FULL PAPER

Two New Flavonoids from *Derris eriocarpa* How

by **Hua-Yong Lou**, **Hong-Guo Wu**, **Yong-Hua Tan**, **Jun-Jie Lan**, **Xiao-Pan Ma**, **Guang-Yi Liang**, **Ping Yi***, and **Wei-Dong Pan***

The Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences, Shachong South Road 202, Guiyang 550002, P. R. China (phone: +86-851-83805348; fax: +86-851-83805081, +86-851-83809055; e-mails: wdpan@163.com; yiping2100@aliyun.com)

Two new flavonoids, **1** and **2**, together with two known flavonoids, tephrosin (**3**) and 12a-hydroxy-α-toxicarol (**4**), were isolated from the whole herb of *Derris eriocarpa* How. The structures and absolute configurations of the new compounds were elucidated on the basis of their MS, NMR, and ECD data. The structures of the known compounds were established by extensive spectroscopic (MS, 1D- and 2D-NMR) analyses and comparison with the literature data. All compounds were isolated from *D. eriocarpa* for the first time. Compound **3** showed modest inhibitory activities against the growth of human cancer cells HEL and A549 with the IC_{50} values of 15.03 ± 0.62 and 13.27 ± 0.39 μM, respectively.

Keywords: *Derris eriocarpa*, Flavonoids, Anticancer activity.

Introduction

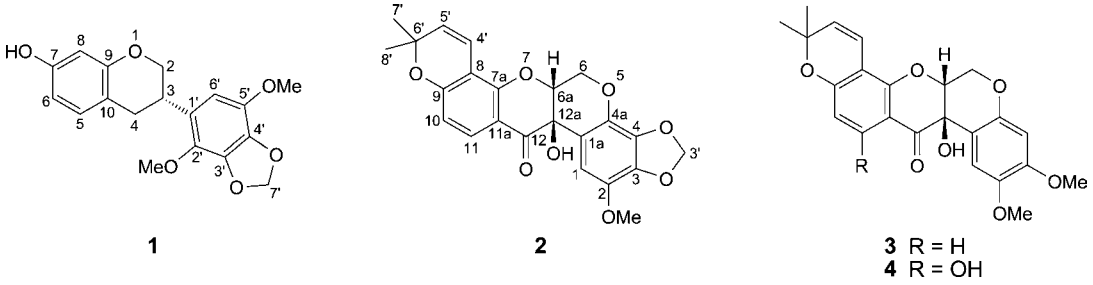
Derris eriocarpa How (Fabaceae) is a traditional Chinese medicinal herb mainly distributed in Guangxi and Yunnan Province [1]. Bioactivity studies on this herb indicated that it had extensive pharmacological activities against nephritis, cystitis, and urethritis [2][3]. Many chemical constituents have been isolated from this plant, such as saponins, steroids [4], stilbenoid [5], flavonoids [5 – 7], and triterpenoids [8]. For the purpose to find further bioactive lead compounds from *D. eriocarpa*, a detailed phytochemical investigation was carried out. As a result, two new flavonoids, **1** and **2**, together with two known flavonoids, **3** and **4** (*Fig. 1*) [9], were isolated from the AcOEt extract of *D. eriocarpa*. All compounds were found from this plant for the first time.

Results and Discussion

Compound **1** was obtained as yellowish oil. Its molecular formula was established as $C_{18}H_{18}O_6$ by HR-ESI-MS (neg.) at *m*/*z* 329.1023 ([*M* − H]$^-$), suggesting ten degrees of unsaturation. The ^{1}H-NMR spectrum of **1** (*Table*) exhibited a typical *ABX* system substitution on benzene with δ(H) 6.93 (*d*, *J* = 8.0, H–C(5)), 6.39 (*dd*, *J* = 8.0, 2.0, H–C(6)), and 6.36 (*d*, *J* = 2.0, H–C(8)). Moreover, the signals for two MeO groups at 3.91 (*s*) and 3.83 (*s*) were observed. The ^{13}C-NMR and DEPT spectra indicated the presence of 18 C-atoms, including two Me, three CH_2, five CH groups, and eight quaternary C-atoms. The –OCH_2O– unit was deduced from the signals at δ(H) 5.97 (*s*, H–C(7′)) and δ(C) 101.6 (C(7′)). All above information revealed that compound **1** might be a flavonoid. This structure was further confirmed by the ^{1}H,^{1}H-COSY and HMBC spectra (*Fig. 2*). The correlations from δ(H) 3.53 (*m*, H–C(3)) to 3.98 (*m*, H–C(2)) and 2.90 (*m*, H–C(4)) in the ^{1}H,^{1}H-COSY spectrum, as well as the correlations from δ(H) 2.90 (*m*, H–C(4)) to δ(C) 70.2 (C(2)), 32.3 (C(3)), 130.3 (C(5)), 155.0 (C(9)), 114.3 (C(10)), and 126.8 (C(1′)) in the HMBC spectrum indicate that **1** was an isoflavane. Further key HMBC correlations from δ(H) 3.91 (*s*) to C(2′) (δ(C) 136.4) and δ(H) 3.83 (*s*) to C(5′) (δ(C) 139.2) revealed that the two MeO groups were linked to C(2′) and C(5′), respectively. The key HMBC correlations from H–C(5) (δ(H) 6.93 (*d*, *J* = 8.0)), H–C(6) (δ(H) 6.39 (*dd*, *J* = 8.0, 2.0)), H–C(8) (δ(H) 6.36 (*d*, *J* = 2.0)) to C(7) (δ(C) 155.0) proved that the OH group was located at C(7) (*Fig. 2*). The HMBC correlations from CH_2(7′) (δ(H) 5.97 (*s*)) to C(4′) (δ(C) 135.6) and C(3′) (δ(C) 138.8) suggested that this group was connected with C(3′) and C(4′) (*Fig. 2*).

Compound **2** was isolated as yellowish oil. Its molecular formula was determined as $C_{23}H_{20}O_8$ from the HR-ESI-MS (*m*/*z* 423.1086 ([*M* − H]$^-$)), indicating 14 degrees of unsaturation, which was further confirmed by the ^{1}H- and ^{13}C-NMR data. The ^{13}C-NMR and DEPT spectra revealed the presence of three Me, two CH_2, six CH groups, and eleven quaternary C-atoms, including one C=O group (δ(C) 190.8) and one MeO group (δ(C) 56.8). The ^{1}H-NMR spectrum (*Table*) showed two aromatic H-atom signals (δ(H) 7.72 (*d*, *J* = 8.8), 6.48 (*d*, *J* = 8.8)) and two C=C bond signals (δ(H) 6.59 (*d*, *J* = 10.1), 5.57 (*d*, *J* = 10.1)). The presence of a –OCH_2O– unit was deduced

 DOI: 10.1002/hlca.201500247



1 **2** **3** R = H
4 R = OH

Fig. 1. Structures of compounds **1** – **4**.

Table. ^{1}H- and ^{13}C-NMR data of compounds **1** – **4**. δ in ppm, J in Hz.

Position	**1**[a])		**2**[b])		**3**[b])		**4**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1			6.27 (*s*)	105.0	6.57 (*s*)	109.8	6.71 (*s*)	109.1
1a				112.9		108.8		108.3
2	3.98 (*m*), 4.26 (*dd*, *J* = 10.0, 1.6)	70.2		138.7		144.2		144.0
3	3.53 (*m*)	32.3		137.8		151.4		151.2
4	2.90 (*m*)	31.1		135.9	6.48 (*s*)	101.3	6.46 (*s*)	101.1
4a				133.5		148.6		148.3
5	6.93 (*d*, *J* = 8.0)	130.3						
6	6.39 (*dd*, *J* = 8.0, 2.0)	108.0	4.70 (*dd*, *J* = 12.2, 2.4), 4.49 (*dd*, *J* = 12.2, 1.2)	63.8	4.63 (*dd*, *J* = 12.0, 2.5), 4.50 (*dd*, *J* = 12.0, 1.0)	64.0	4.61 (*dd*, *J* = 12.2, 2.0), 4.54 (*dd*, *J* = 2.2, 2.0)	63.6
6a			4.57 (*dd*, *J* = 2.4, 1.2)	76.0	4.57 (*dd*, *J* = 2.5, 1.0)	76.5	4.47 (*dd*, *J* = 12.2. 2.2)	75.6
7		155.0						
7a				156.5		156.8		155.5
8	6.36 (*d*, *J* = 2.0)	103.2		109.1		109.3		102.0
9		155.0		160.8		160.9		163.5
10		114.3	6.48 (*d*, *J* = 8.8)	111.9	6.47 (*d*, *J* = 9.0)	112.0	5.98 (*s*)	98.0
11			7.72 (*d*, *J* = 8.8)	128.5	7.73 (*d*, *J* = 9.0)	128.7		164.0
11a				111.0		111.3		99.8
12				190.8		191.5		194.8
12a				67.2		67.6		66.7
1′		126.8						
2′		136.4						
3′		138.8	6.01 (*d*, *J* = 13.0)	102.5				
4′		135.6	6.59 (*d*, *J* = 10.1)	115.3	6.60 (*d*, *J* = 10.0)	115.5	6.51 (*d*, *J* = 10.2)	115.0
5′		139.2	5.57 (*d*, *J* = 10.1)	128.9	5.55 (*d*, *J* = 10.0)	128.9	5.47 (*d*, *J* = 10.2)	126.6
6′	6.27 (*s*)	106.3		78.0		78.1		78.6
7′	5.97 (*s*)	101.6	1.45 (*s*)	28.3	1.45 (*s*)	28.4	1.43 (*s*)	28.4
8′			1.40 (*s*)	28.5	1.39 (*s*)	28.7	1.37 (*s*)	28.6
2-MeO			3.75 (*s*)	56.8	3.78 (*s*)	56.0	3.77 (*s*)	56.3
3-MeO					3.81 (*s*)	56.5	3.83 (*s*)	55.9
2′-MeO	3.91 (*s*)	60.3						
5′-MeO	3.83 (*s*)	57.1						
11-OH							11.63 (*s*)	

[a]) **1** was measured in $CDCl_3$ for ^{1}H (400 MHz) and ^{13}C (100 MHz). [b]) **2** – **4** were measured in $CDCl_3$ for ^{1}H (500 MHz) and ^{13}C (125 MHz).

from the signals at δ(H) 6.01 (*d*, *J* = 13.1) and δ(C) 102.5. All the above information indicated that the structure of compound **2** was similar to that of tephrosin (**3**) (*Table*) [10], which was further confirmed by the ^{1}H,^{1}H-COSY, HMQC, and HMBC spectra (*Fig. 2*). The key HMBC correlations from δ(H) 6.01 (–OCH_2O–) to C(3) (δ(C) 137.8) and C(4) (δ(C) 135.9) suggested that the –OCH_2O– unit was connected with C(3) and C(4) (*Fig. 2*).

The absolute configurations of **1** and **2** were elucidated by electronic circular dichroism (ECD) calculations [11 – 13]. The two enantionmers of **1** ((3*S*)-**1a** and (3*R*)-**1b**) and **2** ((6a*S*,12a*S*)-**2a** and (6a*R*,12a*R*)-**2b**) were calculated for ECD spectra. As a result, the pattern of the calculated ECD spectra of **1a** and **2b** were in good correspondence with the experimental data of **1** and **2**, respectively (*Fig. 3*). Thus, the absolute configurations of the new

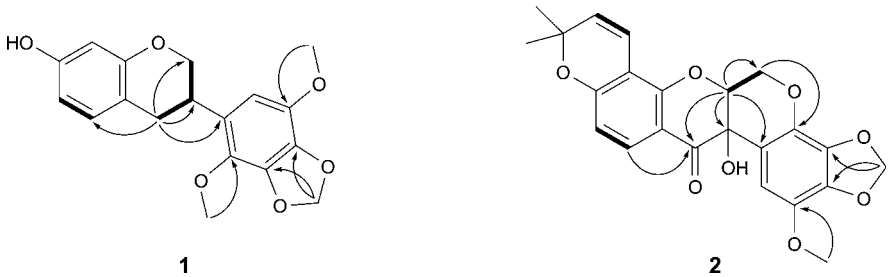


Fig. 2. $^{1}H,^{1}H$-COSY (▬) and key HMBC (H → C) correlations of **1** and **2**.

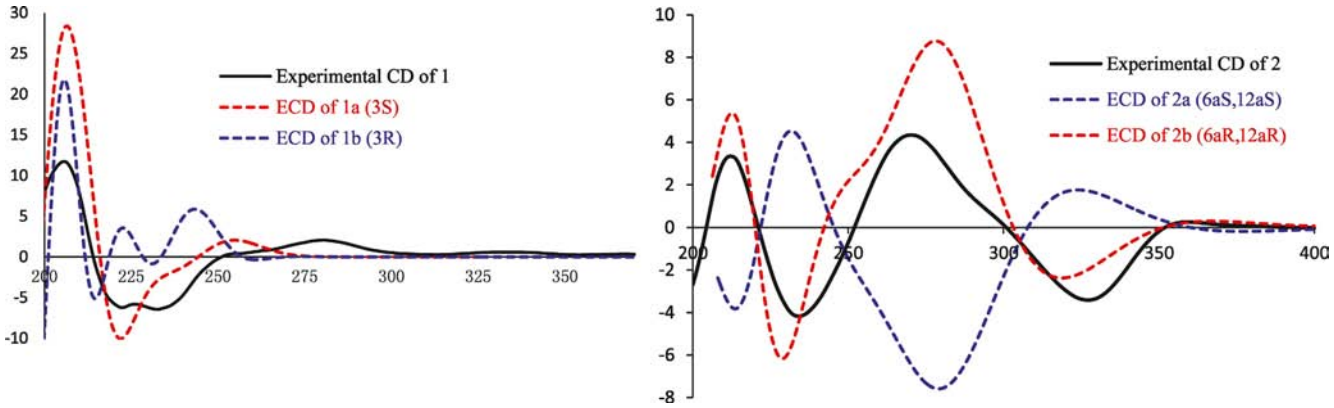


Fig. 3. Calculated and experimental ECD spectra of **1** and **2**.

flavonoids were established as (3*S*) in **1** and (6a*R*,12a*R*) in **2**.

In addition to **1** and **2**, the other two know compounds **3** and **4** were also isolated from *D. eriocarpa*. By comparison of the physical, MS, and NMR data with those of references [9][14], the structures of the two known compounds were identified as tephrosin (**3**) and 12a-hydroxy-α-toxicarol (**4**), respectively.

All compounds were tested for their *in vitro* activity against HEL (human erythro leukemia) and A549 (human lung cancer) cells [15][16]. As a result, compound **3** showed modest inhibitory activities against HEL and A549 cells, with the IC_{50} values of 15.03 ± 0.62 and 13.27 ± 0.39 μM, respectively.

This work was financially supported by the *National Natural Science Foundation of China* (81160390) and the *Science and Technology Department of Guizhou Province* (QKHRCTD [2015]4026) and (QKHLHZ [2015]7400). The authors are grateful to Prof. *Jian-Xin Zhang* and Prof. *Dao-Ping Wang* from the Key Laboratory of Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences for NMR and MS measurements, respectively.

Experimental Part

General

Thin layer chromatography (TLC): silica gel (SiO_2, 200 – 300 mesh; *Qingdao Ocean Chemical Factory*, P. R. China). Column chromatography (CC): SiO_2 (300 – 400 mesh; *Qingdao Ocean Chemical Factory*) and *Sephadex LH-20* (25 – 100 μm; *Amersham Biosciences*, Fairfield, USA). Semi-prep. HPLC: *Waters-600* machine with a *W2489* UV detector, column: ODS (5 μm, 10 × 150 mm; *Waters Co., Ltd.*, U.S.A.). Optical rotations: *Rudolph-IV* polarimeter equipped with a 2.5 cm pathlength cell at 30 °C. UV Spectra: *HP 8453* UV/VIS spectrometer (photodiode array type) in the wavelength range of 190 – 400 nm, in $CHCl_3$ soln.; λ_{max} (log ε) in nm. CD Spectra: *Applied Photophysics Chirascan* spectrometer equipped with a 1 cm pathlength cell; λ_{max} ($\Delta\varepsilon$) in nm. 1D- and 2D-NMR spectra: *Varian Inova-400* and *Wipm-500* spectrometer; δ in ppm rel. to Me_4Si as internal standard, J in Hz. MS: *HP1100-MSD* spectrometer (ESI-MS mode) and *microTOF-QII* instrument (HR-ESI-MS mode), resp.; in m/z. The computational ECD spectra were obtained by using the *Gaussian* 09 software package, the selected conformers were included for full geometry optimization at the B3LYP/6-31G** level in the gas phase. Further ECD calculations were performed at the B3LYP-SCRF (PCM)/6-31G** levels in MeOH soln.

Plant Material

The whole herb of *D. eriocarpa* How were collected from Xingyi, Guizhou Province, P. R. China, in June 2013, and identified by Prof. *Deyuan Chen* (Guiyang College of Traditional Chinese Medicine). A voucher specimen (No. 20130630) was deposited with the Key Laboratory of

Chemistry for Natural Products of Guizhou Province and Chinese Academy of Sciences.

Extraction and Isolation

The air-dried and powdered whole plant of *D. eriocarpa* How (15 kg) was extracted with 95% EtOH (3 × 20 l, 3 h each) by reflux, and after vacuum filtration, the filtrates were condensed using a rotary evaporator under reduced pressure. The residue was then suspended in hot water and extracted with petroleum ether (PE; 3 × 10 l), AcOEt (3 × 10 l) and BuOH (3 × 10 l), respectively. The AcOEt layer was evaporated under reduced pressure to yield a black extract (415 g), which was then subjected to CC on SiO_2 (PE/acetone 50:0 → 0:1) to provide six fractions, *Frs. 1 – 6*. *Fr. 3* (10.2 g) was further separated by CC on SiO_2 with PE/acetone as eluant to afford five fractions, *Frs. 3a – 3e*. *Fr. 3d* (33.5 mg) was purified by CC on *Sephadex LH-20* ($CHCl_3$/MeOH 1:1) to yield compound **1** (9 mg) as yellowish oil. *Fr. 3c* (120.9 mg) was further subjected to semi-prep. HPLC (MeOH/H_2O 60:40) to yield compounds **2** (11 mg), **3** (15 mg), and **4** (22 mg), respectively.

7-Hydroxy-2′,5′-dimethoxy-3′,4′-(methylenedioxy)isoflavane (= **(3*S*)-3-(4,7-Dimethoxy-1,3-benzodioxol-5-yl)-3,4-dihydro-2*H*-1-benzopyran-7-ol**; **1**) [17]. Yellowish oil. $[\alpha]_D^{30} = -13.89$ (c = 0.1, $CHCl_3$). UV ($CHCl_3$): 242 (3.43), 283 (3.31). CD (MeOH): 206 ($\Delta\varepsilon$ + 4.48), 223 ($\Delta\varepsilon$ − 2.78), 281 ($\Delta\varepsilon$ + 0.66). ^{1}H- and ^{13}C-NMR: see the *Table*. ESI-MS (pos.): 331 ($[M + H]^+$), 353 ($[M + Na]^+$), 683 ($[2M + Na]^+$). HR-ESI-MS (neg.): 329.1023 ($[M - H]^-$, $C_{18}H_{17}O_6^-$; calc. 329.1025).

12a-Hydroxy-2-methoxy-3,4-(methylenedioxy)deguelin (= **(5a*R*,13a*R*)-5a,13a-Dihydro-13a-hydroxy-15-methoxy-9,9-dimethyl-9*H*-1,3-dioxolo[7,8][1]benzopyrano[3,4-*b*]pyrano[2,3-*h*][1]benzopyran-13(5*H*)-one**; **2**) [17]. Yellowish oil. $[\alpha]_D^{30} = -13.52$ (c = 0.6, $CHCl_3$). UV ($CHCl_3$): 276 (3.69), 322 (3.23). CD (MeOH): 212 ($\Delta\varepsilon$ + 3.35), 234 ($\Delta\varepsilon$ − 4.17), 270 ($\Delta\varepsilon$ + 4.35), 327 ($\Delta\varepsilon$ − 3.41), 359 ($\Delta\varepsilon$ + 0.26). ^{1}H- and ^{13}C-NMR: see the *Table*. ESI-MS (pos.): 447 ($[M + Na]^+$), 871 ($[2M + Na]^+$). HR-ESI-MS (neg.): 423.1086 ($[M - H]^-$, $C_{23}H_{19}O_8^-$; calc. 423.1080).

Cytotoxicity Assay

The assay was performed to measure the cytotoxicity of the isolated compounds against A549 (human nonsmall cell lung carcinoma) and HEL (human erythro leukemia) cells. The cells were grown in DMEM medium supplemented with 10% FBS, 1% penicillin–streptomycin in a humidified incubator under 5% CO_2 at 37 °C. Cell suspensions (100 μl, containing 1 – 2 × 10^4 cells per well) were placed into 96-well microplates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition. A 100 μl aliquot of the test compounds at concentrations ranging from 0.1 to 128 μM was added to each well. The medium was replaced with one containing the test compounds, and the cells were further cultured at 37 °C. After incubation for 72 h, 10 μl of MTT soln. (*Amresco*) was added to each well, and the cells were incubated under the same conditions for 4 h until a purple precipitate was visible. DMSO (200 μl) was added and the optical density was measured at 490 nm in a microplate reader (*Bio-Tek Synergy HT*). 5-Fluorouracil (5-FU) and DMSO were used as the positive and negative controls, respectively. Each sample was tested in triplicate.

REFERENCES

[1] L.-F. Yang, W. Yu, D.-A. Yang, *J. Chin. Med. Mater.* **2006**, *29*, 1162.
[2] S.-F. Chen, S.-M. Yu, L.-Y. Huang, L.-C. Guo, D.-A. Yang, *Lishizhen Med. Mater. Med. Res.* **2009**, *20*, 2691.
[3] L.-C. Guo, D.-A. Yang, S.-M. Yu, L.-Y. Huang, S.-F. Chen, *Lishizhen Med. Mater. Med. Res.* **2010**, *21*, 154.
[4] H.-G. Wu, H.-Y. Lou, G.-Y. Liang, W.-D. Pan, *Chin. Tradit. Patent Med.* **2014**, *36*, 785.
[5] H.-X. Zhang, P.-K. Lunga, Z.-J. Li, Q. Dai, Z.-Z. Du, *Fitoterapia* **2014**, *95*, 147.
[6] L.-F. Yang, K. Wang, M.-G. Jiang, H.-C. Liu, X. Wang, P.-Y. Qin, Q.-L. Ouyang, *J. Asian Nat. Prod. Res.* **2015**, *17*, 1002.
[7] L.-X. Wang, H.-G. Wu, H.-Y. Lou, G.-Y. Liang, W.-W. Jiang, Z.-C. Yang, H. Zhang, W.-D. Pan, *China. J. Chin. Mater. Med.* **2015**, *40*, 45.
[8] X.-M. Zhang, Z.-R. Li, M.-H. Qiu, *Acta Bot. Yunnanica* **2002**, *24*, 787.
[9] J. N. Vasconcelos, G. M. P. Santiago, J. Q. Lima, J. Mafezoli, T. L. G. Lemos, F. R. L. Silva, M. A. S. Lima, A. T. Á. Pimenta, R. Braz-Filho, A. M. C. Arriaga, D. Cesarin-Sobrinho, *Quim. Nova* **2012**, *35*, 1097.
[10] V. U. Ahmad, Z. Ali, S. R. Hussaini, F. Iqbal, M. Zahid, M. Abbas, N. Saba, *Fitoterapia* **1999**, *70*, 443.
[11] W.-J. Tian, Y. Yu, X.-J. Yao, H.-F. Chen, Y. Dai, X.-K. Zhang, X.-S. Yao, *Org. Lett.* **2014**, *16*, 3448.
[12] J. Xu, Y. Sun, M. Wang, Q. Ren, S. Li, H. Wang, X. Sun, D.-Q. Jin, H. Sun, Y. Ohizumi, Y. Guo, *J. Nat. Prod.* **2015**, *78*, 1563.
[13] Y.-M. Fan, P. Yi, Y. Li, C. Yan, T. Huang, W. Gu, Y. Ma, L.-J. Huang, J.-X. Zhang, C.-L. Yang, Y. Li, C.-M. Yuan, X.-J. Hao, *Org. Lett.* **2015**, *17*, 2066.
[14] C. C. Andrei, P. C. Vieira, J. B. Fernandes, M. F. G. F. da Silva, E. R. Rodrigues-Fo, *Phytochemistry* **1997**, *46*, 1081; E. P. Clark, *J. Am. Chem. Soc.* **1931**, *53*, 313.
[15] H. Lou, S. Zheng, T. Li, J. Zhang, Y. Fei, X. Hao, G. Liang, W. Pan, *Org. Lett.* **2014**, *16*, 2696.
[16] M.-S. Zhang, F.-M. Yang, D.-P. Wang, J.-X. Zhang, Q.-Y. Sun, G.-Y. Liang, W.-D. Pan, *Phytochem. Lett.* **2012**, *5*, 96.
[17] P.-Z. Cong, S.-Y. Li, 'Natural Organic Mass Spectrometry', China Medieo-Pharmaceutical Science and Technology Press, Beijing, 2002.

Received September 28, 2015
Accepted January 20, 2016

