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# Cytotoxic Biotransformed Products from Triptonide by *Aspergillus niger*

Original Paper

**Abstract**

The diterpenoid triepoxides are the major active constituents of *Tripterygium wilfordii* with potent antitumor and immune activities. But the strong toxicity of these compounds has restricted their application to a great extent. In order to find more effective compounds with less toxicity, structural modifications of triptonide (**1**) by *Aspergillus niger* (AS 3.739) were investigated and four biotransformed products were obtained. Based on their chemical and spectral data, their structures were elucidated as 5$\alpha$-hydroxytriptonide (**2**), triptolide (**3**), 17-hydroxytriptonide (**4**), and 16-hydroxytriptonide (**5**), among which **2**, **4** and **5** are new compounds. All the three new transformed products showed cytotoxic activities against the majority of the human tumor cell lines tested, however, they are found to possess less cytotoxic activity when compared with **1**. Both compounds **4** and **5** showed similar cytotoxic activity and their $IC_{50}$ values were 5 – 15 fold less than **1**, while **2** is about 100 times less active than **1**.

**Key words**
Biotransformation · triptonide · *Aspergillus niger* · *Tripterygium wilfordii* · cytotoxic activity

## Introduction

*Tripterygium wilfordii* Hook f. (Celastraceae), also called Lei Gong Teng in Chinese, is a vine-like plant cultivated in many parts of southern China. *T. wilfordii* is used in traditional Chinese medicine for the treatment of various diseases including rheumatoid arthritis, nephritis, systemic lupus erythematosus and skin disorders, as well as in male-fertility control [1], [2]. Previous investigations have shown that the diterpenoid triepoxide, triptolide (**3**), a major active constituent of *T. wilfordii*, is effective in the treatment of autoimmune diseases [3] and has potent antileukemic and antitumor activities [4], [5]. As a biologically active agent, the application of **3** was limited due to its strong toxicity. Triptonide (**1**) is another major active constituent isolated from *T. wilfordii* by Kupchan in 1972 [6] with effective anti-inflammatory and antifertility activities [7], [8]. As a close analogue of **3**, **1** was chosen for structural modification by a biotransformation approach to obtain other potentially bioactive compounds with enhanced activity and decreased toxicity. In this paper, we report the microbial transformation of **1** by *Aspergillus niger* (AS 3.739) for the first time.

## Materials and Methods

### General

Melting points were measured with an XT4A micro-melting point apparatus and are uncorrected. Optical rotation was ob-



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tained on a Perkin-Elmer 243B polarimeter. IR spectra were recorded on an Avatar 360 FT-IR spectrophotometer in KBr pellets. NMR spectra ($^1$H-NMR, $^{13}$C-NMR, DEPT, $^1$H-$^1$H COSY, HMQC, HMBC and NOESY) were recorded 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 carbon multiplicities were obtained by DEPT and HMQC experiments. The chemical shift values ($\delta$) are given in parts per million (ppm), and the coupling constants are in hertz (Hz). High resolution MS were performed on a Bruker Apex II FI-ICR mass spectrometer. Time-of-flight mass spectra (TOFMS) were 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 (10 – 40 $\mu$m). Separation and purification of the extracts were carried out by column chromatography on silica gel (200 – 300 mesh). Silica gel was purchased from Qingdao Marine Chemical Group Co., P. R. China. Triptonide and metabolites were detected on TLC by spraying with Kedde reagent.

### Microorganisms

*Aspergillus niger* (AS 3.739) was purchased from China General Microbiological Culture Collection Center in Beijing, P. R. China.

### Medium

All culture and biotransformation experiments were performed in potato medium. Potato medium was produced by the following procedure: 200 g of minced husked potato were boiled in water for one hour, then the solution was filtered and the filtrate diluted with water to 1 L after addition of 20 g of glucose.

### Substrate

Triptonide (**1**) was purchased from Institute of Medical Sciences of Fujian. Its structure was characterized by $^1$H-NMR, $^{13}$C-NMR and mass spectra. The purity of triptonide was determined to be 95 % by RP-HPLC using methanol : water (65 : 35) as the eluting solvent. The substrate was dissolved in acetone and diluted to 10 mg/mL before use.

### Biotransformations

Among the 30 fungal strains screened, *Aspergillus niger* (AS 3.739) was found to possess better transformed product diversity and high transformation efficiency. The transformation systems were maintained on rotary shakers at 160 rpm at 25 °C. The screening scale biotransformations were performed in 250 mL Erlenmeyer flasks containing 60 mL of potato media. The substrate was added into cultures of *Aspergillus niger* after 48 h of cultivation of the microorganisms. TLC showed that *A. niger* had the ability to biotransform triptonide. Substrate and culture controls were run in parallel to the biotransformation experiment. For preparative scale biotransformation, 1 mL of substrate solution was added into a 1000-mL Erlenmeyer flask containing 300 mL potato medium. In total 100 mg of substrate were added. After 5 days of incubation, the mixtures were filtered under vacuum. The culture filtrates were extracted three times with the

Table **1** NMR data and correlated relations in 2D NMR of **2** (DMSO-$d_6$, 500 and 125 MHz, respectively)[a-c]

| | $^1$H | $^{13}$C | HMBC (C→H) | NOESY |
|---|---|---|---|---|
| 1$\alpha$ | 1.85 (ddd, 6, 12,12) | 24.2 t | H-2$\alpha\beta$ | H-1$\beta$, 2$\alpha$, 11 |
| 1$\beta$ | 1.10 (dd, 5.5, 12) | | | H-1$\alpha$, 2$\alpha\beta$, 11 |
| 2$\alpha$ | 2.13 (br.d) | 16.8 t | H-1$\alpha\beta$ | H-1$\alpha\beta$, 2$\beta$ |
| 2$\beta$ | 2.02 (m) | | | H-1$\beta$, 2$\alpha$, 20 |
| 3 | | 124.5 s | H-1$\beta$, 2$\alpha\beta$, 19 | |
| 4 | | 162.0 s | H-2$\alpha\beta$, 6$\alpha\beta$, 19, 5-OH | |
| 5 | | 69.9 s | H-1$\alpha\beta$, 6$\alpha\beta$, 7, 20, 5-OH | H-1, 6$\alpha\beta$ |
| 6$\alpha$ | 2.22 (m) | 29.7 t | H-7 | H-6$\beta$, 7, 19, 5-OH |
| 6$\beta$ | 2.16 (dd, 14, 15) | | | H-6$\alpha$, 7, 19, 20 |
| 7 | 3.43 (d, 5) | 59.0 d | H-6$\alpha\beta$ | H-6$\alpha\beta$ |
| 8 | | 61.1 s | H-6$\alpha\beta$, 11 | |
| 9 | | 64.3 s | H-1$\alpha\beta$, 7, 11, 12, 20 | |
| 10 | | 40.0 s [d] | H-1$\alpha\beta$, 2$\alpha\beta$, 6$\alpha\beta$, 20, 5-OH | |
| 11 | 4.10 (d, 3) | 56.0 d | H-12 | H-1$\alpha\beta$, 12 |
| 12 | 4.12 (d, 3) | 58.7 d | H-11 | H-11, 15, 16, 17 |
| 13 | | 65.1 s | H-11, 12, 15, 16, 17 | |
| 14 | | 198.4 s | H-7, 15 | |
| 15 | 2.25 (sept, 7) | 25.6 d | H-16, 17 | H-12, 16, 17 |
| 16 | 0.80 (d, 7) | 16.3 t | H-15 | H-12, 15, 17 |
| 17 | 0.89 (d, 6.5) | 18.0 q | H-15, 16 | H-12, 15, 16 |
| 18 | | 173.2 s | H-19 | |
| 19 | 4.90 (q, 18) | 68.7 t | | H-6$\alpha\beta$, 5-OH |
| 20 | 0.93 (s) | 16.0 s | H-1$\beta$ | H-2$\beta$, 6$\beta$ |
| 5-OH | 5.68 (s) | | | H-1$\alpha$, 6$\alpha$,19 |

[a] Chemical shifts in ppm relative to TMS; coupling constant (*J*) in Hz.
[b] Assignments were made by $^1$H-$^1$H COSY, HMBC, HMQC and NOESY data.
[c] Multiplicities were established from DEPT and HMQC data; for $^{13}$C-NMR data s, d, t, and q refer to C, CH, $CH_2$ and $CH_3$.
[d] Overlapped by the solvent peaks of DMSO-$d_6$, but recognizable with HMQC and HMBC technique.

equivalent volume of ethyl acetate. The obtained residue (200 mg) was subjected to chromatography (0.8 × 30 cm) on silica gel (200 – 300 mesh, 20 g), eluted with ethyl acetate-petroleum ether (1 : 1, 2 : 1, 100 mL for each gradient elute) to obtain 5 mg of **2** (5% yield), 3 mg of **3** (3% yield), 10 mg of **4** (10% yield) and 8 mg of **5** (8% yield).

*5α-Hydroxytriptonide* (**2**): Colorless crystals, $[\alpha]_D^{25}$: –157 (*c* 0.14, MeOH), m.p. 269 – 271 °C. IR (KBr): $\nu_{max}$ = 3509, 2961, 1764, 1710, 1679, 1037 $cm^{-1}$; TOFMS: *m/z* = 375 $[M + H]^+$, 392 $[M + NH_4]^+$, 397 $[M + Na]^+$, 766 $[2 M + NH_4]^+$; HRMS: *m/z* = 375.1433 $[M + H^+]$ (calcd. for $C_{20}H_{23}O_7$, 375.1438); $^1$H-NMR (500 MHz, DMSO-$d_6$): see Table **1**; $^{13}$C-NMR (125 MHz, DMSO-$d_6$): see Table **1**.

*17-Hydroxytriptonide* (**4**): Colorless crystals, $[\alpha]_D^{25}$: –180 (*c* 0.1, MeOH), m.p. 219 – 221 °C. IR (KBr): $\nu_{max}$ = 3414, 2938, 2881, 1736, 1674, 1017 $cm^{-1}$; TOFMS: m/z = 375 $[M + H]^+$, 392 $[M + NH_4]^+$, 766 $[2 M + NH_4]^+$; HRMS: *m/z* = 375.1439 $[M + H^+]$ (calcd. for $C_{20}H_{23}O_7$, 375.1438); $^1$H-NMR (500 MHz, DMSO-$d_6$): see Table **2**; $^{13}$C-NMR (125 MHz, DMSO-$d_6$): see Table **2**.

*16-Hydroxytriptonide* (**5**): Colorless crystals, $[\alpha]_D^{25}$: –210 (*c* 0.1, MeOH), m.p. 216 – 218 °C. IR (KBr): $\nu_{max}$ = 3398, 2927, 2855, 1766, 1722, 1678, 1035 $cm^{-1}$; TOFMS: *m/z* = 375 $[M + H]^+$, 392 $[M + NH_4]^+$, 766 $[2 M + NH_4]^+$; HRMS: *m/z* = 375.1441 $(M + H^+)$ (calcd. for $C_{20}H_{23}O_7$, 375.1438); $^1$H-NMR (500 MHz, DMSO-$d_6$): see Table **3**; $^{13}$C-NMR (125 MHz, DMSO-$d_6$): see Table **3**.

**Evaluation of cytotoxic activity**

The cytotoxic activity of the substrate and its biotransformed products was investigated on human cancer cell lines including Bel7402, BGC823, Hela, HL60, KB and MCF-7. The cell lines were maintained in RPMI-1640 (Hyclone) medium supplemented with 10% (v/v) fetal bovine serum, 100 IU/mL penicillin and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere containing 5% $CO_2$. Each tested compound was dissolved in DMSO to the concentrations of 50 mM and diluted to the required concentrations with the medium when used. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide) assay was performed to evaluate the *in vitro* cytotoxic activity of tested compounds. Different cancer cells (180 μL) were seeded at 2,000/mL onto the 96-well plates. After 24 h of incubation at 37 °C, 20 μL compound in serial dilutions (from 1 nM to 100 μM) were added, and 0.1% DMSO was used as the control. Following 48 h of incubation with the drug, 20 μL of 5 mg/mL MTT (Sigma) were added to each well. After additional 4 h of incubation, the medium was discarded and dried in the air, then 200 μL of acid-isopropyl alcohol were added to dissolve the formazan crystals, and the absorbance was measured at 570 nm by a microplate reader. The experiments were run in triplicates. The cytotoxic activity was shown as $IC_{50}$ (see Table **4**), which is the concentration of test compound (μM) to give 50% inhibition of the cell growth. Paclitaxel (Taxol®) was used as the positive control.

Table **2** NMR data and correlated relations in 2D NMR of **4** (DMSO-$d_6$, 500 and 125 MHz, respectively)[a-c]

| | *$^1$H* | *$^{13}$C* | *HMBC (C→H)* | *NOESY* |
|---|---|---|---|---|
| 1 | 1.36 (m) | 29.8 t | H-20 | H-2αβ, 5, 11, 20 |
| 2 | 2.15 (br.d) | 16.6 t | H-1 | H-1, 2β, 20 |
| | 2.01 (m) | | | H-1, 2α, 20 |
| 3 | | 123.1 s | H-1 | |
| 4 | | 161.8 s | H-6β, 19 | |
| 5 | 2.77 | 39.9 d [d] | H-6αβ, 7, 20 | H-1, 6αβ, 11,19,20 |
| 6 | 2.31 (m) | 22.1 t | H-7, | H-5, 6β, 7, 19 |
| | 1.87 (dd, 13, 15) | | | H-5, 6α, 7, 19, 20, |
| 7 | 3.46 (d, 5) | 60.4 d | H-6αβ | H-5, 6αβ |
| 8 | | 61.2 s | H-6αβ, 7 | |
| 9 | | 65.2 s | H-12, 20 | |
| 10 | | 34.6 s | H-1, 20 | |
| 11 | 4.28 (d, 3) | 56.0 d | H-12 | H-1, 5, 12 |
| 12 | 4.19 (d, 3) | 59.6 d | H-11 | H-11, 15, 16, 17-OH |
| 13 | | 64.0 s | H-11, 15, 16, 17 | |
| 14 | | 198.0 s | H-7 | |
| 15 | 2.29 (m) | 33.2 d | H-16, 17 | H-16, 17-OH |
| 16 | 0.76 (d, 7) | 11.5 q | H-15, 17 | H-12, 15,17-OH |
| 17 | 3.26 (octet, 5) | 61.5 t | H-15, 16 | H-12, 15, 16, 17-OH |
| 18 | | 173.1 s | | |
| 19 | 4.82 (q, 18) | 70.2 t | | H- 5, 6αβ |
| 20 | 0.91 (s) | 13.7 q | H-1 | H-1, 2αβ, 5, 6β |
| 17-OH | 4.65 (t, 5) | | | H-12, 15, 16 |

[a] Chemical shifts in ppm relative to TMS; coupling constant (*J*) in Hz.
[b] Assignments were made by $^1$H-$^1$H COSY, HMBC, HMQC and NOESY data.
[c] Multiplicities were established from DEPT and HMQC data; for $^{13}$C-NMR data s, d, t, and q refer to C, CH, $CH_2$ and $CH_3$.
[d] Overlapped by the solvent peaks of DMSO-$d_6$, but recognizable with HMQC and HMBC technique.

Table **3** NMR data and correlated relations in 2D NMR of **5** (DMSO-$d_6$, 500 and 125 MHz, respectively)[a-c]

| | *$^1$H* | *$^{13}$C* | *HMBC (C→H)* | *NOESY* |
|---|---|---|---|---|
| 1 | 1.36 (m) | 29.8 t | H-20 | H-2$\alpha\beta$, 5, 11, 20 |
| 2 | 2.14 (br.d) | 16.6 t | 1-OH | H-1, 2$\beta$ |
| | 2.01 (m) | | | H-2$\alpha$ |
| 3 | | 123.2 s | | |
| 4 | | 161.7 s | H-20 | |
| 5 | 2.77 (br.d) | 39.5 d [d] | H-6$\alpha\beta$, 7 | H-1, 6$\alpha\beta$ |
| 6 | 2.27 (m) | 22.1 t | H-7 | H-5, 6$\beta$, 7 |
| | 1.87 (dd, 14, 15) | | | H-6$\alpha$, 20 |
| 7 | 3.44 (d, 5.5) | 60.6 d | H-6$\beta$ | H-6$\alpha$ |
| 8 | | 61.2 s | H-6$\alpha\beta$, 7, 14 | |
| 9 | | 65.1 s | H-11, 20 | |
| 10 | | 34.9 s | H-6$\alpha$, 20 | |
| 11 | 4.26 (d, 3) | 60.0 d | H-12 | H-1 |
| 12 | 4.16 (d, 3) | 60.3 d | H-11 | H-15, 16, 17 |
| 13 | | 64.7 s | H-12, 14, 15, 16, 17 | |
| 14 | | 197.8 s | H-15 | |
| 15 | 2.07 (sept, 7) | 34.6 d | H-17 | H-12, 16, 17 |
| 16 | 3.41 (quin, 5.5) | 61.3 t | H-15, 17 | H-17 |
| | 3.23 (quin, 5.5) | | | |
| 17 | 0.91 (d, 7) | 12.8 q | H-15, 16 | H-12, 15, 16 |
| 18 | | 173.0 s | | |
| 19 | 4.82 (q, 18) | 70.22 t | | |
| 20 | 0.90 (s) | 13.7 s | | H-1, 6$\beta$ |
| 16-OH | 4.54 (t, 5.5) | | | H-16 |

[a] Chemical shifts in ppm relative to TMS; coupling constant (*J*) in Hz.
[b] Assignments were made by $^1$H-$^1$H COSY, HMBC, HMQC and NOESY data.
[c] Multiplicities were established from DEPT and HMQC data; for $^{13}$C-NMR data s, d, t, and q refer to C, CH, $CH_2$ and $CH_3$.
[d] Overlapped by the solvent peaks of DMSO-$d_6$, but recognizable with HMQC and HMBC technique.

Table **4** Cytotoxicity data of compounds **1 – 5**[a]

| *Compounds* | *Cell lines* | | | | | |
|---|---|---|---|---|---|---|
| | *$Bel_{7402}$* | *$BGC_{823}$* | *Hela* | *HL60* | *KB* | *MCF-7* |
| **1** | NE[b] | 0.85 | 0.03 | 0.02 | 0.01 | 0.10 |
| **2** | NE | 17.50 | 3.07 | 4.82 | 4.16 | 9.37 |
| **3** | NE | 0.09 | 0.04 | 0.03 | 0.03 | 0.07 |
| **4** | NE | 2.87 | 0.34 | 0.34 | 0.37 | 0.85 |
| **5** | NE | 2.44 | 0.33 | 0.34 | 0.32 | 0.68 |
| **Paclitaxel** | 0.52 | NE | 34.25 | 3.5E-4 | not tested | 12.64 |

[a] Results are expressed as $IC_{50}$ in $\mu$M.
[b] NE means $IC_{50}$ >100 $\mu$M

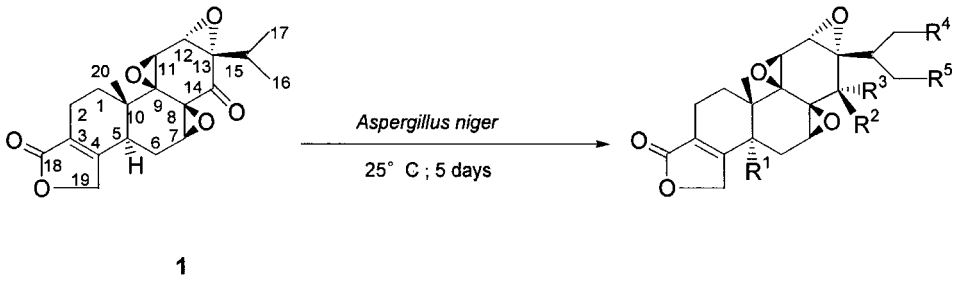
## Results

Triptonide (**1**) was added into the 2-day-old cultures of the microorganisms and four more polar products were isolated by chromatographic methods after an additional 5 days of incubation. On the basis of spectroscopic analysis, their structures were elucidated as 5$\alpha$-hydroxytriptonide (**2**), triptolide (**3**), 17-hydroxytriptonide (**4**), and 16-hydroxytriptonide (**5**), respectively, among which **2**, **4** and **5** are new compounds. The biotransformation reaction is illustrated in Fig. **1**.

The molecular formula of **2** was ascertained as $C_{20}H_{23}O_7$ by HR-MS measurement (*m/z* = 375.1433 $[M + H]^+$; calcd.: 375.1438), which suggested that a hydroxy group had been introduced into the substrate. The IR absorptions at 3509 and 1764 $cm^{-1}$ indicated the presence of hydroxy and carbonyl groups. The $^1$H- and $^{13}$C-NMR spectra of **2** were similar to those of **1** except for the signals of C-5, C-10, C-6 and H-5. The signal of H-5 ($\delta$ = 2.79, brd) disappeared in the $^1$H-NMR spectrum of **2**. In the HMQC spectrum, the new signal ($\delta$ = 69.92ppm) of a quaternary carbon supported the fact that a tertiary carbon was hydroxylated. On the basis of $^1$H-$^1$H COSY and HMBC analyses, the hydroxylated tertiary carbon was assigned to C-5. Additionally, the proton signal at $\delta_H$ = 5.68 ppm (5-OH) showed correlations with the signal at $\delta$ = 1.85 ppm (H1$\alpha$), $\delta$ = 2.22 ppm (H6$\alpha$) and $\delta$ = 4.90 ppm (H19) in the NOESY spectrum. Therefore, **2** was identified as the new compound, 5$\alpha$-hydroxytriptonide.

Compound **3** was obtained as colorless crystals and the TOFMS gave the quasi-molecular ion peak at *m/z* = 361 $[M + H]^+$. After comparison of the spectroscopic data with those reported in the literature [6], **3** was identified as triptolide.

Compounds **4** and **5** are both more polar than the substrate (by TLC analysis). An $[M + H]^+$ ion in the positive high resolution MS of **4** and **5** at 375.1439 and 375.1441, respectively, allowed a molecular formula of $C_{20}H_{23}O_7$ to be assigned to **4** and **5** (calcd.




| | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ |
|---|---|---|---|---|---|
| **2** | OH | O | O | H | H |
| **3** | H | OH | H | H | H |
| **4** | H | O | O | OH | H |
| **5** | H | O | O | H | OH |

**1**

Fig. **1** Biotransformation of triptonide by *Aspergillus niger.*

375.1438). Both IR spectra showed strong hydroxy group absorptions at 3300 – 3500 $cm^{-1}$ and characteristic signals of the carbonyl at 1600 – 1800 $cm^{-1}$ were observed. All the above data suggested that **4** and **5** were hydroxylated products of **1**. DEPT analyses showed the same change in **4** and **5** that the number of methyl carbons changed from 3 to 2, and the number of methylene carbons increased from 4 to 5, indicating that the hydroxy group was introduced at a methyl carbon. On the basis of their HMBC, HMQC and $^1H$-$^1H$ COSY spectra, the hydroxylated methyl carbon of **4** and **5** was assigned to C-16 or C-17. Since C-15 of **1** is a potential chiral carbon, hydroxylation at different methyl groups at C-15 could lead to different transformed products. By comparing the $^1H$- and $^{13}C$-NMR spectra of **4** with those of **5**, it was clear that their structures were essentially similar. Through further comparison of their $^1H$-NMR spectra, the major difference between **4** and **5** was that the two protons of the substituted methyl displayed two signals at 3.41 ppm and 3.23 ppm in the $^1H$-NMR spectrum of **5** but only one signal at 3.26 ppm in that of **4**. The reason of the difference is due to the orientation of the protons indicative of the carbonyl group. The two protons of **5** in which the methyl was substituted at the C-16 position were affected to resonate at different frequencies due to distance differences to the carbonyl group. When the methyl was hydroxylated at C-17, the two protons of **4** were too far from the carboxyl group to be acted on by the average fields. Therefore the two protons of **4** came into resonance at the same frequency. This deduction was also supported by the correlation of 17-OH with H-12 in the NOESY spectrum of **4** while no correlation was observed between 16-OH and H-12 in that of **5**. On the basis of the above analysis, the structure of **4** was elucidated to be 17-hydroxytriptonide and compound **5** to be 16-hydroxytriptonide. The 1D and 2D NMR spectral data of **4** and **5** are summarized in Tables **2** and **3**, respectively.

## Discussion

This is the first report about the biotransformation of triptonide by a biological method and four hydroxylated products were obtained. The cytotoxic activities of the substrate and transformed products against some human cancer cell lines were investigated. Three new transformed products showed potent *in vitro* cytotoxic activities against some human tumor cell lines, but they were found to be less cytotoxic than the two natural products. Compounds **5** and **4** had showed the similar cytotoxic activities and their $IC_{50}$ values were 5 – 15 fold lower than the substrate, while **2** has the lowest cytotoxic activity, and its $IC_{50}$ was 100 fold lower than that of **1**. This might mean that their corresponding toxicity was also decreased, which is desired in the utilization of triptonide- or triptolide-containing drugs. Further investigation concerning the toxicity and other activities of the new biotransformed compounds is currently under way. Biotransformation is now becoming a useful tool for structural modifications of bioactive natural products or synthetic organic compounds by enzymes or cells of microorganisms and plants. Microorganisms are often used to biotransform natural products to obtain novel drug candidates and predict mammalian metabolism *in vitro* [9], [10], [11]. Therefore this study is a good example of an enzyme-mediated biocatalysis reaction by microbial technique which exhibits the great potential and versatility of biotransformation in the structural modification of natural products.

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