 10 flasks containing the suspension cultured cells of *P. tricuspidata* and cultures were incubated at 25°C for five days on a rotary shaker (120 rpm) in the dark. The glycosylation products were extracted and purified according to previously reported methods (Shimoda et al. 2006, 2007a, 2007b, 2007c). After incubation, the cells and medium were separated by filtration with suction. The filtered medium was extracted with ethylacetate. The medium was further extracted with n-butanol. The cells were homogenized and extracted with methanol. The glycoside products were detected in methanol fractions by HPLC analyses. The yields of the glycosylation products were calculated on the basis of the peak area from HPLC using calibration curves prepared by HPLC analyses of the authentic glycosides. The methanol fraction was concentrated and partitioned between water and ethylacetate. The ethylacetate fractions were combined and analyzed by HPLC. The water fraction was applied to a Diaion HP-20 column and the column was washed with water followed...
by elution with methanol. The methanol eluate was subjected to preparative HPLC to give glycoside products.

**RESULTS AND DISCUSSION**

Compounds 2-5 were isolated from cultured cells of *P. tricuspidata*, which had been treated with curcumin (1), by a combination of Diaion HP-20 column chromatography and HPLC.

The molecular ion peaks ([M+Na]+) of compounds 2 and 3 were observed at *m/z* 553.1670 (calcd. 553.1677 for C39H50O21Na) and 715.2205 (calcd. 715.2205 for C53H50O16Na), respectively, indicating the presence of one hexose in 2 and two hexoses in 3. The chemical structures of 2 and 3 were determined as curcumin 4′-O-β-glucoside (2, 9%) and curcumin 4′,4″-O-β-diglucoside (3, 14%) by comparing their 1H and 13C NMR data with previously reported data (Hergenhahn et al. 2002).

Compound 4 was assigned the molecular formula of C39H50O21 (calcd. 877.2724 consistent with a molecular ion peak at *m/z* 877.2724) and was substituted at C-6a. These glucosides were obtained by combination of Diaion HP-20 column chromatography and preparative HPLC to give glycoside products.

Compound 5 was identified as curcumin 4′-O-β-gentiobioside (16%).

The HRFABMS spectrum of 5 gave a pseudomolecular ion [M+Na]+ peak at *m/z* 877.2724 consistent with a molecular formula of C39H50O21 (calcd. 877.2730 for C39H50O21Na), showing the composition of 5 to be one molecule of curcumin and three hexoses. The 1H NMR spectrum of 5 included three anomeric proton signals at δ 4.95 (1H, *d*, J=7.6 Hz), 5.00 (1H, *d*, J=7.6 Hz) and 5.29 (1H, *d*, J=6.9 Hz), indicating the presence of three β- anomers in the sugar moiety. HMBC correlations were observed between the proton signal at δ 5.29 (H-1a) and the carbon signal at δ 150.6 (C-4′e), between the proton signal at δ 5.00 (H-1c) and the carbon signal at δ 150.4 (C-4′e), and between the proton signal at δ 4.95 (H-1b) and the carbon signal at δ 69.8 (C-6a).

A time-course experiment was carried out to investigate the ability of *P. tricuspidata* cell cultures to glycosylate curcumin (1). Cultured cells of *P. tricuspidata* were administered curcumin (1) and harvested at 12 h interval. Fig. 1 shows that the conversion into 2 was observed at 12 h after administration, and that sequential productions of 3, 4, and 5 were found after 12 h. The glycosylation pathway of curcumin (1) in *P. tricuspidata* cell cultures is shown in Fig. 2.

**CONCLUSION**

It was demonstrated that cultured cells of *P. tricuspidata* can glycosylate curcumin stepwise to give the corresponding glucoside, di-glucoside, gentiobioside, and gentiobiosylglucoside. Suspension cell cultures of *P. tricuspidata* are useful for the preparation of higher water-soluble derivatives of curcumin. Curcumin 4′-O-β-glucoside and curcumin 4′,4″-O-β-diglucoside have been chemically synthesized (Hergenhahn et al. 2002). These glucosides were obtained by condensing curcumin with α-d-acetyl-bromogluco-

**REFERENCES**


**JAPANESE ABSTRACT**

ウコン（*Curcuma longa* Linn.）に含まれるcurcuminは、*Parthenocissus tricuspidata*（学名）で培養細胞を触媒する配糖化により、オリゴ糖誘導体へ変換された。*Parthenocissus tricuspidata*（学名）で培養細胞は、curcuminとインキュベートすることにより、curcuminを4'-O-β-glucoside（9%）と4',4''-O-β-diglucoside（14%）の他、二糖類のオリゴ糖である4'-O-β-gentiobioside（16%）と4'-O-β-gentiobiosyl-4''-O-β-glucoside（3%）へ変換する能力を持つことが明らかとなった。