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# Biotransformation of triptonide by cell suspension cultures of *Platycodon grandiflorum**

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*Abstract*: The biotransformation of triptonide by cell suspension cultures of *Platycodon grandiflorum* was investigated. After six days of incubation, five products were obtained. On the basis of chemical and spectral evidence, their structures were elucidated as epitriptolide-14-*O*-β-D-glucoside, 5α-hydroxytriptonide, triptolide, triptodiolide, and 2β-hydroxytriptonide, among which epitriptolide-14-*O*-β-D-glucoside and 5α-hydroxytriptonide are new compounds.

## INTRODUCTION

*Tripterygium* w*ilfordii* Hook.f, Lei Gong Teng in Chinese, has been used in traditional Chinese medicine for the treatment of various diseases including rheumatoid arthritis, nephritis, systemic lupus erythematous, and skin disorders, as well as in male-fertility control [1,2].

Triptolide, a diterpenoid triepoxide, is the major active ingredient of *T. wilfordii* with a characteristic of 18 (4 → 3)abeo-abietane skeleton isolated by Kupchan in 1972 [3]. Previous investigations showed that triptolide is effective in the treatment of autoimmune diseases [4] and has potent antileukemic and antitumor activities [5,6]. The application of triptolide as a biologically active agent is limited by its strong toxicity. To find more effective compounds with less toxicity, chemical modifications of triptolide have been studied [7,8].

Triptonide, as another major active ingredient of *T. wilfordii* with effective anti-inflammatory and antifertility activities [9,10], was chosen for structural modification by a biotransformation approach. In this paper, we report the first successful biotransformation of triptonide by *Platycodon grandiflorum* cell suspension cultures.

## RESULTS AND DISCUSSION

Triptonide (**1**) was incubated for six days with suspension cells of *P. grandiflorum*, whereupon five biotransformation products were isolated from the spent medium. Blank experiments failed to reveal the presence of these or any other conversion products. On the basis of spectroscopic analysis, the structures of two new products were assigned as epitriptolide-14-*O*-β-D-glucoside (**2**) and 5α-hydroxytrip-

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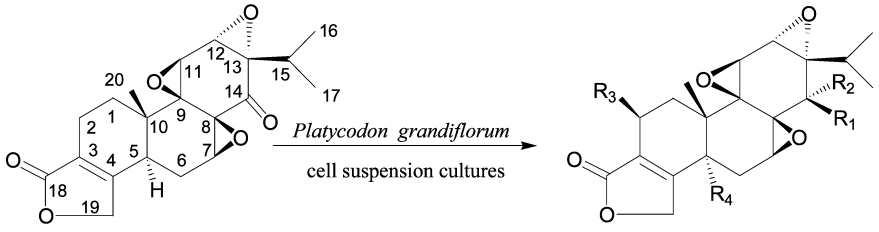
tonide (**3**), and the others identified as triptolide (**4**), triptodiolide (**5**), and 2β-hydroxytriptonide (**6**) (Fig. 1).

Time-of-flight mass spectrometry (TOFMS) of **2** gave a quasi-molecular ion peak at *m/z* 523, and high-resolution secondary-ion mass spectrometry (HRSIMS) of the $[M + H]^+$ ion was consistent with the formula $C_{26}H_{35}O_{11}$ (found: 523.2176; calcd.: 523.2174). A comparison of the $^{13}C$ NMR spectrum of **2** with that of triptonide (**1**) revealed a diagnostic peak for an oxygenated methine carbon at 69.9 ppm in place of a carbonyl resonance. Moreover, $^{13}C$ signals at 102.5 ppm and a $^1H$ doublet ($J$ = 8.0 Hz) at 4.21 ppm consistent with an anomeric proton signal (HMQC), suggested the presence of a β-linked glucosyl residue. This was confirmed, and the sugar moiety was identified as glucose by appropriate signals in $^{13}C$-$^1H$ and $^1H$-$^1H$ COSY spectra and by the MS peak at *m/z* 361, corresponding to $[M + H\text{-glc}]^+$.

A long-range coupling between H1′ and C14 was observed in an HMBC spectrum, suggesting the linkage of the sugar at C14. From the above analysis, one may conclude that triptonide was glucosylated after hydroxylation at C14. The aglycone was identified as epitriptolide on the basis of a $^1H$ singlet at 4.42 ppm for H14, and a $^{13}C$ peak at 69.9 ppm for C14. This deduction was supported by an enzymatic hydrolysis reaction. A solution of compound **1** (4.2 mg) in 0.1 m acetate buffer (pH 4.0, 1.0 ml) was treated with naringinase (5 mg, Sigma Chemical Co.) and the reaction mixture was stirred at 40 °C for 24 h. The reaction mixture was extracted three times with the equivalent volume of ethyl acetate. The ethyl acetate extract was chromatographed on Sephadex HL-20 and eluted with methanol to give the aglycone (1 mg). The aglycone was identified as epitriptolide on the basis of spectral data, which are in good agreement with those reported in literature [11]. The structure of **2** was thus established as epitriptolide-14-*O*-β-D-glucoside.

Compound **3** was obtained as colorless crystals. IR absorption at 3509, 2961, 1764, 1710, 1679, 1037 $cm^{-1}$ indicated the presence of hydroxyl and ketone groups, and a molecular formula of $C_{20}H_{23}O_7$ (*m/z* 375.1433 $[M + H]^+$, calcd. 375.1444) based on its HRSIMS, suggested that a hydroxyl group had been introduced into the substrate molecule. $^1H$ and $^{13}C$ NMR spectra of **3** were similar to those of **1** except for C5, C10, C6, and 5H. In an HSQC spectrum, the new signal (69.92 ppm) for a quaternary carbon supports the fact that a tertiary carbon was hydroxylated. On the basis of $^1H$-$^1H$ COSY and HMBC analysis, this was assigned to C5. Additionally, the proton signal at $\delta_H$ 5.68 ppm (5-OH) showed NOESY correlations with those at δ 1.85 ppm (1α-H), δ 2.22 ppm (6α-H), and δ 4.90 ppm (19-H). Therefore, compound **3** was identified as 5α-hydroxytriptonide.

Three known products were identified as triptolide (**4**) [3], triptodiolide (**5**) [3], and 2β-hydroxytriptonide (**6**) [12], respectively, based on their spectral data, which are in good agreement with those reported in the literature.




**1**

**2** $R_1$=H, $R_2$=O-glu, $R_3$=H, $R_4$=H
**3** $R_1$+ $R_2$=O, $R_3$=H, $R_4$=OH
**4** $R_1$=OH, $R_2$=H, $R_3$=H, $R_4$=H
**5** $R_1$=OH, $R_2$=H, $R_3$=OH, $R_4$=H
**6** $R_1$+ $R_2$=O, $R_3$=OH, $R_4$=H

**Fig. 1** Transformation of triptonide by cell suspension cultures of *P. grandiflorum.*

## EXPERIMENTAL SECTION

### General

Melting points were measured with an XT4A micro-melting point apparatus and are uncorrected. Optical rotations were recorded on a Perkin-Elmer 243B polarimeter using MeOH as solvent with a 1-cm path length. UV spectra were measured on a TU-1901 UV–Vis spectrophotometer. IR spectra were recorded on an Avatar 360 FT-IR spectrophotometer in KBr pellets. 1D and 2D NMR spectra were run on a Bruker DRX-500 spectrometer (500 MHz for $^{1}$H NMR and 125 MHz for $^{13}$C NMR) in DMSO-$d_6$ or $CDCl_3$ with TMS as internal standard. The chemical shift values (δ) were given in parts per million (ppm), and the coupling constants were in hertz (Hz). Abbreviations for NMR signals were as follows: s = singlet, d = doublet, m = multiplet. High-resolution positive SIMS was performed on a Bruker Apex • FI-ICR mass spectrometer. TOFMS was measured with a Perkin-Elmer QSTAR mass spectrometer. All the solvents used for extraction and isolation were of analytical grade. TLC was performed on silica gel G (60 mesh). Separation and purification were carried out by column chromatography on silica gel (200–300 mesh). Silica gels were purchased from Qingdao Marine Chemical Group Co., P.R. China. Triptonide was detected on TLC by spraying with Kedde reagent.

### Substrate

Triptonide (**1**) was purchased from Institute of Medical Sciences of Fujian. Its structure was characterized by $^{1}$H NMR, $^{13}$C NMR, and MS spectra. The purity of triptonide was 95 % determined by RP-HPLC using methanol:water (65:35) as eluting solvent.

### Plant cell cultures

The cell suspension cultures of *P. grandiflorum* were established and maintained in MS medium containing 0.5 mg $l^{-1}$ 6-BA, 0.5 mg $l^{-1}$ NAA, 0.2 mg $l^{-1}$ 2,4-D, and 3 % (w/v) sucrose. The cells were subcultured at intervals of 10 days. About 5 g of fresh cells were inoculated into 300 ml liquid medium in 1000-ml Erlenmeyer flasks on a rotary shaker (110 rpm) in the dark at 25 ± 2 °C.

### Biotransformation procedures

The substrate (**1**, 10 mg) in 1 ml acetone was added to 300 ml of 7-day old cell suspension culture in a 1000-ml Erlenmeyer flask. In total, 500 mg of substrate was inoculated. Incubation was continued for 6 days. Culture controls without substrate, but with the same amount of acetone and substrate controls containing the same amount of substrate without plant cells were carried out under the same conditions.

### Extraction and isolation

After six days of incubation, the culture was filtered and the filtrate was extracted (×3) with the same volume of ethyl acetate. The organic phase was evaporated to dryness *in vacuo*. TLC chromatography of the residue (visualized by Kedde reagent) revealed transformed products more polar than the substrate. No transformation product was detected in either of the controls.

The residue (2.0 g) was subjected to column chromatography over silica gel and gradient eluted with petroleum ether-ethyl acetate (4:1)—ethyl acetate, to give **2** (10 mg), **3** (9 mg), **4** (16 mg), **5** (10 mg), and **6** (5 mg), as well as starting material (200 mg).

Epitriptolide-14-*O*-β-D-glucoside (**2**): colorless crystals in acetone; mp 207–209 °C, $[\alpha]^{25}_{D}$ –112.7 (*c* 0.13, MeOH); UV (EtOH) $\lambda_{max}$ 217 nm; IR (KBr) $\nu_{max}$ 3373, 2967, 2927, 1746, 1672, 1073, 1030 $cm^{-1}$; HRSIMS 523.2176 [M + $H^+$] (calcd. for $C_{26}H_{35}O_{11}$, 523.2174); $^{1}$H NMR (DMSO-$d_6$) δ

4.83 (2H, q, *J* = 18, H19), 4.42 (1H, s, H14), 4.21 (1H, d, *J* = 8, H1′), 4.06 (1H, d, *J* = 6, H7), 3.90 (1H, d, *J* = 3, H11), 3.70 (1H, dd, *J* = 4.5,11, H6′), 3.48 (1H, d, *J* = 3, H12), 3.37 (1H, m, H6′), 3.08 (1H, m, H5′), 3.04 (1H, m, H3′), 2.97 (1H, m, H4′), 2.91 (1H, m, H2′), 2.60 (1H, brd, H5), 2.43 (1H, sept, *J* = 7, H15), 2.16 (1H, m, H6α), 2.10 (1H, brd, H2α), 1.98 (1H, m, H2β), 1.84 (1H, t, *J* = 14, H6β), 1.29 (2H, m, H1), 0.97 (3H, d, *J* = 6.5, H17), 0.95 (3H, s, H20), 0.61 (3H, d, *J* = 7.5, H16); $^{13}$C NMR (DMSO-$d_6$) δ 173.2 (C18), 162.5 (C4), 122.9 (C3), 102.5 (C1′), 77.06 (C3′), 76.66 (C5′), 73.63 (C2′), 70.37 (C4′), 70.28 (C19), 69.91 (C14), 65.46 (C13), 65.41 (C9), 62.36 (C8), 61.41 (C6′), 55.75 (C11), 55.07 (C7), 51.30 (C12), 39.83 (C5), 35.19 (C10), 29.53 (C1), 25.16 (C2), 22.44 (C6), 18.88 (C17), 15.24 (C16), 13.63 (C20).

5α-hydroxytriptonide (**3**): colorless crystals in acetone; mp 269–271 °C, $[\alpha]^{25}_{D}$ –157.1°(*c* 0.14, MeOH); UV (EtOH) $\lambda_{max}$ 218 nm; IR (KBr) $\nu_{max}$ 3509, 2961, 1764, 1710, 1679, 1037 cm$^{-1}$; HRSIMS 375.1433 (M + H$^+$) (calcd. for $C_{20}H_{23}O_7$, 375.1444); $^1$H NMR (DMSO-$d_6$) δ 5.68 (1H, s, 5-OH), 4.90 (2H, s, H19), 4.12 (1H, d, *J* = 3, H11), 4.10 (1H, d, *J* = 2.5, H12), 3.43 (1H, d, *J* = 5, H7), 2.25 (1H, sept, *J* = 7, H15), 2.23 (1H, m, H6α), 2.16 (1H, t, *J* = 14, H6β), 2.13 (1H, brd, H2α), 2.02 (1H, m, H2β), 1.85 (1H, ddd, *J* = 6, 12, 12, H1α), 1.10 (1H, dd, *J* = 5.5, 12, H1β), 0.93 (3H, s, H20), 0.89 (3H, d, *J* = 6.5, H17), 0.80 (3H, d, *J* = 7, H16); $^{13}$C NMR (DMSO-$d_6$) δ 198.4 (C14), 173.2 (C18), 162.0 (C4), 124.5 (C3), 69.91 (C5), 68.73 (C19), 65.13 (C13), 64.29 (C9), 61.08 (C8), 58.99 (C7), 58.70 (C11), 56.02 (C12), 40.01 (C10), 29.72 (C6), 25.57 (C15), 24.23 (C1), 18.01 (C17), 16.78 (C2), 16.35 (C16), 16.02 (C20).

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