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Biotransformation of paeonol by *Panax ginseng* root and cell cultures

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Abstract

Panax ginseng root and cell cultures were shown to biotransform paeonol (**1**) into its 2-*O*-β-D-glucopyranoside (**2**). *P. ginseng* root cultures were also able to biotransform paeonol (**1**) into its 2-*O*-β-D-xylopyranoside (**3**), 2-*O*-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside (**4**) and 2-*O*-β-D-xylopyranosyl(1 → 6)-β-D-glucopyranoside (**5**), and its demethylated derivate, 2′,4′-dihydroxyacetophenone (**6**). Compounds **3** and **4** are new glycosides. It is the first example that the administrated compound was converted into its xylopyranoside by plant biotransformation.

Keywords: *Panax ginseng*; Biotransformation; Glycosylation; Paeonol

1. Introduction

Biotransformation using plant cell or organ cultures, which involved the reactions of oxidation, reduction, esterification, hydroxylation, glycosylation, etc., has become an important method for production of useful compounds [1–3]. In recent years, production of biological active glycosides by plant biotransformation has attracted much attention, because the glycosides are bio-synthesized under mild conditions, and the protection and deprotection processes are not necessary for glycosylation as those in the chemical synthesis. This method is also considered economic, as the reaction is carried out without expensive purified enzymes or UDP-sugars. Some plant cell and organ cultures, such as *Panax ginseng*, *Eucalyptus perriniana*, *Nicotiana tabacum*, *Gardenia jasminoides*, etc., have been reported the ability to convert administrated compounds into their corresponding glycosides [3–10]. Paeonol (**1**) is a representative bioactive compound in *Paeonia suffruticosa* and *Paeonia lactiflora* (Paeoniaceae), and its glycosides have been reported the radical scavenging effects [11]. On continuing our interest in the production of useful glycosides by plant cell and organ cultures, we have investigated the biotransformation of paeonol (**1**) by *Panax ginseng* root and cell cultures.

2. Experimental

2.1. General experimental procedures

The IR spectra were measured with a JASCO FT/IR-300E (by a KBr disk method) spectrometer. Optical rotations were measured with a JASCO DIP-370 digital polarimeter in a 0.5-dm cell. The ESIMS was taken on an LCQ mass analyzer. FABMS and HRFABMS were conducted using a JEOL JMS-700 MStation mass spectrometer. The ^{1}H and ^{13}C NMR spectra were measured with a JEOL ECP-500 spectrometer in methanol-d_4 solution with TMS as the internal reference, and chemical shifts are expressed in δ (ppm). For HPLC, a Jasco PU-2080 HPLC system, equipped with a Shimadzu SPD-M10 Avp Diode Array detector was used for analysis, and a JASCO PU-2080 HPLC system, equipped with a Shodex RI-101 Differential Refractometer detector was used for preparation. Diaion HP-20 (Mitsubishi Chemical Corporation, Tokyo, Japan) and ODS (Chromatorex, 100–200 mesh, Fuji Sylisia Chemical, Ltd., Aichi, Japan) were used for column chromatography.

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TLC was conducted in Kieselgel 60 F_{254} plates (E. Merck).

2.2. Cultivation

The root cultures, which were established as described in a previous paper [12], were subcultured on B5K liquid medium (Murashige and Skoog's basal medium [13] containing 5 mg/l indole-3-butyric acid (IBA), 0.1 mg/l kinetin and 30 g/l sucrose) at 25 °C in the dark at 140 rpm on a rotary shaker at 4-week intervals. The cell cultures (Pg-3 cell line) were established as described in a previous paper [14]. The cells were subcultured on B2K agar medium (Murashige and Skoog's basal medium containing 2 mg/l IBA, 0.1 mg/l kinetin, 30 g/l sucrose and 9 g/l agar) at 25 °C in the dark at 4-week intervals. Prior to use in the biotransformation, the callus were transferred to 300 ml Erlenmeyer flask containing 120 ml B2K liquid medium, and cultured at 25 °C in the dark at 140 rpm on a rotary shaker.

2.3. Biotransformation of *1* by P. ginseng root cultures

Fifty mg of paeonol (**1**) was administered to the roots precultured for 3 weeks in a 300 ml Erlenmeyer flask containing 120 ml B5K liquid medium and then cultured for 1 week. The cultures not administered paeonol (**1**) were used as negative controls. The cultures were separated into roots and medium by filtration through a filter paper. The roots (6 g, fresh weight) were extracted with 60 ml methanol under ultrasonic treatment three times for 1 h each at room temperature. The methanolic extract was concentrated by evaporation in vacuo to give the residue (PGRR, 950 mg). The media were passed through a Diaion HP-20 column, and washed with water to remove sugars, then methanol to give the fraction (PGRM, 94 mg). PGRR and PGRM were analyzed by HPLC under the following conditions: column, YMC ODS-A (4.6 mm × 150 mm); solvent, 35% MeOH; flow rate, 0.8 ml/min; column temperature, 40 °C; detection, UV 274 nm. Two products (**2** and **5**) in PGRR and five products (**2**–**6**) in PGRM were found with the peaks for **2** at 4.6 min, **3** at 7.2 min, **4** at 3.2 min, **5** at 3.8 min and **6** at 8.2 min. Further separation by an ODS open column and then HPLC to give the products **2** (9 mg, biotransformation yield, 9.1%), **3** (1 mg, 1.1%), **4** (4 mg, 2.7%), **5** (3 mg, 2.2%) and **6** (1 mg, 2.2%).

Paeonol 2-*O*-β-D-glucopyranoside (**2**): colorless amorphous solid, $[\alpha]_D$ -69° (*c* 1.1, MeOH, 22 °C); ESIMS (positive) *m/z*: 351.1 $[M + Na]^+$; ^1H and ^{13}C NMR (see Table 1).

Paeonol 2-*O*-β-D-xylopyranoside (**3**): colorless amorphous solid, $[\alpha]_D$ −17° (*c* 0.10, MeOH, 22 °C); IR υ_{max} cm^{-1}: 3570, 1798, 1652, 1540, 1462; ESIMS (positive) *m/z*: 321.1 $[M + Na]^+$; ^1H and ^{13}C NMR (see Table 1).

Paeonol 2-*O*-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside (**4**): colorless amorphous solid, $[\alpha]_D$ −30° (*c* 0.31, MeOH, 22 °C); IR υ_{max} cm^{-1}: 3565, 1797, 1652, 1546, 1462; FABMS (positive) *m/z*: 513.2 $[M + Na]^+$; HRFABMS (positive): observed 513.1570, calcd for $C_{21}H_{30}O_{13}Na$ $[M + Na]^+$, 513.1584. ^1H and ^{13}C NMR (see Table 1). Acid hydrolysis of **4** and determination of the absolute configuration of the glucose were carried out same as previously reported [4].

Paeonol 2-*O*-β-D-xylopyranosyl (1 → 6)-β-D-glucopyranoside (**5**): colorless amorphous solid, $[\alpha]_D$ −20° (*c* 0.19, MeOH, 22 °C); ESIMS (positive) *m/z*: 483.2 $[M + Na]^+$; ^1H and ^{13}C NMR (see Table 1). Acid hydrolysis of **5** and determination of the absolute configuration of the glucose and xylose were carried out same as previously reported [4].

2′,4′-Dihydroxyacetophenone (**6**): colorless amorphous solid; ESIMS (negative) *m/z*: 151.1 $[M–H]^-$; ^1H NMR (see Table 1).

2.4. Biotransformation of *1* by P. ginseng cell cultures

Fifty mg of paeonol (**1**) was administered to suspension cells precultured for 3 weeks in a 300 ml Erlenmeyer flask containing 120 ml B2K liquid medium and then culture for 1 week. The cultures not administered paeonol (**1**) were used as negative controls. The extraction process was carried out same as those on the roots. Analysis on the methanolic extract of the cells (PGCC, 188 mg) and the medium (PGCM, 116 mg) by HPLC with the same condition as in roots resulted in the observation of a product (**2**) in PGCM with the peak at 4.6 min. Further separation of PGCM by an ODS open column and then HPLC to give the product (**2**, 2 mg, biotransformation yield, 2.0%).

3. Result and discussion

3.1. Biotransformation of *1* by P. ginseng root cultures

Paeonol (**1**) was administrated to the roots precultured for 3 weeks in B5K liquid medium, and then cultured for 1 week. The cultures not administered **1** were used as negative controls. Each culture was separated into roots and medium by filtration. The methanolic extracts of the roots and the medium were analyzed by HPLC. Two biotransformation products (**2** and **5**) were found in the roots, five (**2**–**6**) in the medium, and none in the negative controls. Further separation by ODS column chromatography and preparative HPLC gave the products **2**–**6**.

Product **2** was obtained as a colorless amorphous solid. It revealed an $[M + Na]^+$ ion peak at *m/z* 351.1 in the positive-ion ESIMS spectrum, suggesting that **2** is larger than **1** by a hexose unit. In the ^1H and ^{13}C NMR spectra of **2**, besides the signals for a paeonol moiety, they showed a set of β-glucopyranosyl signals, with the resonance for the anomeric proton at δ 5.05 (1H, d, J = 7.6 Hz). Further comparison of the NMR and $[\alpha]_D$ data of **2** with those in the reference showed that it is paeonol 2-*O*-β-D-glucopyranoside [15].

Product **3** was obtained as a colorless amorphous solid. Positive-ion ESIMS data show an $[M + Na]^+$ ion peak at *m/z* 351.1, suggesting that **3** is larger than **1** by a pentose unit.

Table 1
^{13}C and ^{1}H NMR data for compounds **1–6**

	1		**2**		**3**		**4**		**5**		**6**
	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{13}C NMR	^{1}H NMR	^{1}H NMR
Aglycon											
1	114.8		122.5		122.4		122.9		122.8		
2	165.8		160.7		159.6		160.4		160.4		
3	101.6	6.37 d (2.4)	102.6	6.85 d (2.4)	102.5	6.74 d (2.5)	103.4	6.88 d (2.5)	103.3	6.87 d (2.3)	6.24 d (2.3)
4	167.3		166.4		165.6		166.3		166.3		
5	108.1	6.45 dd (8.8, 2.4)	109.5	6.65 dd (9.0, 2.4)	108.3	6.67 dd (8.9, 2.5)	109.1	6.67 dd (8.7, 2.5)	109.1	6.67 dd (8.9, 2.3)	6.36 dd (8.9, 2.3)
6	133.7	7.72 d (8.8)	133.0	7.74 d (9.0)	132.6	7.75 d (8.9)	133.0	7.74 d (8.7)	133.1	7.74 d (8.9)	7.71 d (8.7)
COCH3	26.4	2.52 s	32.2	2.64 s	31.3	2.63 s	32.1	2.63 s	32.1	2.63 s	2.51 s
COCH3	204.0		200.6		200.8		200.7		200.6		
OCH3	56.1	3.81 s	56.2	3.85 s	55.5	3.85 s	56.3	3.87 s	56.3	3.87 s	
Sugar											
1′			102.6	5.05 d (7.6)	102.5	5.04 d (7.3)	102.5	5.05 d (7.6)	102.5	5.02 d (7.6)	
2′			75.0	3.55 dd (8.9, 7.6)	73.9	3.53 dd (8.7, 7.3)	74.9	3.54 dd (9.0, 7.6)	74.8	3.54 dd (9.2, 7.6)	
3′			78.6	3.48 t (8.9)	77.0	3.45 t (8.7)	78.2	3.48 t (9.0)	78.2	3.47 t (9.2)	
4′			71.4	3.37 dd (9.5, 8.9)	70.3	3.58 m	71.4	3.40 t (9.0)	71.5	3.37 t (9.2)	
5′			78.4	3.51 m	66.3	3.94 dd (11.5, 5.3) 3.39 dd (11.5, 9.9)	77.4	3.75 m	77.4	3.71 m	
6′			62.7	3.91 dd (12.0, 2.2) 3.69 dd (12.0, 6.4)			70.4	4.15 dd (11.6, 2.1) 3.83 dd (11.6, 6.4)	70.5	4.10 dd (11.7, 2.3) 3.80 dd (11.7, 6.6)	
1″							105.0	4.32 d (7.8)	105.7	4.27 d (7.5)	
2″							75.2	3.20 dd (9.0, 7.8)	74.9	3.19 dd (9.0, 7.5)	
3″							78.1	3.33[a]	77.7	3.28[a]	
4″							71.7	3.27[a]	71.1	3.47 m	
5″							78.0	3.22 m	66.9	3.84 dd (11.5, 5.7) 3.14 dd (11.5, 10.1)	
6″							62.8	3.85 dd (11.6, 2.3) 3.64 dd (11.6, 5.7)			

[a] Overlapping signals.

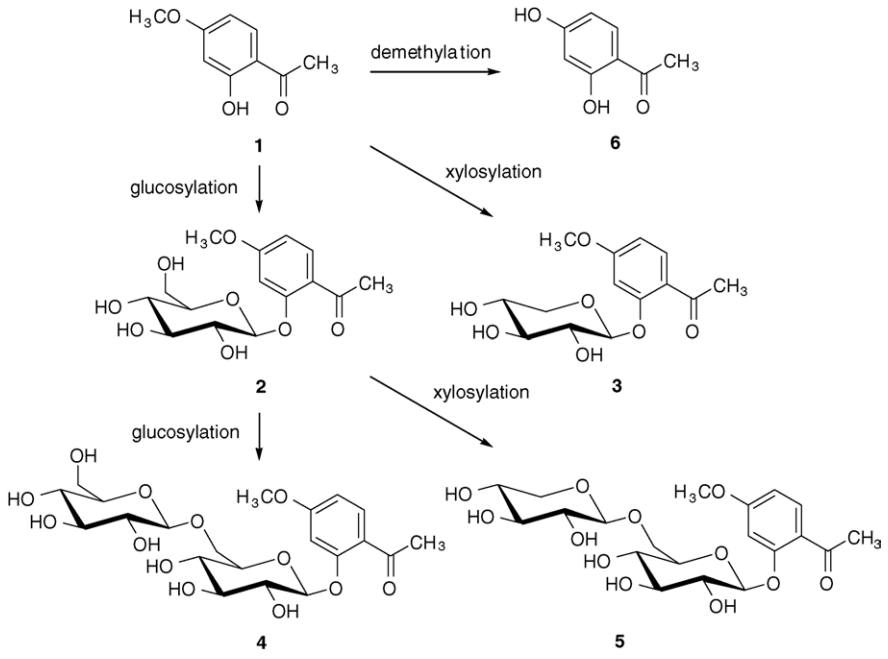

Fig. 1. Biotransformation of paeonol (**1**) by *P. ginseng* root cultures.

The ^{1}H and ^{13}C NMR spectra, in combination with detailed analysis of the DQFCOSY and HMQC data, suggested the presence of a xylopyranosyl moiety in **2**. The β-anomeric configuration for the xylopyranose was determined from its large $^{3}J_{H1,H2}$ coupling constant (7.3 Hz). Due to the limited amount of the compound available, the absolute configuration of the xylose was not corrected by a chemical method. However, it was considered as D-form in keeping with those obtained in our previous biotransformation study carried out in *P. ginseng* root cultures [4]. Thus, the structure of **3** was determined to be paeonol 2-*O*-β-D-xylopyranoside.

Product **4** was obtained as a colorless amorphous solid. Positive high-resolution FABMS analysis provided a molecular formula of $C_{21}H_{25}O_{13}Na$ [$(M + Na)^{+}$], suggesting that **4** is larger than **2** by a hexose unit. Acid hydrolysis of **4** gave only D-glucose as component sugars. In the ^{1}H NMR spectrum, two anomeric proton signals at δ 5.05 (1H, d, J = 7.6 Hz) and 4.32 (1H, d, J = 7.8 Hz) suggested the β-configurations of the glucoses. The position of the sugar moiety attached to the aglycon and the interglycosidic linkage were confirmed by observing the correlations between H-1 (δ 5.05) of the inner glucose and C-2 (δ 160.4) of the paeonol moiety, H-1 (4.32) of the terminal glucose and C-6 (δ 69.0) of the inner glucose in the HMBC spectrum. Thus, the structure of **4** was elucidated as paeonol 2-*O*-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside.

Product **5** was obtained as a colorless amorphous solid. It revealed an $[M + Na]^{+}$ ion peak at m/z 483.2 in the positive-ion ESIMS spectrum, suggesting that **5** is larger than **2** by a pentose unit. In comparison of the ^{1}H and ^{13}C NMR spectra of **5** with those of **4**, the signals due to paeonol and the inner glucose moieties were superimposable, while some differences were observed for the signals due to the terminal sugar. Further analysis of the signals due to the terminal sugar by ^{1}H, ^{13}C NMR, DQFCOSY and HMQC spectra, suggested it must be β-xylopyranose. The D-form of glucose and xylose was confirmed by the result of acid hydrolysis. Thus, the structure of **5** was identified as paeonol 2-*O*-β-D-xylopyranosyl (1 → 6)-β-D-glucopyranoside [15].

Product **6** was obtained as a colorless amorphous solid. It revealed an $[M–H]^{-}$ ion peak at m/z 151.1 in the negative-ion ESIMS spectrum, suggesting that **6** is smaller than **1** by a methyl unit, which was further confirm by the ^{1}H NMR spectrum. Thus, the structure of **6** was identified as 2′,4′-dihydroxyacetophenone.

Compounds **3** and **4** are new glycosides. Based on the structures of biotransformation products, the possible pathway for the metabolism of paeonol (**1**) in *P. ginseng* root cultures is shown in Fig. 1. **1** was glucosylated to produce the main product **2**, and xylation and demetylation of **1** gave two minor products **3** and **5**. Further glucosyltion and xylation of the main product **2** at glc-C-6 produced the disaccharide compounds **4** and **5**.

3.2. Biotransformation of *1* by P. ginseng cell cultures

Paeonol (**1**) was administrated to the suspension cells precultured for 3 weeks in B2K liquid medium, and then cultured for 1 week. The cultures not administered **1** were used as negative controls. Each culture was separated into cells and medium by filtration. The methanolic extracts of the roots and the medium were analyzed by HPLC. A biotransformation product was found in the medium but none in the roots or negative controls. Further separation by ODS

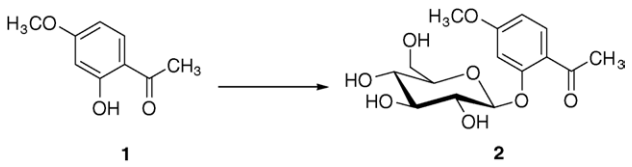

Fig. 2. Biotransformation of paeonol (**1**) by *P. ginseng* cell cultures.

column chromatography and preparative HPLC gave product **2**, which was identified as paeonol 2-*O*-β-D-glucopyranoside Fig. 2.

4. Conclusions

In summary, biotransformation of paeonol (**1**) by *P. ginseng* root and cell cultures was studied. Both the root and cell cultures were shown to biotransform **1** into its 2-*O*-β-D-glucopyranoside (**2**). *P. ginseng* root cultures were also able to biotransform paeonol (**1**) into its 2-*O*-β-D-xylopyranoside (**3**), 2-*O*-β-D-glucopyranosyl(1 → 6)-β-D-glucopyranoside (**4**) and 2-*O*-β-D-xylopyranosyl (1 → 6)-β-D-glucopyranoside (**5**), and its demethylated derivate, 2′,4′-dihydroxyacetophenone (**6**). Compounds **3** and **4** are new glycosides. Glycosylation, esterification, and hydroxylation had been reported to be involved in the biotransformation studies by *P. ginseng* cell, root or hairy root cultures [4–10]. The product (**6**) demonstrated a new metabolic reaction of demethylation occurring in the biotransformation of paeonol (**1**) by *P. ginseng* root cultures. To our knowledge, it is the first example that the administrated compound was converted into its xylopyranoside by plant biotransformation.

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