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Production of Curcumin Oligosaccharides by Glycosylation with *Parthenocissus tricuspidata*

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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**, heterobuclear multiple-bond correlation; **HPLC**, high performance liquid chromatography; **HRFABMS**, high resolution fast atom bombardment mass spectrometry; **NMR**, nuclear magnetic resonance; **TMS**, tetramethylsilane

INTRODUCTION

Turmeric derived from the rhizome of *Curcuma longa* Linn. has been widely used as a spice. In addition, it has been used for 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 (Hergenhahn *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 (Nanji *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*.

MATERIALS AND METHODS

General experimental procedures

The substrate curcumin (**1**) and Diaion HP-20 were purchased from Sigma-Aldrich Co. CD_3OD was purchased from Tokyo Kasei Kogyo Co. Ltd. YMC-Pack R&D ODS column was from YMC Co. Ltd. ^1H NMR (400 MHz), ^{13}C NMR (100 MHz), H-H COSY, C-H COSY, and HMBC spectra were recorded using a Varian XL-400 spectrometer in CD_3OD 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 cultured suspension cells of *P. tricuspiddata* 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

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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 $C_{27}H_{30}O_{11}Na$) and 715.2205 (calcd. 715.2205 for $C_{33}H_{40}O_{16}Na$), 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 ^{1}H and ^{13}C NMR data with previously reported data (Hergenhahn *et al.* 2002).

Compound **4** was assigned the molecular formula of $C_{33}H_{40}O_{16}$ (calcd. 715.2205 for $C_{33}H_{40}O_{16}Na$) from the HRFABMS (*m/z* 715.2199 $[M+Na]^+$), indicating that two molecules of hexose were introduced to the substrate. The ^{1}H NMR spectrum of **4** had two signals at δ 4.98 (1H, *d*, *J*=7.6 Hz) and 5.33 (1H, *d*, *J*=7.6 Hz) corresponding to their attachment to the anomeric carbons with β-configuration. The connectivity of anomeric carbons was established by an HMBC experiment. The HMBC spectrum included correlations between the anomeric proton signal at δ 5.33 (H-1a) and the carbon signal at δ 150.9 (C-4'), and between the anomeric proton signal at δ 4.98 (H-1b) and the carbon signal at δ 69.7 (C-6a). These findings confirmed that the inner β-D-glucopyranosyl residue was attached to the 4'-hydroxyl group of curcumin (**1**), and that the second β-D-glucopyranosyl residue and the inner β-D-glucopyranosyl residue were 1,6-linked. Thus, the structure of **4** was determined to be 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 $C_{39}H_{50}O_{21}$ (calcd. 877.2730 for $C_{39}H_{50}O_{21}Na$), showing the composition of **5** to be one molecule of curcumin and three hexoses. The ^{1}H NMR spectrum of **5** included three anomeric proton signals at δ 4.95 (1H, *d*, *J*=7.6 Hz), 5.00 (1H, *d*, *J*=8.0 Hz), and 5.29 (1H, *d*, *J*=7.6 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'), between the proton signal at δ 5.00 (H-1c) and the carbon signal at δ 150.4 (C-4''), and between the proton signal at δ 4.95 (H-1b) and the carbon signal at δ 69.8 (C-6a). These data established that both phenolic hydroxyl groups at C-4' and C-4'' of **1** were attached by β-D-glucopyranosyl residues, one of which (at C-4' of **1**) was substituted at C-6a position by β-D-glucopyranosyl residue. Thus, compound **5** was identified as curcumin 4'-*O*-β-gentiobiosyl-4''-*O*-β-glucoside (3%).

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**.

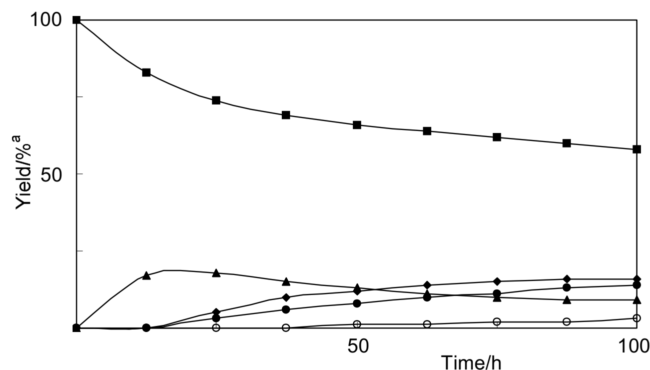

Fig. 1 Time-course of the glycosylation of curcumin (1) by cultured cells of *P. tricuspidata*. [a]Yield is expressed as relative percentage to the total amount of whole reaction products. Yields of **1** (■), **2** (▲), **3** (●), **4** (♦), and **5** (○) are plotted.

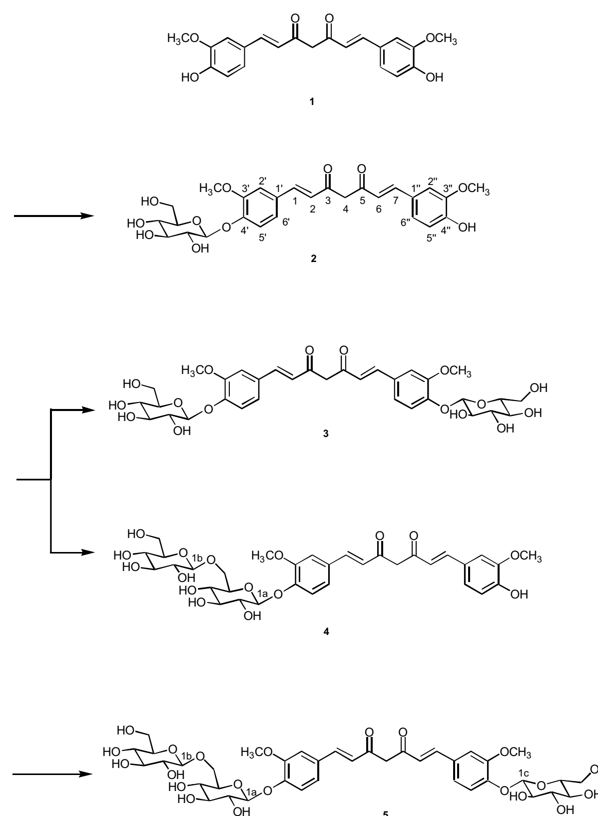

Fig. 2 Glycosylation of curcumin (1) by cultured cells of *P. tricuspiddata*.

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-bromoglucose followed by deacetylation in only 8% for curcumin 4'-*O*-β-glucoside and 3% for curcumin 4',4''-*O*-β-diglucoside. One step biocatalytic glycosylation by cultured plant cells of *P. tricuspidata* is more convenient than chemical glycosylation which requires tedious protection-deprotection steps.

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JAPANESE ABSTRACT

ウコン（*Curcuma longa* Linn.）に含まれるcurcuminは、*Parthenocissus tricuspidata*培養細胞が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*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、二種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。