

## New antioxidant phenylethanol glycosides from *Torenia concolor*

Yu-Shiang Chou<sup>a</sup>, Yu-Ling Ho<sup>b</sup>, Chih-Wen Ding<sup>a</sup> and Yuan-Shiun Chang<sup>ac\*</sup>

<sup>a</sup>Graduate Institute of Chinese Pharmaceutical Sciences, China Medical University, Taichung, Taiwan;

<sup>b</sup>Department of Nursing, Huangkuang University, Taichung, Taiwan; <sup>c</sup>Chinese Crude Drug Pharmacy, China Medical University Hospital, Taichung, Taiwan

(Received 3 April 2008; final version received 30 September 2008)

Two new phenylethanol glycosides, phenylethyl-*O*- $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-glucopyranoside (torenoside A, **1**) and 2'-*O*-3,4-dihydroxy- $\beta$ -phenylethoxy-*O*- $\alpha$ -L-rhamnopyranosyl-(1''  $\rightarrow$  3')-(4'-*O*-caffeoyl)- $\beta$ -D-glucopyranoside (torenoside B, **2**), along with the 17 known compounds (**3**–**19**) were isolated from *Torenia concolor*. Those structures were established on the basis of spectroscopic analysis including NMR spectroscopic techniques (<sup>13</sup>C, <sup>1</sup>H, <sup>1</sup>H–<sup>1</sup>H COSY, HMQC, HMBC, TOCSY, and NOESY). Moreover, phenylethanol glycosides **3**–**6** exhibited significant antioxidant activities in DPPH radical scavenging assay.

**Keywords:** *Torenia concolor*; Scrophulariaceae; phenylethanol glycosides; torenoside A; torenoside B

### 1. Introduction

*Torenia concolor* Lindl. (Scrophulariaceae) is a perennial prostrate herb found in sunny meadows at low elevation, which are widely distributed in India, Ryukyus, southern China, and Taiwan [1]. All parts of the plant have been used as folk medicine for expectorant, relieving cough, detoxification, defervescence, etc., in Taiwan [2]. Previous phytochemical studies of the family Scrophulariaceae have identified several different types of constituents, including flavonoids, iridoid glycosides, lignan glycosides, phenylethanol glycosides, and triterpenoids [3–6]. In continued studies on the bioactive constituents of this herb, we report herein the isolation and structural elucidation of two new phenylethanol glycosides, torenoside A (**1**) and torenoside B (**2**), along with the 17 known compounds, acetoside (**3**) [7], alphitolic acid (**9**) [8], augustic acid (**10**) [9], betulin (**11**), betulinic acid (**12**) [10], (2*R*,3*R*)-2,3-bis(3,4-dihydroxy)butyrolactone

(**7**) [11], campneoside II (**5**) [7], 3-epimaslinic acid (**13**) [12], isoacetoside (**4**) [7], jionoside C (**6**) [13], lupeol (**14**) [10], maslinic acid (**15**) [14], oleanolic acid (**16**) [15], succinic acid (**8**) [16], as a mixture of stigmasterol (**17**) and  $\beta$ -sitosterol (**18**) [17], and  $\beta$ -sitosterol-3-*O*-D-glucoside (**19**) [18], which have not been described previously, from genus *Torenia*. Compounds **1**–**6** (Figure 1) were tested for their antioxidant activities. Structural elucidation of the new isolates was based on the spectroscopic analysis, including 1D and 2D NMR techniques (<sup>1</sup>H–<sup>1</sup>H COSY, HMQC, HMBC, TOCSY, and NOESY) and chemical hydrolysis.

### 2. Results and discussion

Two new phenylethanol glycosides named torenoside A (**1**) and torenoside B (**2**) were obtained from the MeOH extract of *T. concolor*. The molecular formula of **1** was determined to be C<sub>20</sub>H<sub>30</sub>O<sub>10</sub> by HR-FAB-MS,

\*Corresponding author. Email: yschang@mail.cmu.edu.tw

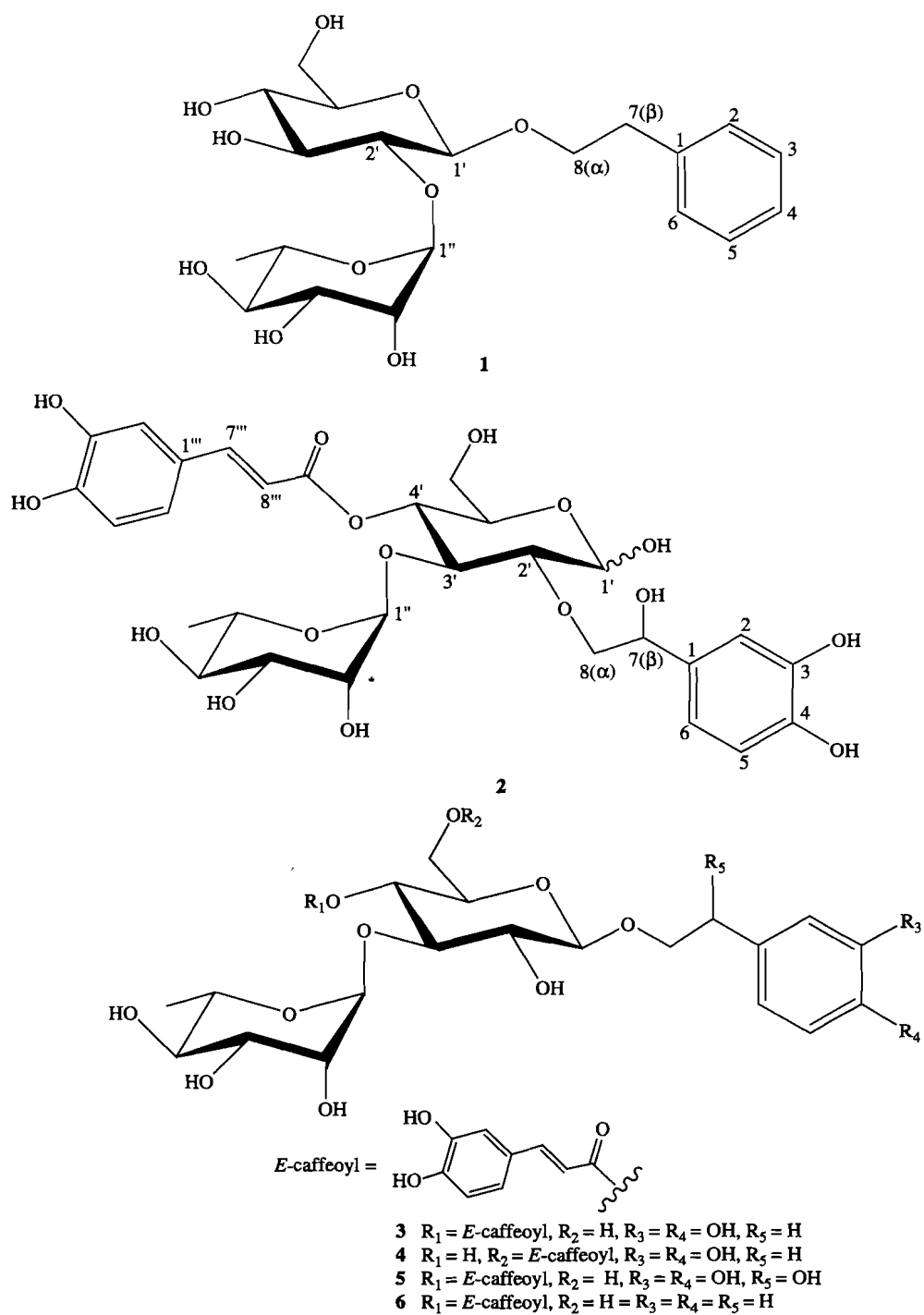


Figure 1. Structures of compounds 1–6.

which exhibited a *quasi*-molecular ion peak at  $m/z$  453.1733  $[M + Na]^+$ . The IR spectrum showed absorptions at 3398 (OH), 1458 (C=C), and 1085 (C—O—C)  $cm^{-1}$ . The  $^1H$ ,  $^{13}C$  NMR, and DEPT spectra showed two anomeric signals at  $\delta_H$  4.38 (1H, d,  $J = 7.5$  Hz) and 5.22 (1H, br s);  $\delta_C$  100.8 and 101.7, which were identified as  $\beta$ -D-glucopyranose and  $\alpha$ -L-rhamnopyranose, in addition to eight carbon signals for the aglycone of phenylethanol, suggesting that **1** is a phenylethanol glycoside [19] (Table 1). Acid hydrolysis of **1** with 10% HCl gave

glucose and rhamnose as the component sugars, which were identified by TLC comparison with authentic samples. Inspection of the HMBC spectrum of **1**, the correlations of H-1' of Glc and C-8( $\alpha$ ) of the aglycone and H-1'' of Rha and C-2' of Glc were conspicuously observed (Figure 2). On the basis of these results, **1** was assigned as phenylethyl-*O*- $\alpha$ -L-rhamnopyranosyl-(1  $\rightarrow$  2)- $\beta$ -D-glucopyranoside and named as torenoside A.

Compound **2** was determined to have an elemental composition of  $C_{29}H_{36}O_{16}$ , based

Table 1.  $^{13}C$  and  $^1H$  NMR spectral data of torenoside A (**1**) and torenoside B (**2**).

	<b>1</b>		<b>2</b>	
	$^1H$	$^{13}C$	$^1H$	$^{13}C$
Aglycone				
1		138.4		129.8
2	7.27 (1H, d, $J = 8.0$ Hz)	128.6	6.82 (1H, br s)	114.4
3	7.28 (1H, d, $J = 8.0$ Hz)	127.9		146.2
4	7.19 (1H, t, $J = 8.0$ Hz)	125.8		146.3
5	7.28 (1H, d, $J = 8.0$ Hz)	127.9	6.72 (1H, d, $J = 6.5$ Hz)	116.2
6	7.27 (1H, d, $J = 8.0$ Hz)	128.6	6.69 (1H, dd, $J = 6.5, 1.5$ Hz)	118.8
7 $\beta$	2.96 (2H, m)	35.9	4.60 (1H, dd, $J = 8.5, 2.0$ Hz)	78.4
8 $\alpha$	4.10 (1H, dd, $J = 17.5, 9.0$ Hz)	70.3	3.98 (1H, m)	72.9
	3.76 (1H, dd, $J = 17.5, 9.0$ Hz)		3.65 (1H, m)	
Glc				
1'	4.38 (1H, d, $J = 7.5$ Hz)	101.7	4.55 (1H, d, $J = 6.5$ Hz)	99.0
2'	3.43 (1H, dd, $J = 9.0, 7.5$ Hz)	79.2	3.45 (1H, dd, $J = 8.5, 6.5$ Hz)	81.9
3'	3.49 (1H, t, $J = 9.0$ Hz)	77.8	4.13 (1H, t, $J = 8.5$ Hz)	77.3
4'	3.39 (1H, t, $J = 9.0$ Hz)	70.3	5.09 (1H, t, $J = 8.5$ Hz)	70.4
5'	3.27 (1H, m)	76.4	3.76 (1H, m)	77.8
6'	3.88 (1H, dd, $J = 12.0, 2.0$ Hz)	61.3	3.62 (1H, m)	62.0
	3.67 (1H, dd, $J = 12.0, 5.5$ Hz)		3.58 (1H, m)	
Rha				
1''	5.22 (1H, br s)	100.8	5.17 (1H, br s)	102.1
2''	3.95 (1H, dd, $J = 3.0, 2.0$ Hz)	70.8	3.77 (1H, m)	72.0
3''	3.70 (1H, dd, $J = 9.0, 3.0$ Hz)	70.9	3.52 (1H, dd, $J = 8.0, 2.5$ Hz)	71.9
4''	3.39 (1H, t, $J = 9.0$ Hz)	72.6	3.28 (1H, m)	73.5
5''	4.08 (1H, dd, $J = 9.0, 6.0$ Hz)	68.4	3.58 (1H, m)	70.4
6''	1.25 (1H, d, $J = 6.0$ Hz)	16.6	1.21 (1H, d, $J = 6.0$ Hz)	18.3
Caffeoyl				
1'''				127.6
2'''			7.06 (1H, d, $J = 1.5$ Hz)	115.2
3'''				146.8
4'''				148.2
5'''			6.78 (1H, d, $J = 6.5$ Hz)	116.4
6'''			6.96 (1H, dd, $J = 6.5, 1.5$ Hz)	123.2
7'''			7.60 (1H, d, $J = 13.5$ Hz)	149.8
8'''			6.29 (1H, d, $J = 13.5$ Hz)	114.4
C=O				167.9

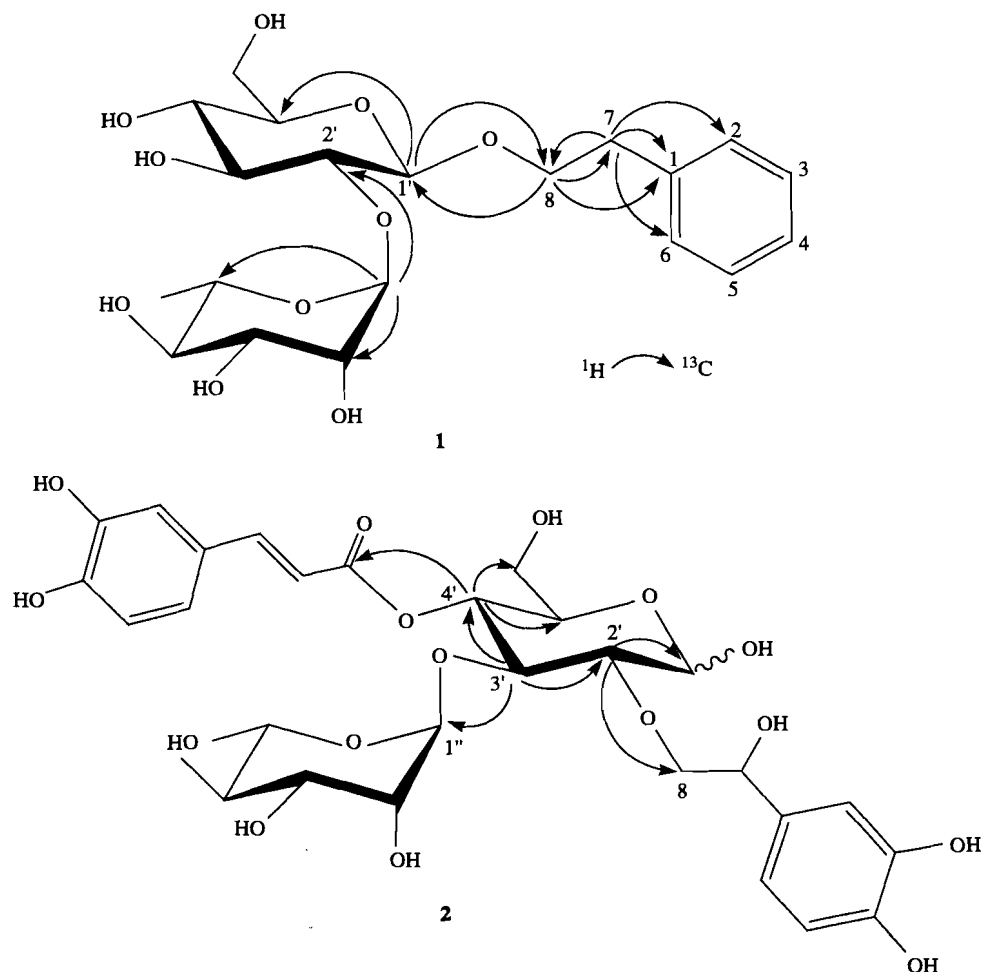


Figure 2. The key HMBC correlations of **1** and **2**.

on the results of HR-FAB-MS at  $m/z$  663.1901  $[M + Na]^+$ . The IR spectrum showed absorptions at 3397 (OH) and 1690 (C=O of COOH), 1450 (C=C), and 1048 (C–O–C)  $\text{cm}^{-1}$ . The  $^1\text{H}$ ,  $^{13}\text{C}$  NMR, COSY, and HMQC spectra of **2** showed the presence of two ABX system signals at  $\delta_{\text{H}}$  6.72 (1H, d,  $J = 6.5$  Hz), 6.82 (1H, br s), and 6.69 (1H, dd,  $J = 6.5, 1.5$  Hz) for the 3,4-dihydroxy- $\beta$ -phenyl moiety; and 6.78 (1H, d,  $J = 6.5$  Hz), 6.96 (1H, dd,  $J = 6.5, 1.5$  Hz), and 7.06 (1H, d,  $J = 1.5$  Hz) for the caffeoyl moiety, two *trans* olefinic protons as AB-type signals at  $\delta_{\text{H}}$  6.29 (1H, d,  $J = 13.5$  Hz) and 7.60 (1H, d,  $J = 13.5$  Hz), together with two anomeric protons at  $\delta_{\text{H}}$  4.55 (1H, d,  $J = 6.5$  Hz)/ $\delta_{\text{C}}$

99.0 and  $\delta_{\text{H}}$  5.17 (1H, br s)/ $\delta_{\text{C}}$  102.1. As in **1**, glucose and rhamnose in **2** were verified by TLC analysis after acid hydrolysis. The HMBC spectrum of **2** showed correlations between H-4' and C-3', C-5', C-6', C=O; between H-3' and C-2', C-4', C-1''; and between H-2' and C-1', C-4', C-8 $\alpha$ ; which suggested that the linkage of C-2', C-3', and C-4' of glucose were directly connected to C-8 of aglycone moiety, C-1'' of rhamnose, and C=O group of caffeoyl moiety, respectively (Figure 2). Thus, the structure of **2** was elucidated to be 2'-*O*-3,4-dihydroxy- $\beta$ -phenylethoxy-*O*- $\alpha$ -L-rhamno-pyranosyl-(1''  $\rightarrow$  3')-(4'-*O*-caffeoyl)- $\beta$ -D-glucopyranoside and named as toreno-side B.

Compounds **1–6** were tested for their antioxidant scavenging effects on DPPH. Our results demonstrated that **3–6** exhibited significant antioxidant effects with  $IC_{50}$  values of 5.34, 9.98, 10.81, and 5.97  $\mu\text{g/ml}$ , respectively, whereas compounds **1** and **2** were found inactive. It was noted that the compounds **3** and **6** had profound potent effect among these isolates. However, since **3** and **6** were more effective than those of **5**, the methene group at C-7 seemed to play a crucial role for the antioxidant activity.

### 3. Experimental

#### 3.1 General experimental procedures

The IR spectra were measured on a Mattson Genesis II spectrophotometer using a KBr matrix. The UV spectra were obtained on a Shimadzu UV-160A instrument. The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were performed on a Bruker NMR (Avance 400 MHz) and Varian NMR spectrometers (Unity Plus 500 MHz) using  $\text{CDCl}_3$ ,  $\text{CD}_3\text{OD}$ , and  $\text{C}_5\text{D}_5\text{N}$  as the solvent for measurement. FAB-MS data were performed on a Jeol SX-102A instrument. High-resolution FAB-MS was measured on a Finnigan/Thermo Quest MAT mass spectrometer. Silica gels (70–230 and 230–400 mesh, Merck, Darmstadt, Germany) were used for column chromatography and pre-coated silica gel (60 F-254, Merck) plates were used for TLC. The spots on TLC were detected by spraying with 5%  $\text{H}_2\text{SO}_4$  and then heating at  $100^\circ\text{C}$ . Preparative HPLC was performed using a reverse-phase column (Cosmosil 5SL-II column, 250 mm  $\times$  20 mm i.d., Kyoto, Japan) on a Shimadzu LC-6AD series apparatus with a RID-10A refractive index detector.

#### 3.2 Plant material

The whole *T. concolor* plants were collected in July 2006 in the mountain of Nantou County, Taiwan, and identified by Prof. Chung-Chuan Chen, School of Chinese Medicine Resources, China Medical University. A voucher specimen (No. CMU200607B1) had been

deposited in the Graduate Institute of Chinese Pharmaceutical Sciences, China Medical University, Taichung, Taiwan.

#### 3.3 Extraction and isolation

The air-dried *T. concolor* (3.89 kg) was extracted with methanol thrice at  $50^\circ\text{C}$ . The combined extracts were evaporated under a reduced pressure and yielded a residue (744.14 g), which was suspended in water and successively extracted with *n*-hexane,  $\text{CHCl}_3$ , and ethyl acetate (EtOAc), yielding *n*-hexane (129.07 g),  $\text{CHCl}_3$  (71.25 g), EtOAc (41.59 g), and aqueous (502.23 g) fractions. The  $\text{CHCl}_3$  layer was chromatographed over a silica gel column and eluted with  $\text{CHCl}_3$ –MeOH (1:0  $\rightarrow$  0:1) to get 10 fractions, C1–C10. Fraction C5 (8 g) was rechromatographed over Sephadex LH-20, eluted with  $\text{CHCl}_3$ –MeOH (1:2) to give six fractions, C5.1–C5.6. Fraction C5.3 was further separated and purified by silica gel, eluted with  $\text{CH}_2\text{Cl}_2$ –MeOH (1:0  $\rightarrow$  0:1) to give **14** (14.8 mg), **15** (7.9 mg), **16** (12.1 mg), and a mixture of **17** and **18** (30 mg). Fraction C5.4 (3 g) was chromatographed on silica gel, eluting with  $\text{CH}_2\text{Cl}_2$ –MeOH and then purified by HPLC (Cosmosil 5C<sub>18</sub>-AR II) to obtain **9** (2.3 mg), **10** (5.4 mg), **11** (6.9 mg), and **19** (19.6 mg) eluting with 65% MeOH and **12** (22.2 mg), **13** (14.0 mg), **14** (10.2 mg), **15** (9.0 mg) with 70% MeOH. The EtOAc layer was subjected to silica gel column chromatography eluting with increasing amount of EtOAc and then MeOH in *n*-hexane to give fractions E1–E9. Fraction E5 (5 g) was further separated by chromatography on a Sephadex LH-20 with MeOH to yield five fractions, E5.1–E5.5. Fraction E5.2 (750 mg) was further purified by HPLC (Cosmosil 5C<sub>18</sub>-AR II) with 55% MeOH to afford **2** (6.0 mg), **3** (7.0 mg), **4** (20.0 mg), and **5** (22.0 mg). Fraction E5.3 (300 mg) was chromatographed using HPLC (Cosmosil 5C<sub>18</sub>-AR II) with 45% MeOH to afford **1** (9.8 mg), **6** (3.6 mg), and **7** (17.0 mg). By using HPLC with 70% MeOH, **8** (23.0 mg) was obtained from fraction E5.5 (100 mg).

### 3.3.1 *Torenoside A (1)*

Brown solid (7.6 mg); UV (MeOH)  $\lambda_{\max}$  (nm): 229, 260, 290, and 330; IR (KBr)  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3398, 1672, 1460, and 1085;  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data: see Table 1; HR-FAB-MS  $m/z$ : 453.1733  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{20}\text{H}_{30}\text{O}_{10}\text{Na}$ , 453.1737).

### 3.3.2 *Torenoside B (2)*

Brown solid (5.3 mg); UV (MeOH)  $\lambda_{\max}$  (nm): 204, 266, 298, and 331; IR (KBr)  $\nu_{\max}$  ( $\text{cm}^{-1}$ ): 3397, 1690, 1450, and 1048;  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectral data: see Table 1; HR-FAB-MS  $m/z$ : 663.1901  $[\text{M} + \text{Na}]^+$  (calcd for  $\text{C}_{29}\text{H}_{36}\text{O}_{16}\text{Na}$ , 663.1907).

### Acknowledgements

The authors are thankful to Drs Yao-Haur Kuo and Hui-Chi Huang for the bioassay test in the National Research Institute of Chinese Medicine, Taipei, Taiwan.

### References

- [1] H.Y. Liu, *Flora of Taiwan*, 2nd ed. (Editorial Committee of the Flora of Taiwan, Taipei, 1993), p. 629.
- [2] W.S. Gan, *Medical Botany* (National Research Institute of Chinese Medicine, Taipei, 1993), p. 504.
- [3] Y.J. Zhang, D.L. WittDe, S. Murugesan, and M.G. Nair, *Life Sci.* **77**, 3222 (2005).
- [4] H. Franzyk, C.E. Olsen, and S.R. Jensen, *J. Nat. Prod.* **67**, 1052 (2004).
- [5] I. Saracoglu, M. Varel, U.S. Harput, and A. Nagaytsu, *Phytochemistry* **65**, 2379 (2004).
- [6] E. Kalpoutzakis, N. Aligiannis, S. Mitakou, and A.L. Skaltsounis, *J. Nat. Prod.* **62**, 342 (1999).
- [7] J. Wu, J. Huang, Q. Xiao, S. Zhang, Z. Xiao, Q. Li, L. Long, and L. Huang, *Magn. Reson. Chem.* **42**, 659 (2004).
- [8] L.H. Xie, T. Akao, K. Hamasaki, T. Deyama, and M. Hattori, *Chem. Pharm. Bull.* **51**, 508 (2003).
- [9] M.S. Alam, N. Chopra, M. Ali, and M. Niwa, *Phytochemistry* **41**, 1197 (1996).
- [10] S. Mochammad, Y. Kazuo, K. Ryoji, and T. Osamu, *Chem. Pharm. Bull.* **28**, 1006 (1980).
- [11] L.H. Xie, T. Akao, K. Hamasaki, T. Deyama, and M. Hattori, *Chem. Pharm. Bull.* **51**, 508 (2003).
- [12] K. Hisashi and O. Haruo, *Phytochemistry* **25**, 729 (1986).
- [13] H. Sasaki, H. Nishimura, M. Chin, and H. Mitsuhashi, *Phytochemistry* **28**, 875 (1989).
- [14] T. Yamagishi, D.C. Zhang, J.J. Chang, D.R. McPhail, A.T. McPhail, and K.H. Lee, *Phytochemistry* **27**, 3213 (1988).
- [15] J.S. Lai, J.Y. Huang, and K.F. Huang, *Chin. Pharm. J.* **48**, 451 (1996).
- [16] C.Y. Wang, A.M. Pamukcu, and G.T. Bryan, *Phytochemistry* **12**, 2298 (1973).
- [17] P.C. Kuo, C.C. Chiu, L.S. Shi, C.Y. Li, S.J. Wu, A.G. Damu, P.L. Wu, C.S. Kuo, and T.S. Wu, *J. Chin. Chem. Soc.* **49**, 113 (2002).
- [18] R.B. Anna, M. Jeannette, and M. Ivano, *Pharm. Acta Helv.* **71**, 191 (1996).
- [19] M. Ono, A. Yoshida, Y. Ito, and T. Nohara, *Phytochemistry* **51**, 819 (1999).