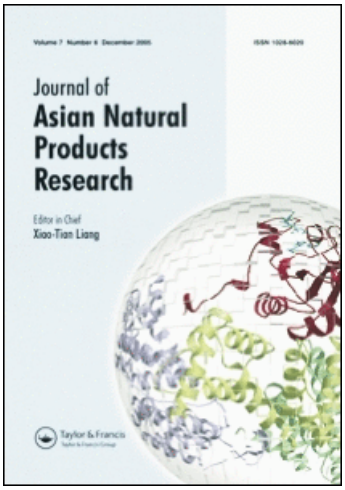

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BIOTRANSFORMATION OF TRIPTOLIDE AND TRIPTONIDE BY CELL SUSPENSION CULTURES OF *CATHARANTHUS ROSEUS*

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Catharanthus roseus cell suspension cultures were used to bioconvert both triptolide (**1**) and triptonide (**2**). The same reaction path was followed in both biotransformations. Two biotransformed products were obtained and their structures identified as triptriolide (**3**) and 12β,13α-dihydroxytriptonide (**4**), respectively, from **1** and **2**. Product **4** is a new compound.

Keywords: Biotransformation; Triptolide; Triptonide; Cell suspension cultures; *Catharanthus roseus*

INTRODUCTION

Tripterygium wilfordii Hook. f, a vine-like plant cultivated in many parts of southern China, has served as an important herb in traditional Chinese medicine for the clinical treatment of rheumatoid arthritis, skin disorders, in male-fertility control as well as in the treatment of cancer and inflammatory diseases [1]. Triptolide (**1**) and triptonide (**2**), diterpenoids with triepoxide structures, were isolated in 1972 by Kupchan from *Tripterygium wilfordii* and found to have potent antileukemic and antitumor activities [2]. This discovery stimulated worldwide interest, and subsequent studies have shown that **1** is effective in treating autoimmune diseases [3] and has potent antileukemic and antitumor activities [4,5], and that **2** has effective anti-inflammatory and antifertility activities [6,7].

As biologically active compounds, the application of both compounds was limited due to their toxicity. To find more effective compounds with less toxicity, structural modifications by chemical synthetic methods have been studied in depth [8–10]. Biotransformation techniques are becoming a useful tool for structural modifications of bioactive natural

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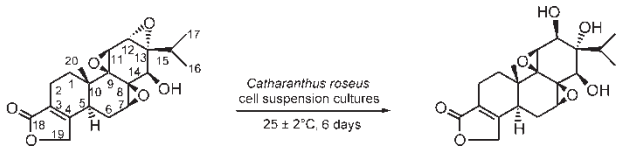


1 3

FIGURE 1 Transformation of triptolide by cell suspension cultures of *Catharanthus roseus*.

products. However, structural modifications of **1** and **2** by biological methods have not been reported so far.

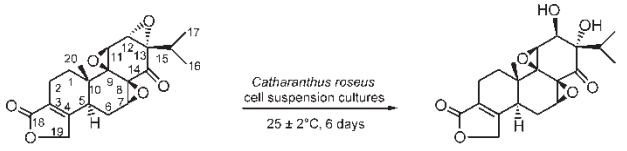
In continuation of our endeavors to optimize the structures of bioactive natural products [11–17], we report here the first successful biotransformations of **1** and **2** by *Catharanthus roseus* cell suspension cultures. One new product and one known product were obtained. On the basis of their IR, 1D and 2D NMR, and mass spectroscopic data the new product was identified as 12β,13α-dihydroxytriptonide (**4**).

RESULTS AND DISCUSSION

The biotransformations of both triptolide and triptonide by *Catharanthus roseus* cell suspension cultures were performed, and one product was obtained for each precursor. According to TLC analysis the biotransformed products were more polar than their precursors. The same reaction path was followed in the biotransformation process of both **1** and **2**, as illustrated in Figs. 1 and 2.

Compound **3** was obtained as colorless crystals, and the TOFMS gave the quasi-molecular ion peak at *m/z* 377 $[M + H]^+$. After comparison of the spectroscopic data with those reported in the literature [18], compound **3** was identified as triptriolide. It is a derivative of **1**, formed by opening of the 12,13-epoxy ring and the introduction of two hydroxyl groups at C-12 and C-13, which is a known structure.

The TOFMS spectrum of **4** showed a quasi-molecular ion at *m/z* 377 $[M + H]^+$, 394 $[M + NH_4]^+$, and 770 $[2M + NH_4]^+$. The IR spectra showed strong hydroxyl group absorptions at 3300–3500 cm^{-1}. HRMS measurement gave a molecular formula of $C_{20}H_{25}O_7$, 18 units greater than that of **2**. The above data suggested that **4** was derived from the opening of one epoxy ring of **2** followed by the introduction of a H_2O molecule (as with compound **3**). This was further confirmed by comparing with the ^{13}C NMR spectrum of **2** [10] with that of **3**, which showed two new peaks at δ 70.7 and 81.4 ppm, and that two peaks had disappeared between 55 and 65 ppm. On the basis of DEPT, ^1H-^1H COSY, HMQC and HMBC analyses, we concluded that the 12,13-epoxy ring was opened in **2** and that two hydroxyl groups were introduced at C-12 and C-13. The signal at δ 70.7 was assigned to C-12 since its corresponding proton signal (δ 4.18) was coupled with H-11. The signal at δ 81.4



2 4

FIGURE 2 Transformation of triptonide by cell suspension cultures of *Catharanthus roseus*.

TABLE I NMR chemical shift assignments of compound **4** (500 MHz for ^{1}H and 125 MHz for ^{13}C NMR in CD_3OD)

C	δ_H	δ_C	*HMBC*
1	1.47 ddd (12.0, 12.0, 6.0) 1.57 dd (12.0, 5.0)	30.5	20-CH_3
2	2.13 m 2.29 m	16.5	H-1
3		124.3	H-1, H-19
4		162.2	H-6, H-19
5	3.06 brd	39.9	H-1, H-6, H-7, 20-CH_3
6	1.98 t (14.0) 2.31 m	22.3	H-7
7	3.33 d (6.0)	60.2	H-5, H-6
8		63.7	H-6, H-7, H-11
9		69.3	H-1, H-6, 20-CH_3
10		34.7	H-1, H-6, 20-CH_3
11	4.06 d (5.0)	58.1	
12	4.18 d (5.0)	70.7	H-11
13		81.4	H-11, H-12, H-15, 16-CH_3, 17-CH_3
14		205.1	H-7, H-12
15	2.34 sept (7.0)	26.4	H-12, 16-CH_3, 17-CH_3
16	0.78 d (7.0)	15.6	H-15, 17-CH_3
17	0.93 d (7.0)	14.1	H-15, 16-CH_3
18		174.6	
19	4.86 q (12.5)	70.7	H-1
20	1.01 s	13.1	

was assigned to C-13 due to its long-range coupling with H-11, H-12, H-15, H-16 and H-17 in the HMBC spectrum. The orientation of 12-OH was identified as a β configuration according to the coupling constant of H-12 (d, $J_{12,11} = 5.0$ Hz). If 12-OH was in an α configuration, the dihedral angle between H-11 and H-12 would be close to 90°, due to distortion, with no coupling between them. On the contrary, 12-OH has a β configuration, and the dihedral angle between H-11 and H-12 is somewhere between 0° and 30°, which gives a coupling constant of 5–7 Hz. From the above deduction, compound **4** was identified as 12β,13α-dihydroxytriptonide, which is a new compound. All the ^{13}C and ^{1}H NMR data of **4** were unambiguously assigned by extensive NMR techniques (DEPT, ^{1}H-H COSY, HMQC, HMBC and NOESY). The 1D and 2D NMR spectral data of **4** were summarized in Table I. The bioactivities of the transformed products are under investigation.

EXPERIMENTAL

General Experimental Procedures

Melting points were measured with a XT4A micro-melting point apparatus and are uncorrected. Optical rotations were recorded on a Perkin–Elmer 243B polarimeter using methanol 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 as KBr pellets. The 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 CD_3OD with TMS as internal standard. The chemical shift values (δ) are in parts per million (ppm), and the coupling constants were in hertz (Hz). High-resolution positive SIMS were performed on a Bruker Apex II FI-ICR mass spectrometer. TOFMS were measured with a Perkin–Elmer QSTAR mass spectrometer. All solvents used for extraction and isolation were of analytical

grade. TLC was performed on silica gel G. (10–40 μm). 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., China. Triptolide and triptonide were detected on TLC by spraying with Kedde reagent.

Substrate

Triptolide (**1**) and triptonide (**2**) were purchased from the Institute of Medical Sciences of Fujian. Their structures were characterized by ^{1}H and ^{13}C NMR and MS spectra. The purity of triptolide and triptonide were 98% and 95%, respectively, as determined by RP-HPLC using methanol–water as eluting solvent.

Plant Cell Cultures

The cell suspension cultures of *Catharanthus roseus* were previously established in the laboratory and maintained in MS medium containing 6-BA ($0.5\,mg\,l^{-1}$), NAA ($0.5\,mg\,l^{-1}$), 2,4-D ($0.2\,mg\,l^{-1}$) and 3% (w/v) sucrose. The cells were sub-cultured at 10 day intervals. About 5 g of fresh cells were inoculated into a liquid medium (300 ml) in 1000 ml Erlenmeyer flasks on a rotary shaker (110 rpm) in the dark at 25 ± 2°C [16].

Biotransformation Procedures

The substrate (**1**, 10 mg) in acetone (1 ml) was added to suspension cells (300 ml) pre-cultured for 7 days in a 1000 ml Erlenmeyer flask. In total 60 mg of **1** was added. The incubation was continued for six additional days. The biotransformation of **2** was performed under the same conditions as those for **1**. Culture controls without substrate but with the same amount of acetone, and each substrate control containing the same amount of substrate without plant cells, were carried out under the same transformation conditions.

Extraction and Isolation

After 6 days of incubation, the two cultures were filtered and the filtrates extracted with the same volume of ethyl acetate (3 ×). The organic phase was evaporated to dryness *in vacuo*. The residues were dissolved in acetone. The two solutions were spotted on silica gel plates developed by light petroleum (60–90°C)–ethyl acetate (1:3) and visualized by spraying with Kedde reagent. TLC chromatography showed that *Catharanthus roseus* had the ability to biotransform triptolide and triptonide. The transformed products were more polar than the substrates. No transformation product was detected in either of the controls.

Some 80 mg of residue was obtained from the cultivation of **1** and 70 mg of residue was obtained from that of **2**. The residues were chromatographed on silica gel columns. Both columns were eluted with light petroleum–ethyl acetate in a gradient manner. By the same cell suspension culture, biotransformation of **1** resulted in 8 mg of **3**, while the biotransformation of **2** resulted in 12 mg of **4**.

Compound **4**: 12β,13α-dihydroxytriptonide, colorless crystals. $[\alpha]_D^{25}$ −79.1 (*c* 0.11, MeOH); mp 237–239°C. IR (KBr) ν_{max} (cm^{-1}): 3450, 2969, 2932, 1735, 1672, 1085, 1022; TOFMS *m/z* 377 $[M + H]^+$, 394 $[M + NH_4]^+$, 770 $[2M + NH_4]^+$; HRMS *m/z* 377.1596 $[M + H]^+$ (calcd for $C_{20}H_{25}O_7$, 377.1595); ^{1}H and ^{13}C NMR see Table I.

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